A FATAL CASE OF CEREBRAL MALARIA COMPLICATED WITH GEMELLA BERGERI BACTEREMIA: ROLE OF PLASMODIUM MITOCHONDRIAL COX3 GENE AND MALDI-TOF MASS SPECTROMETRY FOR SPECIES IDENTIFICATION

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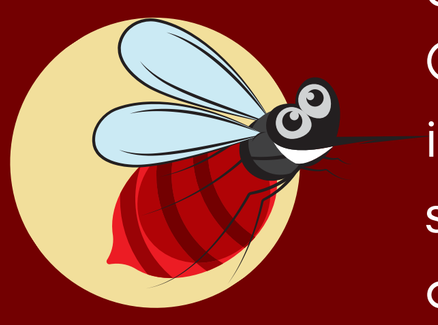
BACKGROUND

Here, we described a fatal case of a 37-year-old returning traveller from Burkina Faso, West Africa, who presented with an acute fitting episode later diagnosed as cerebral malaria induced by Plasmodium falciparum based on microscopic examination and Plasmodium mitochondrial cytochrome c oxidase III (cox3) gene PCR target. Unfortunately, the patient passed away due to severe malaria with multiorgan failure complicated with Gemella bergeri bacteremia. G. bergeri was identified using MALDI-TOF mass spectrometry.

METHODOLOGY

Preliminary evaluation of blood film malaria parasite showed coinfection of P. falciparum and Plasmodium malariae. Further PCR-based detection analysis using the Plasmodium mitochondrial cox3 gene was used to identify the malaria species. The patient's blood culture revealed gram-positive cocci in clusters, and further identification using MALDI-TOF Mass Spectrometry was done.

RESULTS & DISCUSSION



The patient blood sample was positive for P. falciparum based on analysis using a PCR assay targeting the cox3 gene. Further, analysis done at Makmal Kesihatan Awam Kebangsaan confirmed P. falciparum infection. His blood culture revealed gram-positive cocci in clusters, which was later identified as G. bergeri using MALDI-TOF mass spectrometry. Despite the initiation of intravenous artesunate and subsequent parasite count responding and showing parasite clearance, the complication is detrimental. As shown in our case, cerebral malaria induced by P. falciparum causes coma, long-term neurological consequences and death. The patient also had concomitant fatal G. bergeri bacteremia. Initial examination revealed that the patient had poor oral hygiene and a missing tooth which could be the source of Gemella infection, which is common in the oral cavity and a causative organism of septic shock.

CONCLUSION

This report is the first fatal case of cerebral malaria caused by P. falciparum coinfection with G. bergeri bacteremia. Therefore, it is critical to determine the exact aetiology for appropriate medical management. Plasmodium mitochondrial cox3 gene and MALDI-TOF Mass Spectrometry are useful for species identification, as illustrated in this case.

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NOTIFICATION OF ABSTRACT ACCEPTANCE

30 September 2022

Dear Dr Ummu Afeera Zainulabid,

We are delighted to inform you that your abstract has been accepted for e-poster session.

P-37		
Title	:	A fatal case of cerebral malaria complicated with *Gemella bergeri* bacteremia: Role of Plasmodium mitochondrial cox3 gene and MALDI-TOF mass spectrometry for species identification
Authors	:	Ummu Afeera Zainulabid, Nor Diyana Dian, Jauhary Effendy Juma'at, Petrick Periyasamy, Najma Kori, Cheah Saw Kian, Asrul Abdul Wahab and Zulkarnain Md Idris

Please refer to our website www.mdbd.usm.my for the presentation guidelines and delegate session/schedule. Please also let us know if you need further assistance.

Thank you in advance for your consideration in participating in MDBD 2022, and we very much look forward to meeting you soon.

Regards,

6th MDBD 2022 Scientific Committee

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THE 6TH INTERNATIONAL CONFERENCE ON

MOLECULAR DIAGNOSTICS & BIOMARKER DISCOVERY

BUILDING RESILIENCE IN BIOMEDICAL RESEARCH

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Welcoming remarks from Conference Advisor

Associate Prof. Dr. Aziah Ismail

Greetings and a warm welcome to the 6th International Conference on Molecular Diagnostics & Biomarker Discovery (MDBD 2022). Academics, medical and industry professionals, postdoctorates, post-graduates, researchers, scientists, and all other relevant individuals are welcome to attend this virtual conference. The theme of this conference, "Building Resilience in Biomedical Research," is co-organized with the Ministry of Higher Education (MOHE), Malaysia. The COVID19 pandemic has taught us a valuable lesson in emphasising the importance of knowledge and research in addressing a global health challenge. Building resilience in biomedical research is critical for addressing current health issues as well as planning for the future. We can identify pathogens, their transmission routes, and novel treatment and prevention strategies by generating and sharing knowledge.

In keeping with the theme, this conference will cover a wide range of topics including molecular diagnostics, infectious diseases, biomarker discovery, therapeutics, nanotechnology, clinical and companion diagnostics, genomics, proteomics, genetics, oncology, and tumour microenvironment. This conference is also intended to demonstrate the university's commitment to science and technology, as well as the spirit of knowledge-sharing. We hope you enjoy our comprehensive programme, which includes world-leading plenary speakers, early career researchers, academic, medical, and industrial experts, as well as oral and poster presentations. Please take advantage of this opportunity to boost knowledge sharing and networking among local and international delegates.

The conference programme is the result of many people's efforts. I would like to thank the Organizing Committee for their months of hard work, dedication, and commitment. We are also thankful to all of our sponsors for their kind support and contributions. Finally, I would like to thank all of the delegates who came to share their research. Please enjoy the conference.

Aziah Ismail

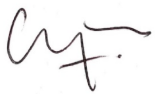
Message from MDBD Conference Chairperson

Dr. Tye Gee Jun

On behalf of the Organizing Committee of the 6th International Conference on Molecular Diagnostics & Biomarker Discovery (MDBD 2022), I would like to extend my heartiest and warmest welcome to all delegates from local and international universities, speakers and sponsors. My deepest appreciation to our distinguished speakers for kindly accepting our invitation; we are indeed honoured and fortunate to have outstanding international and local experts' present among us. We would also like to extend our deepest appreciation to Kementerian Pendidikan Tinggi Malaysia (KPT) under the HICoE programme for INFORMM who have been supportive of the MDBD conference series as our co-organizer. This conference is carried out to share advances in the biomedical field with emphasis in biomarker discovery, infectious diseases, nanotechnology, diagnostics, therapeutics, and many other health related fields especially research resilience after the COVID-19 pandemic. It is very challenging to pursue research in the current pandemic era, but it is an opportunity for research systems to improve and advocate the importance of their existence by being accountable in this crisis. Multidisciplinary collaboration is needed to ensure continuous biomedical research that offers solutions and development of effective interventions to achieve a better and more sustainable future for the entire humanity. Thus, the theme of the 6th MDBD conference is "Building Resilience in Biomedical Research".
This conference is structured to put together a multitude of keynote talks, symposia sessions, oral and poster communication sessions in both diagnostics and therapeutics. We hope that this three-day virtual conference will be a great platform for participants to connect with colleagues, share and exchange knowledge, generate new ideas and establish collaboration.

Finally, I'd like to extend my gratitude to the wonderful and extremely hardworking team of committee members that ensured that this conference would be a great experience for all of us. We hope everyone would have a great time here and gain as much as possible from it!

With best wishes,



PROGRAMME

DAY 1
11 OCTOBER 2022

0845 – 0900		**OPENING CEREMONY** *Associate Professor Dr Aziah Ismail, Director of INFORMM, Advisor of 6th MDBD Organizing Committee*
0900 – 0945		**CHAIRPERSON** *Associate Professor Dr Aziah Ismail* **KEYNOTE ADDRESS** **Emerita Professor Datuk Dr Asma Ismail *FASc*** *President of Academy of Sciences Malaysia*
0945 – 1030	Room Link (Main Hall)	**CHAIRPERSON** *Dr Fatin Hamimi Hamat@Mustafa* **PLENARY 1** **Professor Dr Mohd Adzir Bin Mahdi** *Lab-on-fiber Biophotonic Sensors*
1030 - 1045		**BREAK**
1045 - 1115		**CHAIRPERSON** *Dr Leow Chiuan Herng* **Technical Talk 1** **Dr Marshall Feteri (Genomax)** *Nanostring's Complete Spatial Solution*

1115 - 1300	**SYMPOSIUM 1A** **Diagnostic Platform** Room Link (Hall 1)	**SYMPOSIUM 1B** **Therapeutic Platform** Room Link (Hall 2)
	CHAIRPERSON *Prof Dr Gurjeet Kaur*	**CHAIRPERSON** *Assoc Prof Dr Sasidaran Sreenivasan*
	Professor Peter Hoffmann *Statistical methods and machine learning in the diagnosis of endometrial cancer*	**Dr Azhar Rasul** *Malic Enzyme 2 (ME2): Novel and selective target for cancer therapy*
	Dr Arutha Kulasinghe *Spatial genomics in the era of immunotherapy and COVID-19*	**Dr Rangsima Reantragoon** *Osteoarthritis in the immunological context*
	Mr Tang Kok Mun *Aptamers – Digitalizing Biotechnology for Global Diagnostics*	**Professor Ian Charles Paterson** *Sphingosine 1-phosphate signaling in cancer*
	Q&A	Q&A
1300 – 1400	**BREAK** **[e-POSTER DISPLAY & Q/A]**	

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Dr Noor Fatmawati Mokhtar

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Professor Dr Cheong Sok Ching
Cancer therapeutics: Drug repurpose</td></tr>
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Dr Khairul Mohd Fadzli Mustafa

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Dr Hui Foon Tan (Next Gene – Genscript) Vaccine development for emerging infectious diseases</td></tr>
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DAY 2
12 OCTOBER 2022

0845 - 0930	Room Link (Main Hall)	**CHAIRPERSON** *Dr Ong Ming Thong* **PLENARY 3** **Emeritus Professor Satvinder Singh Dhaliwal** *Logistic regression and predictive models in biomedical research, and its validity*		
0930 - 1000		**CHAIRPERSON** *Dr Leow Chiuan Herng* **Technical Talk 3** **Dr Lee Wei Kang (Codon Genomics)** *Digital Solutions and Platforms for Biomedical Research*		
1000 - 1015	**BREAK**			
1015 - 1215	**ORAL PRESENTATION 2A** Room Link (Hall 7)	**ORAL PRESENTATION 2B** Room Link (Hall 8)	**ORAL PRESENTATION 2C** Room Link (Hall 9)	
	CHAIRPERSON *Dr Nurulhasanah Othman*	**CHAIRPERSON** *Associate Prof Dr Choong Yee Siew*	**CHAIRPERSON** *Dr Daruliza Kernain*	
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1215 - 1245	Room Link (Main Hall)	**CHAIRPERSON** *Dr Khairul Mohd Fadzli Mustafa* **Technical Talk 4** **Ms Yolanda (3S Scientific)** *CytoFLEX SRT: The CytoFLEX That Sorts*		
1245 - 1345		**BREAK** **[e-POSTER DISPLAY & Q/A]**		
1345 - 1415		**CHAIRPERSON** *Dr Khairul Mohd Fadzli Mustafa* **Technical Talk 5** **Dr Tam Yew Joon (Biogenes)** *Rapid Synthesis of Oligonucleotides using Benchtop Synthesizer*		
1415 - 1700	**SYMPOSIUM 2A** **Diagnostic Platform:** Room Link (Hall 10)	**Symposium 2B** **Therapeutic Platform** Room Link (Hall 11)		

	CHAIRPERSON *Dr Tye Gee Jun*	**CHAIRPERSON** *Dr Eugene Ong Boon Beng*
	Professor Dr Mehmet Özsöz *CRISPR powered electrochemical biosensors*	**Associate Professor Dr Pongrama Ramasoota** *Therapeutic Antibody against E and Ns1 proteins of dengue virus*
	Dr Klaus Hochleitner *Selection of lateral flow test reagents using surface plasmon resonance*	**Dr Lai Hung Wei** *Role of transporters in cancer phototheragnostics*
	Dr Shimaa Hassan Eisaa *Aptamers in diagnostic electrochemical biosensors*	**Dr Mina Aminlou** *How ATMP helps the incurable diseases?*
	Q&A	**Q&A**
	End of Day 2	

DAY 3 13 OCTOBER 2022		
0845 – 1100	Room Link (Main Hall)	**FORUM** *Building Resilience in Biomedical Research* **Moderator** **Dr Tye Gee Jun** *INFORMM, Universiti Sains Malaysia MALAYSIA* **Panel** 1. **Professor Dr Norazmi Mohd Noor** *Universiti Sains Malaysia MALAYSIA* 2. ***Professor Ir. Dr Fatimah Binti Ibrahim*** *Universiti Malaya, MALAYSIA* 3. **Mr Ivan Hoh** *Founder & Chief Executive Officer, Codon Genomics, MALAYSIA* 4. ***Dr Low Ley Hian*** *Director of Clinical and Science Operations, InterVenn Biosciences, MALAYSIA*
1100 - 1130	Room Link (Main Hall)	**CHAIRPERSON** *Dr Leow Chiuan Herng* **Technical Talk 6** **Ms Lydiana Ng (Neoscience)** *Digital PCR: Next Generation Solution for Molecular Diagnostic*
1130 – 1200		**CHAIRPERSON** *Prof Dr Gurjeet Kaur* **ANNOUNCEMENT OF THE WINNERS** *Best poster and oral presenters, winner, and runner-up*
		CLOSING CEREMONY *Dr Tye Gee Jun Chair of the 6th MDBD Organizing Committee*
	End of Conference	

SPEAKERS

Keynote Speaker's Profile

Professor Emerita Datuk Dr. Asma Ismail, FASc
President, Academy of Sciences
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asmainformm@yahoo.com

Summary

Prof. Emerita Datuk Dr. Asma binti Ismail is the President of Academy of Sciences Malaysia, the Ibnu Sina Chair for Medicine at IIUM and has been appointed as the National Science, Technology and Innovation Advisor with effect from 23 June 2022. She has also been appointed on the Consultatative and Anti-Corruption Panel for the Malaysian Anti-Corruption Commission as of 1st July 2022.

Prof. Emerita Datuk Dr. Asma binti Ismail holds a BSc (Biology) from the University of Nevada, Reno, USA, M.A. (Microbiology) from Indiana University, USA and a Ph.D (Cellular and Molecular Biology) from University of Nevada, Reno, USA. She also received her Honorary Doctor of Science from the University of Glasgow, Honorary Degree Doctor of the University, Keele University and Honorary Doctorate in Literature from Kyoto University of Foreign Studies (KUFS).

Besides being the first female Vice-Chancellor of Universiti Sains Islam Malaysia (USIM) and Universiti Sains Malaysia (USM), Prof. Emerita Datuk Dr. Asma binti Ismail is also Malaysia's first female Director-General of Higher Education, first female President of Academy of Sciences Malaysia, first female to be appointed as the Chairperson of the Malaysian Qualifications Agency (MQA) and the first woman to be the National Science Advisor to the country. In addition, she was elected to the Academy of Sciences Malaysia in 2003, The Academy of Sciences for the Developing World (TWAS) in 2010, The Islamic World Academy of Sciences in 2016, and was elected as an Honorary Member of the Iranian Academy of Medical Sciences in 2017.

Prof. Emerita Datuk Dr. Asma binti Ismail's contribution to higher education and science development ecosystem are internationally recognised. She has initiated scientific discoveries which have led to the attainment of 16 patents that are commercialised worldwide, and the rapid diagnostic test for typhoid called TYPHIDOT, advocated by WHO. She had also obtained numerous awards and recognitions for her achievements at National and International levels. At the moment, she has shared her knowledge via more than 130 papers, 540 invited talks and more than 80 keynotes at national and global levels.

Prof. Emerita Datuk Dr. Asma binti Ismail has been elected as a member of several international boards including the Board of Governors for Commonwealth of Learning (COL) based in Vancouver, Canada, member of the Directing Board and Executive Committee for Islamic Citation Center based in Shiraz, Iran for the OIC, Member of the College of Fellows, Keele University and as a Governing Advisory Board Member for Ritsumeikan Asia Pacific University, Japan. She has been appointed as Co-Chair, for the Inter Academy Partnership Board comprising of 149 science academies world-wide in 2022 and elected as a member of the International Science Council Standing Committee for Science Planning in 2022.
At the national level she serves as a board member for CREST (Collaborative Research in Engineering, Science and Technology) Center to move STI based companies in the country since 2017, board member of IIUM Holdings Sdn. Bhd, member of the National Science Council chaired by the Prime Minister

and the High-Tech Nation Council chaired by the Minister of MOSTI (Ministry of Science, Technology and Innovation) and member of the National Action Council on Shared Prosperity Vision under the Distinguished Educator category.

She has also been a part of the selection panel for various international awards and recognitions such as TWAS membership (Medical and Health Sciences) and the Islamic Development Bank (IDB) Prize for Women's contribution to development. She was also part of the jury panel for the Merdeka Award, Rhodes Scholarship to select Malaysians to Oxford University, the Loreal Women in Science Award, the Anugerah Akademik Negara (National Academic Award), MOHE Entrepreneurial Award (MEA) and Best Managed Companies, Asia by Deloitte.

Prof. Emerita Datuk Dr. Asma binti Ismail was also awarded as "Tokoh Srikandi" National Award (Academic) in 2018, and "Tokoh Maulidul Rasul" (national level) in 2019, "Tokoh Akademik Negara "in 2022 for her outstanding contributions in the field of higher education, research and innovation as well as policy in the Science and Technology, industry locally and abroad.

Prof. Emerita Datuk Dr. Asma binti Ismail's contributions to Malaysia's higher education system include the establishment of the prestigious National Academic Award (Anugerah Akademik Negara), the establishment of Research Universities in Malaysia and in developing and implementing The Malaysian Education Blueprint (Higher Education) 2013 to 2025.

Conference talk title Building resilience in biomedical research

Speaker's Profile

Professor Mohd Adzir Mahdi
Universiti Putra Malaysia
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mam@upm.edu.my

Summary

Mohd Adzir Mahdi received the Bachelor degree with first class honors in Electrical, Electronics and Systems Engineering from the Universiti Kebangsaan Malaysia, Selangor, Malaysia in 1996. Later, he received the Master and Ph.D. degrees with distinctions in Optical Fiber Communications from the Universiti Malaya, Kuala Lumpur, Malaysia in 1999 and 2002, respectively. In January 2003, he joined the Faculty of Engineering, Universiti Putra Malaysia, Selangor, Malaysia where he was an Associate Professor and is now a full Professor. Prior to the current appointment, Adzir was an optical design engineer at IOA Corporation, Sunnyvale, USA and a research officer at Research and Development Division, Telekom Malaysia Berhad. Since 1996, he has been involved in photonics research specializing in optical amplifiers and lasers. He has authored/co-authored over 470 scientific papers in journals. His research interest includes optical fiber lasers, optical sensors and nonlinear optics.

Conference talk title

Lab-on-fiber Biophotonic Sensors

Abstract

The emergence of photonic sensors in distinguished fields of physical, biological, and chemical sensing has been attracting intensive research interest in recent decades. Due to their high sensitivity, immunity to electromagnetic interference, low loss, and simplicity, sensors utilizing optical components have been proven to be reliable for structural monitoring, disease detection and material identification. Among the preferred alternatives is a non-uniform cylindrical waveguide known as microfiber. The fabrication of a microfiber is based on a heat and pull mechanism that is applied simultaneously on a standard optical fiber to create a tapered segment. As light travels along this region, a portion will be excited to form evanescent waves that can be manipulated for the benefit of optical sensing. The interaction of this wave and its surrounding material is very unique and measurable in terms of either light absorption or wavelength shift. Moreover, with the addition of bio-receptor layer deposited onto the microfiber surface, the specificity of the sensor towards a precise target of interest can be greatly enhanced. With such advantages, this "Lab-on-fiber" microfiber-based sensor has displayed promising performance in biosensing applications such as Dengue virus and Leptospira bacteria.

Speaker's Profile

Professor Sok Ching Cheong, FASc
Cancer Research Malaysia
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Summary

Professor Cheong leads the Translational Cancer Biology and Digital Health Research Units at Cancer Research Malaysia. She is also the Deputy Chief Scientific Officer at Cancer Research Malaysia and the current Dr. Siti Hasmah Mohd Ali Professorial Chair at the University of Malaya, Kuala Lumpur. Her research aims to improve the management and survival of cancer patients through the understanding of the underlying molecular changes, and through the development of novel treatment approaches, focusing on head and neck cancers. These include the development of immunotherapy based on head and neck cancer antigens. Further, using CRISPR-Cas9 functional screens, her team has identified novel targets for head and neck cancer that are currently being studied. Professor Cheong has received grants from national and international funding bodies including the Newton-Ungku Omar Fund, Newton Fund Impact Scheme, Global Challenges Research Fund and funds from the Ministry of Science, Technology and Innovation, amongst others. In recognition of her research contributions, she has received several national and international scientific awards, most recently the President's Award by the International Association of Oral Maxillofacial Pathologists (IAOP).

Conference talk title

Identifying essential genes in oral cancer development

Abstract

Oral cancers affect more than 350,000 individuals annually and the majority of the cases are diagnosed in Asia. Whilst the characterization of the cancer genome can reveal key actionable mutations that have impacted the clinical treatment of some cancers, such mutations are not commonly found in oral cancers. Hence, the development of targeted therapies for head and neck cancers has been lagging behind compared to other cancers. Although the epidermal growth factor receptor (EGFR) inhibitor (cetuximab) and immunotherapy (pembrolizumab and nivolumab) have been approved for the treatment of OSCC, the response is seen only in a limited subset of patients underscoring the need for more effective therapies. One way of identifying potential therapeutic targets in cancer is to understand the genetic vulnerabilities within a cancer cell. We used high-throughput CRISPR/Cas9 screens to identify the genetic vulnerabilities of oral cancer using unique Asian oral cancer cell lines. I will share our findings on oral cancer essential genes with different degrees of tractability. Combining this with high-throughput drug response data on the same cell lines, we identified candidate drugs for drug repurposing for oral squamous cell carcinoma.

Speaker's Profile

Emeritus Professor Satvinder Singh Dhaliwal
Institute for Research in Molecular Medicine (INFORMM)
UNIVERSITI SAINS MALAYSIA (USM)
satvinder.dhaliwal@usm.my

Summary

Professor Dhaliwal has worked as a Biostatistician for the past 30 years and has accumulated extensive experience, both nationally and internationally, on the practical application of Statistics/Biostatistics in a wide variety of situations. Prof Dhaliwal's research interest includes the application of Biostatistics in the fields of Public Health Research and Clinical Research. He specializes in the development of Clinical Prediction Models in Medical Research. Prof Dhaliwal has published in excess of 200 journal articles (Google Scholar H-index is 50 with over 9,400 citations). His publications have had a significant impact and were often the result of successful national and international research collaborations. Prof Dhaliwal is an Emeritus Professor of Biostatistics and Health Research at Curtin University, Australia. Prof Dhaliwal also holds and Adjunct Professor position with Duke-NUS Medical School in Singapore and is a Professor (Research and Statistics) at Singapore University of Social Sciences. Prof Dhaliwal is also commissioned as a Consultant Statistician to various private and government organizations in Australia and Singapore.

Conference talk title

Logistic regression and predictive models in biomedical research, and its validity

Abstract

Regression techniques are versatile in their application to biomedical research because they can measure associations, predict outcomes, and control for confounding effects of variables. Logistic Regression, in particular, is an efficient and powerful way to analyze the effect of a group of independent variables on a binary outcome variable. The strongest linear combination of variables with the greatest probability of detecting the observed outcome can be identified. Important considerations when conducting logistic regression include selecting independent variables, ensuring that relevant assumptions are met, and choosing an appropriate model building strategy. The fit of the resulting logistic regression will also have to be assessed. Results for independent variables are typically reported as odds ratios (ORs) with 95% confidence intervals (CIs). Sensitivity, Specificity and Area Under the ROC curve are also computed. Prior to reaching definitive conclusions from the results of any of these methods, one should formally quantify the model's internal validity (i.e., replicability within the same data set) and external validity (i.e., generalizability beyond the current sample).

Speaker's Profile

Dr Rangsima Reantragoon
Chulalongkorn University
THAILAND
rangsima.reantragoon@gmail.com

Profile summary

Rangsima Reantragoon, M.D., Ph.D. is an associate professor at the Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Thailand. She graduated with a Bachelor's Degree in Medicine, Chulalongkorn University and went on to further her studies as a PhD in Microbiology and Immunology from the University of Melbourne, Australia. Her previous working experiences include demonstrations at the Gene Technology Access Centre (GTAC) and postdoctoral training at the Peter Doherty Institute for Infection and Immunity. She has much researched in MAIT cells as an immunologist and have now further implemented T cell immunology research into mainly osteoarthritis (OA).

Conference talk title

Osteoarthritis in the immunological context

Abstract

Osteoarthritis pathogenesis encompasses a multidisciplinary approach to its understanding. These include biomechanics, biochemical changes and inflammation of the host. Here, we focus on the immune responses that occur within osteoarthritic patients, covering both innate and adaptive immunity and inflammation within the host.

Speaker's Profile

Dr Arutha Kulasinghe
University of Queensland (Diamantina Institute)
AUSTRALIA
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Profile summary

Dr Arutha Kulasinghe is a Peter Doherty NHMRC Research Fellow and leads the 'Clinical-oMx Lab' at the University of Queensland. Dr Kulasinghe has pioneered spatial transcriptomics using digital spatial profiling approaches in the Asia-Pacific region, contributing to world-first studies for lung cancer, head and neck cancer, and COVID-19. His research aims to understand the underlying pathobiology by using an integrative multi-omics approach. Dr Kulasinghe has published his research in 60 manuscripts and is supported by the NHMRC, Australian Academy of Sciences, Cancer Australia, Cure Cancer, MRFF and numerous philanthropic and hospital foundations.

Conference talk title

Spatial genomics in the era of immunotherapy... and COVID-19

Abstract

Lung cancers remain the leading cause of cancer related mortality and have a poor 5-year survival. Immunotherapies have led to durable benefit in a cohort of non-small cell lung cancer (NSCLC) patients. Identifying those patients likely to achieve benefit remains a clinical unmet need. Whilst predictive biomarkers such as PD-L1 and tumour mutation burden (TMB) have shown utility, the underlying tumour-immune biology is unlikely represented. The composition and activation status of the cellular milieu contained within the tumour microenvironment (TME) is becomingly increasingly recognised as a driving factor dictating response to immunotherapies. In this study, we employed multiplex IHC (mIHC), and digital spatial profiling (DSP) to capture the targeted immune proteome and transcriptome of tumour and TME compartments from ICI-treated (n=41) and standard of care (n=47) NSCLC patient cohorts. Oncotopix® Discovery was also used to analyse the highplex imagery. The analysis pipeline consisted of tissue segmentation (tumor, stroma, necrosis, etc), nuclear detection using a deep-learning algorithm for DAPI, a threshold-based cellular phenotyping step, and spatial analyses. We demonstrate by mIHC that the interaction of CD68+ macrophages with PD1+, FoxP3+ cells is enriched in ICI refractory tumours (p=0.012). Patients sensitive to ICI therapy expressed higher levels of IL2 receptor alpha (CD25, p=0.028) within the tumour compartments, which corresponded with increased IL2 mRNA (p=0.001) within their stroma. IL2 mRNA levels within the stroma positively correlated with the expression of pro-apoptotic markers cleaved caspase 9 (p=2e-5) and BAD (p=5.5e-4) and negatively with levels of memory T cells (CD45RO) (p=7e-4). Immuno-inhibitory markers CTLA-4 (p=0.021) and IDO-1(p=0.023) were supressed in ICI-responsive patients. Tumour CD44 (p=0.02) was depleted in the response group and corresponded inversely with higher stromal expression of one of its ligands, SPP1(osteopontin, p=0.008). Cox survival analysis indicated tumour CD44 expression was associated with poorer prognosis (HR=1.61, p=0.01), consistent with its depletion in ICI sensitive patients. The SOC cohort paralleled similar roles for immune checkpoints and pro-apoptotic markers, with LAG3 (HR=3.81, p=0.04) indicating poorer outcome, and BIM (HR=0.16, p=0.014) with improved outcome. Through multi-modal approaches, we have dissected the characteristics of NSCLC treatment groups and provide evidence for the role of several markers including IL2, CD25, CD44 and SPP1 in the efficacy of current generations of ICI therapy. The signatures are being validated in prospective larger cohort studies.

Speaker's Profile

Dr Klaus Hochleitner
Global Lead Technology Product Specialist, Cytiva (formerly known as GE)
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Profile summary

Dr. Klaus Hochleitner is Global Technology Product Specialist at Cytiva in Germany. He is working on lateral flow test systems for about 30 years, and currently responsible for supporting new test developments in collaboration with different academic and professional clients.

Conference talk title

Selection of lateral flow test reagents using surface plasmon resonance

Abstract

Lateral flow tests are different from most other immunodiagnostic test systems by the very short interaction times the capture and detector reagents have to bind to their analyte. This requires the selection of reagents that have very fast on-rates and off-rates that are compatible with the intended test duration time. These kinetic data can be generated using surface plasmon resonance. The technology allows for a label-free analysis of reagent properties, and also for selecting antibody pairs for sandwich assays as well as lateral flow membrane family selection. We will present examples of reagent and membrane selection experiments.

Speaker's Profile

Dr Lai Hung Wei
Kochi University
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Summary

Dr. Lai is a USM alumni currently working as a Senior Scientist in the Center for Photodynamic Medicine (CPDM), Kochi University. For seven years, Lai has been working on aminolevulinic acid (ALA)-mediated phototheragnostics – the treatment and diagnosis of cancer using light. He identified protein transporters responsible in the uptake of ALA, which leads to false positive incidence and cytotoxicity towards normal cells. He was recently awarded the "Research Excellence Award" for his research in identifying new method to enhance the efficacy of phototheragnostics. Lai's current affiliation, CPDM, is the pioneer in the field and is the first to treat bladder cancer patients using photodynamic diagnosis with orally administered ALA in Japan. Lai is also the Head Coordinator of Sakura Science Club, Japan Science & Technology, where he is actively involved in organizing various exchange programmes in Japan for students abroad since 2017.

http://www.kochi-u.ac.jp/kms/CPDM/greetings-en.html

Conference talk title

Role of transporters in cancer phototheragnostics

Abstract

Phototherapy, the use of light as a therapy for various diseases, have been introduced since the ancient civilizations and underwent ages of prosperity and decline throughout history. Today, with the addition of photosensitizer such as aminolevulinic acid (ALA), light is being used for cancer diagnosis and therapy as an alternative for standard cancer treatment regimen such as chemotherapy due to its high specificity and low side effect nature. This method for treating cancer is known as phototheragnostics, which comprises of photodynamic therapy (PDT) and photodynamic diagnosis (PDD). Despite its effectiveness in treating cancer, several studies have suggested potential false positive incidence and undesirable phototoxicity towards surrounding normal cells. The culprit behind these phenomena was found to be the high expression of protein transporters involved in uptake of ALA in normal cells. The authors suppressed the expression of these uptake transporters through the usage of common drugs, such as ibuprofen and tryptophan, without affecting the effectiveness of ALA-PDT and PDD towards cancer. On the other hand, novel drug that could enhance the effectiveness of ALA-PDT through the inhibition of transporters responsible in the efflux photosensitizers, particularly towards PDT-resistant cancers, has been identified. Based on these findings, it is believed that the roles of uptake and efflux transporters are pivotal to maximize the effectiveness and specificity of PDT and PDD towards cancer.

Speaker's Profile

Dr Mina Aminlou
CellTech Pharmed Company
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Profile summary

Dr Mina Aminlou MD. PhD is the Medical Director and CRA of CellTech Pharmed company, the largest manufacturer of the stem cell therapy products in middle east and west Asia. The principal goal of this company is the mass production of Allogeneic mesenchymal stem cells specially derived from umbilical cord for various purposes such as Cerebral Palsy, GvHD, Osteoarthritis, ALA, MS, Autism, post MI heart failure and so on. Most of these diseases are incurable and surprisingly MSCs have shown remarkable therapeutic and palliative effect on them. As these products are investigational so for every indication every phase of clinical trials must be performed and the national FDA approval is mandatory before entering the market. Aminlou as a CRA is responsible for coordination and checking the integrity of clinical trials under regulatory guidelines and inspections. Email: aminlou@celltech.co

Conference talk title

How ATMP helps the incurable diseases?

Abstract

Advanced Therapeutic Medicinal products (ATMPs) have received a lot of attention in recent years. They include gene-therapy medicines, cell- therapy medicines and tissue engineered products. They may be used in combination forms. These are very different in comparison to chemical medicined from the aspect mechanism and activity and also the guidelines and regulations of the administration. Like other pharmaceutical products, cGMP production is necessary and the investigators who are prescribing the IMPs should be GCP certified. Although most of the patients in need of these products are struggling with incurable diseases but the administrations must be monitored by the national FDA regulatory in order to avoid any misuse or abuse. As mentioned above cell- therapy is a classified as ATMPs. Somatic cells specially the mesenchymal stem cells derived from different sources are the main cell therapy products. Several sources have been introduced but in recent years the umbilical derived MSC are getting more attention because of their valuable characteristics. These cells are obtained from the umbilical cord of healthy neonates and after cell extraction, processing and expansion they are cryopreserved and stored in the banks. While a patient needs the product, a physician orders the approved amount of the cell therapy product. All steps are performed in sterile and standard condition and only the pre-determined safe and efficient products are preferred to use.

Speaker's Profile

Dr. Azhar Rasul
Government College University Faisalabad
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Profile summary

Dr. Azhar Rasul is an Assistant Professor at Faculty of Life Sciences, Government College University Faisalabad. He received MSc and M. Phil degree in Biology from Bahauddin Zakariya University, Multan, Pakistan. He obtained PhD fellowship jointly awarded by Ministry of Education (MOE), Pakistan and China Scholarship Council (CSC), China and completed his Ph.D. in Cell Biology (Chemical Cancer Biology) from Northeast Normal University, China. He later received China postdoctoral fellowship in 2012, Japanese Society for Promotion of Science (JSPS) Postdoctoral fellowship in 2013, and subsequently Tokyo Biochemical Research Foundation (TBRF) fellowship in 2015. He has published over 153 peer-reviewed articles with cumulative impact factor over 336 and with
over 3482 citations. He also has published eleven book chapters. He has presented several invited talks at National and International level. He has attended more than 20 International conferences in Japan, China, South Korea, Dubai, Sri Lanka, Qatar, Turkey, Thailand, Malaysia and France. Under his supervision, thirteen MPhil students completed their research work. Three PhD and two MPhil students are currently working under his supervision. He has obtained several national and international research grants (HEC-Pak Turk Mobility Grant, ISSESCO RG, HEC-NRPU RG(s), The Nagai Foundation Tokyo RG(s) and COMSTECH-TWAS RG). His lab is actively engaged in interdisciplinary research on novel tumor biomarkers, cancer-related health disparities, identification of non-toxic anticancer compounds for various hallmarks of cancer (cancer stem cells, cancer cell metabolism, and tumor hypoxia) from natural sources and development of highly efficient green extraction methodologies for preparation of active constituent enriched-bioactive extracts libraries. He is reviewer and editorial board member of several well-reputed journals.

Conference talk title

Malic Enzyme 2 (ME2): Novel and selective target for cancer therapy

Abstract

The development of cancer-specific therapeutics has been limited because most of healthy cells and cancer cells depend on common pathways. The recent evidence suggests that targeting the cancer specific metabolic and mitochondrial remodelling may offer selectivity in cancer treatment. Malic enzyme 2 (ME2) is predominantly overexpressed in a number of tumor types and inhibition of ME2 results in decreased tumor growth. Reversing the mitochondrial suppression and the increased glucose consumption in cancer cells is an important step and has great potential for therapeutic drug developments. Therefore, we performed library screen to discover novel inhibitors of tumor metabolic enzyme, ME2, for cancer treatment. Here, progress regarding screening for ME2 inhibitors will be reported.

Speaker's Profile

Dr Shimaa Eissa
Alfaisal University
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Profile summary

Dr. Shimaa Eissa obtained her Ph.D. degree in Science of Energy and materials from INRS-EMT, Varennes, Canada. Her research is focused on the development of electrochemical biosensors for various foodborne, diagnostic, and environmental applications exploiting aptamers as novel biorecognition receptors as well as nanomatrials as transduction elements. Dr. Shimaa was a visiting researcher at Université du Québec à Montréal (UQAM) for 2 years. In 2016, she joined the Chemistry Department, Alfaisal University, Riyadh KSA as an assistant research professor. Dr. Shimaa has published over 65 articles with more than 2300 citations. She received several awards including the L'Oréal-UNESCO "For Women In Science"-Middle East award 2016. She supervised several graduate and undergraduate students in various research projects. She is a member in different scientific organizations including the Arab, German young academy of Sciences (AGYA).

https://scholar.google.ca/citations?user=jlAURowAAAAJ&hl=en

Conference talk title

Aptamers in diagnostic electrochemical biosensors

Abstract

Aptamers are single stranded DNA or RNA molecules selected in vitro from large libraries of synthetic random sequences. The first aptamers have been reported in 1990 and much progress has been made to date. Many aptamers have been selected against wide range of targets, including proteins, small molecules, viruses and cells. Aptamers as recognition receptors showed several advantages over antibodies particularly in disease diagnosis. Aptamers are prepared by in vitro selection procedure without using experimental animals and they can be developed even against low molecular weight metabolic biomarkers which is hardly achievable with antibodies. Aptamers can be selective to different parts of the target molecules and can be easily synthesized with high purity, reproducibility at very low cost, in contrast to antibodies that needs experimental animals and suffers from batch-to-batch reproducibility. The aptamers can be chemically modified easily by various chemical tags allowing the immobilization of aptamers onto various solid supports and they are also highly stable at different conditions. Due to these advantages, aptamers as recognition receptors have received considerable attention as novel capturing agents to replace antibodies in biosensors for various diagnostic applications. Aptamer-based sensors (aptasensors) have been designed using different strategies, particularly the conformation change of the aptamer upon binding with the target. Moreover, the binding of aptamers to their complementary nucleic acids can be exploited in sensing schemes. Aptamers had been integrated in various electrochemical Biosensors for diagnostic applications combining the high selectivity of the biorecognition processes with the high sensitivity, possibility of multiplexing, capability of miniaturization and low cost of electrochemical transducers. Moreover, unlike optical biosensors, electrochemical biosensors are not affected by sample turbidity, or interference from fluorescing compounds commonly found in biological samples. Here, we discuss the utilization of aptamers in various electrochemical biosensors for diagnostic applications.

Speaker's Profile

Tang Kok Mun
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Profile summary

Mr. Tang Kok Mun is the co-founder and CEO of Biogenes Technologies, a Malaysian-based company focusing on development and commercialisation of aptamer-based molecular diagnostic technologies for applications in human healthcare, animal healthcare, agricuture, aquaculture, food safety and pollution monitoring. Mr Tang have over 15+ years of experience in bringing technologies from R&D stage to commercial markets. Some of his earlier startups and projects are in technology areas such as smart water monitoring system, biomass utilization for renewable energy and bio-based chemicals, as well as genomics in aquaculture. Mr. Tang majors in Chemical Engineering and Master in Business Administration from University of Malaya, as well as having graduated from Stanford University Center of Professional Development in Entrepreneurship. He also holds the position of Industrial Fellow in Universiti Sains Malaysia under Institute for Research in Molecular Medicine (INFORMM), and serving as one of panel experts in Malaysian Rubber Council (MRC).

Conference talk title

Aptamers – Digitalizing Biotechnology for Global Diagnostics

Abstract

The recent COVID-19 has highlighted the grave inequality in access to healthcare diagnostics between rich and poor nations. Biogenes believes that the adoption of a digital form of biotechnology is able to expand the access of healthcare diagnostic to all corners of the world. This is made possible with the development and use of oligo-based aptamers instead of antibodies as the capturing element in diagnostic products. By bringing aptamer design, development and synthesis into the digital realm, we attempt to create an eco-system of researchers, entrepreneurs, healthcare practitioners, global health bodies and governments of nations to enable everyone in this world to have affordable and rapid access to healthcare diagnostics

Speaker's Profile

Professor Ian Paterson
University of Malaya
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Profile summary

Ian Paterson is a professor of cancer biology at the University of Malaya. He obtained his BSc degree in biology from the University of London and his PhD in biochemistry from the University of Bath. From 1992 to 2010 he worked at the University of Bristol, UK, where he developed his interest in cancer biology. Prof Paterson has studied the molecular pathogenesis of head and neck cancer, and oral squamous cell carcinoma in particular, for more than 20 years and has published more than 100 articles in ISI journals. For the past 10 years, he has worked at the University of Malaya where he continues to focus on the molecular basis of head and neck cancers, including oral and nasopharyngeal carcinomas.

https://umexpert.um.edu.my/ipaterson.html

Conference talk title

Sphingosine 1-phosphate signaling in cancer

Abstract

Sphingosine 1-phosphate (S1P) is a small bioactive lipid that is formed when sphingosine is phosphorylated by sphingosine kinase 1 and 2 (SK1/2). S1P exerts its effects following binding to a family of five G protein-coupled receptors, known as S1P1–5. Following receptor activation, multiple signalling cascades are activated, allowing S1P to regulate a range of cellular processes, such as proliferation, apoptosis, migration and angiogenesis. Over the past 15 years, evidence has accumulated to demonstrate the involvement of SKs and S1P receptors in cancer progression; the oncogenic effects of S1P can result from alterations in the expression of one or more of the S1PRs and/or the enzymes that regulate the levels of S1P. More recently, S1P signalling has also been shown to influence the behaviour of cells within the tumour microenvironment to create a permissive environment for tumour growth. Consequently, components of the S1P signalling pathway represent targets for potential therapeutic intervention in the treatment of cancer. The role of S1P signalling in cancer will be discussed along with opportunities to target this pathway therapeutically.

Speaker's Profile

Professor Mehmet Özsöz
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Profile summary

Prof Dr. Mehmet Özsöz received the BS degree from the Department of Chemical Engineering, Middle East Technical University,Ankara, Turkey, PhD in Analytical Chemistry from Faculty of Pharmacy, Ege University. He has been as a post doc for two years in the area of Electrochemical Biosensor in USA. He has been as visiting scientist in Lancaster University of Chemistry Department. He has authored or co-authored over 150 scientific publications with h factor of 54 and he has given over 50 invited lectures and conference contributions world-wide. Some of his publications have appeared in very reputable scientific journals such as ACS Langmiur, ACS Analytical Chemistry, ACS Synthetic Biology, RSC Analyst, and Biosensors & Bioelectronics. Recipient of "Science Award" (Chemistry) of the Scientific and Technological Research Council of Turkey (TUBITAK) on the area of Electrochemical Biosensors. His name appeared in the 45th rank among the "100 Turks Guiding Science" book published by Sanko Holding in May 2017. He is selected in Stanford University List of Top 2% Scientists Worldwide 2021. His research interest is Electrochemical Biosensors, Crispr Cas systems, Artificial Intelligence

Conference talk title

CRISPR powered electrochemical biosensors

Abstract

There has been increasing interest in electrochemical biosensors over the past decade (1). The discovery of CRISPR-Cas (clustered regularly interspaced short palindromic repeats and CRISPR-associated proteins) systems revolutionized genome engineering (2). In addition, it opened up new possibilities in electrochemical biosensing technologies (3, 4). CRISPR-associated (Cas) endonucleases enable the targeting of specific nucleic acid sequences based on Watson-Crick base pairing between the CRISPR-RNA (crRNA) and the target nucleic acid. Cas9, Cas12, and Cas13 are endonucleases of Class 2 CRISPR-Cas systems and they are the most frequently used Cas enzymes in biosensing studies (5). While Cas9 binds and cuts the target dsDNA, the nuclease-deficient version of it, namely dead Cas9, only binds to the target DNA. Cas12 and Cas13 bind DNA and RNA, and they perform the indiscriminate cleavage of ssDNAs and ssRNAs in solution, respectively (5). This type of nuclease activity is called collateral (trans) cleavage and has been harnessed to cleave electrochemical reporter nucleic acids consist of ssDNA or ssRNA (6, 7). In this way, a Cas endonuclease sense the presence of a target nucleic acid in a solution and its activation results in the cleavage of electrochemical reporter ssDNA or ssRNA which eventually generate a detectable signal. Beyond CRISPR-based biosensors, CRISPR-Cas-mediated genomic mutations can be detected using electrochemical biosensors. Detection of CRISPR-Cas9-mediated mutations has been performed using a carbon nanotube-modified pencil graphite electrode (8). After creating a double-strand break with Cas9 in murine cells, 98 nt single-stranded oligodeoxynucleotide (ssODN) was introduced for Homology Directed Repair (HDR)-based gene editing. An ssDNA probe was designed to verify the mutated sequence electrochemically. After performing PCR for the target sequence, PCR products were hybridized with the probe. The difference between the electrochemical oxidation signal of the ssDNA probe and dsDNA formed by the hybridization of the target sequence with the probe informed us about the presence of mutant DNA and the success of genome editing.

Speaker's Profile

Professor Peter Hoffmann
Lloyd Sansom Chair
President Australasian Proteome Society
Treasurer international HUPO
Clinical Health Sciences
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Profile summary

Professor Peter Hoffmann is President of the Australasian Proteomics Society, Conference Chair for the National Meeting of the Australasian Proteomics Society and is Treasurer of the international Human Proteome Organisation (HUPO). For fifteen years, Peter's research has delved into the identification of low abundant proteins and the mapping of their posttranslational modifications and their use as potential biomarkers for early detection of diseases, one of the biggest challenges in proteomics. He is leader in the field of Mass Spectrometry Imaging and his research group was the first to use this technology in Australia. He is Director of the NCRIS Bioplatforms Australia Node for Tissue Imaging Mass Spectrometry. He has experience transforming research into industry-relevant outcomes, forming OncoDX to commercialise patented biomarkers for the early detection of ovarian and gastric cancer.

https://people.unisa.edu.au/?homepageid=Peter.Hoffmann

Conference talk title

Statistical methods and machine learning in the diagnosis of endometrial cancer

Abstract

Classic histopathological examination of tissues remains the mainstay for cancer diagnosis and staging. In some cases, histopathologic analysis yields ambiguous results, leading to inconclusive disease classification. Here, we set out to explore the diagnostic potential of mass spectrometry-based imaging for tumour classification based on proteomic fingerprints. Supervised machine learning (ML) approach was applied to large MALDI MSI datasets from endometrial cancer (EC) TMAs consisting of 302 unique patients. The pathologist labels for these patients included 43 patients of primary tumour with lymph node metastasis (LNM), 214 patients with no LNM, and 45 patients that were unclassified/not tested. Combining mass spectrometry with ML, we were able to predict the presence of LNM in primary tumour of EC with an overall accuracy of 80% (90% sensitivity and 69% specificity). In addition, this approach was able to distinguish colorectal tumour from normal tissue with an overall accuracy of 98% (98.2% sensitivity and 98.6% specificity). Using supervised ML of EC MALDI MSI data, in conjunction with pathologist annotation and patient meta data, this study set to establish approaches for binary sample classification problems. This is achieved based on the MALDI MSI data alone, without any need to identify the m/z values. Overall, these results highlight the potential of this technology to determine the optimal treatment for cancer patients to reduce morbidity and improve patients' outcomes.

Speaker's Profile

Professor Pongrama Ramasoota
Mahidol University
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Profile summary

Dr. Pongrama Ramasoota is working as Director of Center of Excellence for Antibody Research (CEAR) and Head, department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University. He is lead investigator in the research project "Neutralizing human monoclonal antibodies (NhuMAbs) against 4 serotypes of Dengue virus" funded by Science and Technology Research Partnership for Sustainable Development (JST-JICA-SATREPS), Japan Society for the promotion of Sciences (JSPS). Now this NhuMAb is under licensing by Bio-Pharma Company from USA. and will go for phase-I trial in 2024 and plan to sell in 2028. He also led other research project on development of therapeutic human monoclonal antibodies against virus, such as Sar-Cov 2 and Rabies. Dr. Ramasoota holds Doctor of Veterinary Medicine (DVM) from Kasetsart University, Master of Public Health, Mahidol University, Thailand and Master of Science and Ph.D. in Molecular biology from Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. He was awarded Thailand Outstanding Researcher 2020, Thailand outstanding Veterinarian 2016, Outstanding Research and Invention Awards from National Research Council of Thailand (NRCT) in the years 2008-2010, 2014 and 2016, Gold medal award from 2014 Taipei Invention show & Techno mart, 2008 Korea inventor Promotion Association (KIPA), 2008 International Federation of Inventor Association (IFIA), and. He has published more than 70 publications with 4 international patents.

https://www.tm.mahidol.ac.th/tropmed-staff/Pongrama-Ramasoota.php

Conference talk title

Therapeutic Antibody against E and Ns1 proteins of dengue virus

Abstract

Dengue hemorrhagic fever (DHF) cause by the mosquito borne Dengue virus (DENV) has become the world public health problem due to global warming and globalization. Each year, 100 million Dengue cases required hospitalization. World Health Organization aim to reduce Dengue mortality to be at 50% in the year 2020. But until present, there is no specific drug for Dengue treatment. By using SPYMEG myeloma cell fused with peripheral blood mononuclear cell (PBMC) obtained from Dengue patients, the hybridoma cell producing neutralizing human monoclonal antibodies (NhuMAbs) anti envelope protein against 4 serotypes of DENV has been established at CEAR. Two candidates NhuMAb (clones 19 and 54) were successfully pre-clinically tested in vitro by 95-100 % neutralized all 4 serotypes of 20 clinical isolates DENV and in vivo tested by significantly decreased mortality of prior DENV intra-cranially mice and eliminated DENV (from 1010 to be 0) in blood of DENV challenged Marmoset monkeys within 2 days. To make NhuMAb without causing ADE, FC-modified at LALA position of both NhuMAb clones was established. Industrial scale production of NhuMAbs using the Food and drug administration (FDA) accepted method of Stable expressed Chinese Hamster Ovary (CHO) cell produced at GMP facility was prepared at the licensing company. AG129 mice that 24 hrs. prior subcutaneously injected with 105 FFU DENV followed by treated with Fc-modified NhuMAb clone 54 showed almost 100 % survival. The dose finding was also performed in AG129 mice and found that the dose for first in human trial are 4 - 6 mg/kg. The licensing company is ongoing to test this Fc-modified NhuMAb clone 54 for further PK, PD and viral clearance in nonhuman primate tests in the year 2023, followed by Phase 1 clinical trial in the year 2024.

PANELIST

Forum Panelist's Profile

Professor Dr Norazmi Mohd Noor, FASc
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Profile summary

Norazmi Mohd Nor, is a Professor of Molecular Immunology at the School of Health Sciences, Universiti Sains Malaysia. He obtained his B.Sc (Hon) from Monash University and PhD from Flinders University, Australia. He has held many important administrative positions in USM, including the Director of the Institute for Molecular Medicine, and Dean of the School of Health Sciences. Norazmi's main research interest is in the development of vaccines against cholera, tuberculosis and COVID-19. He has secured about RM20m (about USD5m) in research funding as principal investigator from national and international agencies throughout his career. Norazmi has research collaborations with several international institutions and frequently sits in national and international review panels for research grant proposals. A few years ago, he initiated a global project to compile opinions papers from renowned experts in the field of TB vaccine development, in a book entitled "The Art & Science of Tuberculosis Vaccine Development", that was published by Oxford University Press (Malaysian branch) which is freely downloadable. Norazmi has been involved in many strategic planning for research in Malaysia, the latest being the National Vaccine Development Roadmap in 2021. Norazmi was the Founding Senior VP (Strategic & Planning) for the Malaysian Biotechnology Corporation and currently advises several biotechnology companies. Based on his scientific contributions, Norazmi was inducted as a Fellow of the Academy of Sciences, Malaysia in 2012.

Forum Panelist's Profile

Professor Ir. Dr Fatimah Binti Ibrahim
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Profile summary

Professor Ir. Dr. Fatimah Ibrahim received her B.Sc.E.E. from Marquette University, USA in 1989. She obtained her M.Sc. Electronics (Medical Systems) from University of Hertfordshire, UK in 1994 and Ph.D. in Biomedical Engineering from University of Malaya (UM), Malaysia in 2005. She is a Professor of Biomedical Engineering, UM, the head of Centre for Innovation in Medical Engineering (CIME), and advisor for the Center of Printable Electronics, UM. She is the senior member of IEEE, fellow of Institute of Engineer Malaysia (IEM), and fellow of Academy Science Malaysia (ASM). Her research interests are in physiological measurement and modeling, biosensors, BioMEMS, bioinstrumentation, and artificial intelligence in medicine. She owned 16 patents including 3 commercialised patents. In recognition of her research innovations, she has received 8 top awards (Gold medal/best award/special award), 3 Silver Medals, and 2 bronze medals at various international and national invention exhibitions. She has been awarded the Top Research Scientist Malaysia (TRSM 2019) by the Academy of Sciences Malaysia (ASM). Recently, she received the IFMBE Laura Bassi Award 2022 (an award attributed to a female biomedical engineer for her outstanding research contributions in the field of medical and biological engineering) by the International Federation of Medical and Biological Engineering (IFMBE), USA.

Forum Panelist's Profile

Dr Low Ley Hian
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InterVenn Biosciences (Malaysia)
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Profile summary

Ley Hian obtained his PhD (Neuroscience) from The Florey Institute of Neuroscience and Mental Health, The University of Melbourne in 2013 and relocated to San Francisco for his postdoctoral fellowship at the University of California San Francisco (UCSF). In 2017, Ley Hian crossed path with InterVenn Biosciences and became an early team member of InterVenn Biosciences. Ley Hian currently serves as the Director of Business Development at InterVenn.

Forum Panelist's Profile

Mr Ivan Hoh
Founder & Chief Executive Officer, Codon Genomics
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Profile summary

"Harnessing the power of scientific knowledge for the future of Malaysia – Scientific breakthroughs by Malaysians for Malaysia"

For Ivan Hoh, science has always been sparked curiosity and fascination, even as a teen. Born and bred in Ipoh, Perak, Ivan nurtured his love for science and discovering new knowledge by reading everything he could get his hands on and asking incessant questions to anyone who would answer. By the time he enrolled in Universiti Kebangsaan Malaysia (UKM) as a fresh-faced molecular biology student, he was firmly convinced that his path in life lies in the study of science.

In his university years as an undergraduate and Masters student, he seized the opportunity to work under inspiring local scientists at the then-newly founded Malaysia Genome Institute (MGI), learning all he could about high throughput sequencing, management and analysis in the Lates calcarifer transcriptome project. Having gotten a taste of what the study of genomics and bioinformatics has to offer, Ivan embarked on a new journey to prove to the world that Malaysian scientists can contribute a whole lot to the world.

In 2010, two years after graduating with a MSc in Genomics from UKM and having gained some experience as a Sequencing Project Manager, Ivan founded Codon Genomics with fellow Malaysian scientists with his vision firmly set on providing powerful genomic applications to every possible industry. Today, Codon Genomics is one of the fastest growing bioinformatics company in South East Asia and the de facto partner for digitization and data analytics.

As a young man, Ivan was inspired and motivated by fellow scientists he has met and he believes the future lies in humans' will and ability to innovate science. To ensure his vision for science in Malaysia to continue moving forward, Ivan is actively participating in efforts to invest in young Malaysian scientists. Currently, he is on the various committees and advisory boards in local institutions like UKM, Universiti Putra Malaysia (UPM), and Management and Science University (MSU). As a member of the International Rubber Research Development Board associate member, Ivan is also part of the committee for the Malaysia Science, Technology and Innovation (STI) Masterplan Planning.

Ivan, with more than 35 Tier One scientific publications and 750 citations, has set his sights on steering Codon Genomics towards contributing further in advancing science and society by utilising biological data and analytics. In this age of Big Data analytics making leaps and bounds in harnessing the power of information for the understanding our world better through science, Ivan with his team is set on the path that leads to more breakthroughs and solutions to today's scientific questions.

ORAL

CHAIRPERSON :	Dr Daruliza Kernain (USM, INFORMM)
JUDGES :	1. Dr Nik Yusnoraini Yusof (USM, INFORMM)
	2. Dr Farizan Ahmad (USM)
WEBEX LINK :	(Hall 3)

	ORAL PRESENTATION 1A [11 Oct 2022, 1515 - 1715pm]
O-1	**Tissue-based proteomics: insight into molecular mechanisms in cervical carcinogenesis** *Gaayathri Kumarasamy, Mohd Nazri Ismail, Sharifah Emilia Tuan Sharif, Christopher Desire, Parul Mittal, Peter Hoffmann and Gurjeet Kaur*
O-2	**The expression of apoB and 4HNE in overweight-and obese-related colorectal carcinoma tissues** *Ng Phei Ying, Siti Norasikin Mohd Nafi, Nur Asyilla Che Jalil, Kueh Yee Cheng, Lee Yeong Yeh and Anani Aila Mat Zin*
O-3	**Quantitative proteomics analysis of insecticide resistant *Ae. aegypti*** *Abubakar Shettima, Intan H Ishak, Benjamin Lau, Hadura Abu Hasan and Noorizan Miswan and Nurulhasanah Othman*
O-4	**Clarithromycin resistance in *Helicobacter pylori* is associated with genetic polymorphism of virulence factors** *Anis Rageh Al-Maleki and Naim Rosli*
O-5	**Metabolome analysis of induced-resistance *Helicobacter pylori* against clarithromycin** *Naim Rosli and Anis Rageh*
O-6	**The antimicrobial effect of vinegar on *Escherichia coli* O157:H7 isolated from lettuce** *Yu Xiang Soo and Seow Hoon Saw*
O-7	**Raman-based spectroscopic techniques for Leptospira DNA detection** *Anis Athirah Abdul Razak, Fatin Hamimi Mustafa, Hui Yee Chee, Mohd Adzir Mahdi and Fariza Hanim Suhailin*
O-8	**Autophagy modulation as the potential target in the combination treatment of BZD9L1 and regorafenib in colorectal cancer** *Deepa Rajendran, Yi Jer Tan, Yeuan Ting Lee and Chern Ein Oon*

CHAIRPERSON :	Dr Anizah Rahumatullah (USM, INFORMM)
JUDGES :	1. Dr Nor Hayati Yusof (USM)
	2. Dr Agustine Nengsih Binti Said @Fauzi
WEBEX LINK :	(Hall 4)

	ORAL PRESENTATION 1B [11 Oct 2022, 1515 - 1715pm]
O-9	**Determination of solubility and dissolution profile of BZD9L1 sirtuin inhibitor** *Pei Yi Mok, Kah Hay Yuen and Chern Ein Oon*
O-10	**Performance evaluation of nested polymerase chain reaction (nPCR), light microscopy and Plasmodium falciparum histidine rich protein 2 rapid diagnostic test (pfhrp-2 RDT) in the detection of falciparum malaria in Akure, Nigeria** *Oluwaseun Bunmi Awosolu, Zary Shariman Yahaya and Meor Termizi Farah Haziqah*
O-11	**Association of single nucleotide polymorphisms on iron regulating genes with iron metabolising parameter** *Liew Jin Rou, Loo Keat Wei and Teh Lai Kuan*
O-12	**Stable expression of anti-BmR1 IgG4 antibody** *Jacqueline Kar Kei Mark and Gee Jun Tye*
O-13	**The role of the protein phosphatase 4 regulatory subunit 1 (PP4R1) in leukaemia** *Maryam Behjat and Mirna Mourtada-Maarabouni*
O-14	**X-chromosome wide association in Thai SLE patients** *Krisana Jaiwan, Yao Lei, Manon Boonbangyang, Punna Kunhapan, Nusara Satproedprai, Surakameth Mahasirimongkol, Prapaporn Pisitkun, Nattiya Hirankarn, Wang Yong Fei and Pattarin Tangtanatakul*
O-15	**The effect of mesenchymal stem cells-mediated macrophages activation on breast cancer progression** *Nur Ramziahrazanah Jumat, Muhammad Amir Yunus, Badrul Hisham Yahaya, Rafeezul Mohamed*
O-16	**Therapeutic properties of Malaysian stingless bee pollen and its protective effect against DNA damage** *Nurdianah Harif Fadzilah and Wan Adnan Wan Omar*

CHAIRPERSON :	Associate Prof Dr Khoo Boon Yin
JUDGES :	1. Dr. Norsyahida Ariffin (USM)
	2. Dr Norhahani Mohd Redzuan (USM)
WEBEX LINK :	(Hall 5)

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O-17	**Novel thermoresponsive Chitosan/PEG based hydrogels and their prospective biomedical applications** *Aniqa Junaid, Murtaza Najabat Ali, Mariam Mir and Sadia Hassan*
O-18	**Acute and sub-chronic toxicological evaluation of probiotic strain Lactobacillus rhamnosus GG in Sprague Dawley rats** *Venkata Kanthi Vaishnavi Vedam, Arun Kumar Adhikary and Parasuraman Subramani*
O-19	**In-Depth Investigation of microRNA Methylome Signature in Colorectal Cancer** *Nurul Qistina Rus Bakarurraini, Rashidah Baharudin, Imilia Ismail, Nadiah Abu, Siti Aishah Sulaiman, Learn-Han Lee and Nurul Syakima Ab Mutalib*
O-20	**Identification of LRRC17 as colonic fibroblast activation marker and its potential role in colorectal cancer progression** *Sahira Syamimi Ahmad Zawawi and Marahaini Musa*
O-21	**Effect of polyphenolic-rich fraction of cornsilk (*Stigma maydis*) in streptozotocin-induced diabetic rats** *Siti Azhani, Nor Syamim, Nur Fatihah and Sabreena Safuan*
O-22	**Phytochemical, anti-microbial activity and anti-proliferation test against human cancer-origin cell lines using water extracts of *Momordica cochinchinensis* (Gac fruit)** *J T Priscilla, Ming-Thong Ong and Sreeramanan S*
O-23	**Conjugates between *P. marcocarpa* aqueous extract and TiO2 exhibited a synergistic antimicrobial effect** *Fanne Yeoh Fern Nii, Ong Ming Thong, Lim Gin Keat and Srimala Sreekantan*
O-24	**Cytotoxicity, proliferation and migration assessment of BHMC, the curcuminoid analogue on human liver cancer cells, HepG2** *Muhammad Aminuddin Mohd Shafiee, Mohd Ashraf Muhamad Asri, Nurul Asyikin Mahbud, Nur 'Aqilah 'Inani Hanapi, Marwah Salaebing, Zulkefley Othman, Armania Nurdin and Sharifah Sakinah Syed Alwi*

CHAIRPERSON :	Dr. Sylvia Annabel Dass (USM)
JUDGES :	1. Dr. Chew Ai Lan (USM)
	2. Dr. Noor Fatmawati Mokhtar (USM)
WEBEX LINK :	(Hall 6)

	ORAL PRESENTATION 1D [11 Oct 2022, 1515 - 1715pm]
O-25	**Unraveling the tumour-regulatory role of miR-3934 in human breast cancer** *Zhi-Xiong Chong, Swee-Keong Yeap, Chee-Mun Fang and Wan-Yong Ho*
O-26	**Whole-genome-scale identification of novel non-protein-coding RNAs controlling cell proliferation and survival through a functional forward genetics strategy** *Daniel P Tonge, David Darling, Farzin Farzaneh and Gwyn Williams*
O-27	**Selection of T-cell receptor (TCR) like antibody against Human Leukocyte Antigen A-2 (HLA-A2) for cervical cancer diagnostics** *Rehasri Selva Rajan, Sylvia Annabel Dass, Tye Gee Jun and Venugopal Balakrishnan*
O-28	**Development of T-cell receptor-like antibody against human leukocyte antigen A-11 (HLA-A11) human papillomavirus (HPV) type 16 & 18 oncoprotein E7 for the diagnosis of cervical cancer** *Azrin Syazana Zulcafli, Sylvia Annabel Dass, Tye Gee Jun and Venugopal Balakrishnan*
O-29	**High throughput molecular profiling of bacterial diversity in Johor mangroves** *Roshan Mascarenhas, Oh Bi Han, Ng Xin Yan, Dominic Kay, Kwa Yee Chu, Pang Kok Lun, James Sy-Keen Woon, Nadine Nograles and Mahibub Mahamadsa Kanakal*
O-30	**Ranking of the *Mycobacterium tuberculosis* T-cell epitopes using tabulated immune properties for potential Tuberculosis diagnosis and vaccine candidates** *Nurul Syahidah Shaffee, Ezzeddin Kamil Mohamed Hashim, Armando Acosta Dominguez, Maria Elena Sarmiento Garcia San Miguel, Siti Suraiya Md Noor and Norazmi Mohd Nor*
O-31	**Desiccation tolerance mediates ST239-SCCmec type III-SCCmercury to ST22-**

	SCCmec type IV MRSA Clonal Replacement in Hospital Settings *Nurul Amirah binti Mohamad Farook, Raja Mohd Fadhil Raja Abd Rahman, Sharifah Azura Salleh, Muttaqillah Najihan Bin Abdul Samat and Hui-min Neoh*
O-32	**IL-21 gene silencing suppressed the proliferative activity of HCT116 and HT-29 colorectal cancer cells** *Ong Ching Yi, Ong Ming Thong and Khoo Boon Yin*

CHAIRPERSON :	**Dr. Nurulhasanah Othman**
JUDGES :	1. Associate Professor Dr. Mohd Nazri Bin Ismail
	2. Dr Yazmin Binti Bustami
WEBEX LINK :	(Hall 7)

	ORAL PRESENTATION 2A [12 Oct 2022, 1015 am – 1215pm]
O-33	**Ligand-based pharmacophore modeling, molecular docking and molecular dynamics study targeting prolyl oligopeptidase enzyme for effective treatment for Parkinson's disease: computational approach** *Yahaya Sani Najib, Yusuf Oloruntoyin Ayipo, Waleed Abdullah Ahmad Alananzeh and Mohd Nizam Mordi*
O-34	**Inhibitory effects of andrographolide in PC-3 cell line and the induction of apoptosis via the involvement of caspases activity** *Janany Manimaran and Daruliza Kernain Mohd Azman*
O-35	**Lentiviral modification of hard-to-transduce NK-92MI cells** *Chin Ding Sheng and Tye Gee Jun*
O-36	**Higher wheal sizes of *Dermatophagoides farinae* sensitization exhibit worse nasal symptoms in allergic rhinitis patients** *Siti Nur Husna Muhamad, Norasnieda Md Shukri, Hern-Tze Tina Tan, Noor Suryani Mohd Ashari and Kah Keng Wong*
O-37	**Immunoinformatics analysis on human coronavirus spike protein for universal immunogen discovery** *Chin Peng Lim, Boon Hui Kok, Hui Ting Lim, Chiuan Yee Leow and Chiuan Herng Leow*
O-38	**Andrographolide induced apoptosis by enhancing c-Myc/p53 in human glioblastoma DBTRG-05MG cell line** *Nurul Syamimi Othman and Daruliza Kernain*
O-39	**In vitro uptake and activation of human dendritic cells by liposomes derived from total lipid of *Mycobacterium smegmatis*** *Nurfatihah Azlyna Ahmad Suhaimi, Nor Asyikin Nordin, Siti Suraiya, Rohimah Mohamud and Ramlah Kadir*
O-40	**Identification of medicinal fungi by molecular analysis** *Eng Wei Keat, Leow Chiuan Yee, Lai Ngit Shin, Sasidharan Sreenivasan and Leow Chiuan Herng*

CHAIRPERSON :	**Associate Prof Dr Choong Yee Siew**
JUDGES :	1. Associate Professor Dr. Darlina Binti Md Naim
	2. Dr Shazana Hilda Binti Shamsuddin
WEBEX LINK :	(Hall 8)

	ORAL PRESENTATION 2B [12 Oct 2022, 1015 am – 1215pm]
O-41	**Effect of Andrographolide on proliferation, migration, and invasion of MDA-MB-231 and MCF-7 cell lines** *Deeza Syafiqah Mohd Sidek and Daruliza Kernain Mohd Azman*
O-42	**Culture and biochemical testing versus 16S rRNA Next-Generation Sequencing for bacterial identification from clinical samples: Practicability, cost and turn-around time in a Malaysian laboratory** *Nurnabila Syafiqah Muhamad Rizal, Hui-min Neoh, Ramliza Ramli, Petrick@Ramesh A/L K Periyasamy, Alfizah Hanafiah, Muttaqillah Najihan Abdul Samat, Toh Leong Tan, Kon Ken Wong, Sheila Nathan, Sylvia Chieng, Seow Hoon Saw and Bee Yin Khor*
O-43	**Isolation and production of recombinant monoclonal antibody proteins against a *Toxocara canis* antigen using phage display technology** *Zamrina Baharudeen and Anizah Rahumatullah*
O-44	**Optimization of transient expression of recombinant IgG binding protein, FcγRIIa**

	Shin Yi Gan, Gee Jun Tye, Ai Lan Chew and Ngit Shin Lai
O-45	**Cloning of IgM Fc receptor for mammalian expression system** *Hai Shin Pung, Gee Jun Tye and Ngit Shin Lai*
O-46	New β-carboline compound as a promising anticancer agent in chronic myelogenous leukemia (CML) *Meroshine Nageswara Rao, Mazlin Mohideen, Leong Sze Wei, Thiruventhan Karunakaran and Nur Azzalia Kamaruzaman*
O-47	**Discovery of potent small molecule inhibitor for dengue through *in silico* and *in vitro* approaches** *Norshidah Harun, Leow Chuan Herng, Kamarulzaman Ezatul Ezleen, Abdul Wahab Habibah, Ramachandran Vignesh and Lai Ngit Shin,*
O-48	**Systematic review on preclinical reports: Titania Nanotube Arrays technology for medical orthopaedic screw implant application** *Tan Chia Yi, Rabiatul Basria S M N Mydin and Mohd Sharizal Abdul Aziz*

CHAIRPERSON :	Dr Daruliza Kernain
JUDGES :	1. Dr. Eugene Ong Boon Beng (USM)
	2. Dr. Noor Fatmawati Mokhtar (USM)
WEBEX LINK :	(Hall 9)

	ORAL PRESENTATION 2C [12 Oct 2022, 1015 am – 1215pm]
O-49	**Clinicopathological association of chronic rhinosinusitis with nasal polyp (CRSwNP) and periostin expression** *Sakinah Mohamad, Baharudin Abdullah, Wan Faiziah Wan Abdul Rahman and Najib Majdi Yaacob*
O-50	**Detection of *Herpes simplex* virus-1 by direct immunofluorescence and viral isolation from cerebrospinal fluid** *Ummu Salamah Faisal, Ummu Afeera Zainulabid and Wong Kong Ken*
O-51	**Development of cisplatin-resistant urothelial cancer cells using pulse-shock treatment** *Siti Farizan Mansor, Abhi Veerakumarasivam and Badrul Hisham Yahaya*
O-52	**Biochemical and biophysical characterization of leishmanial histidyl tRNA synthetase** *Fouzia Nasim and Insaf Ahmed Qureshi*
O-53	**Isolation and characterization of ssDNA aptamers against HlyE of *Salmonella Typhi*** *Ahmad Najib Mohamad, Khairul Mohd Fadzli Mustaffa, Eugene Boon Beng Ong, Muhammad Fazli Khalid, Mohd Syafiq Awang, Nor Syafirah Zambry, Asrulnizam Abd Manaf, Yazmin Bustami, Hairul Hisham Hamzah and Ismail Aziah*
O-54	**Development of DNA aptamers for the detection of the *Burkholderia pseudomallei* towards the diagnosis of melioidosis** *Kasturi Selvam, Muhammad Fazli Khalid, Khairul Mohd Fadzli Mustaffa, Azian Harun, Habibah A Wahab and Ismail Aziah*
O-55	**Epigenetic oncogenesis, biomarkers and emerging chemotherapeutics for breast cancer** *Yusuf Oloruntoyin Ayipo, Abdulfatai Temitope Ajiboye, Wahab Adesina Osunniran, Akeem Adebayo Jimoh and Mohd Nizam Mordi*
O-56	**Selection of ssDNA aptamers against Programmed Death -Ligand 1 (PD-L1)** *Muhammad Najmi Mohd Nazri, Khairul Mohd Fadzli Mustaffa and Noor Fatmawati Mokhtar*

O-1

Tissue-based proteomics: insight into molecular mechanisms in cervical carcinogenesis

Gaayathri Kumarasamy[1], Mohd Nazri Ismail[1,2], Sharifah Emilia Tuan Sharif[3], Christopher Desire[4], Parul Mittal[4], Peter Hoffmann[4] and Gurjeet Kaur[1]

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Tissue-based proteomics is an evolving tool used in cancer research to characterize the pathophysiology of disease. However, the proteome alterations involved in cervical carcinogenesis are not extensively studied. This study aims to elucidate the differentially expressed proteins and offer insights into the cellular processes and pathways involved in the development of cervical cancer.

Methodology

The pathological regions of interest in the cervical squamous epithelium were microdissected from formalin-fixed paraffin-embedded (FFPE) tissue sections of six normal cervix cases, five HPV-associated squamous intraepithelial lesion (SIL), and six squamous cell carcinomas (SCC). The samples were trypsin digestion and subjected to high throughput liquid chromatography-electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS) and trapped ion mobility time-of-flight-mass spectrometry (timsTOF-MS), followed by quantification with MaxQuant and Perseus software. Bioinformatics analyses were carried out using DAVID, ConsensusPathDB, and STRING.

Results and Discussion

We identified a total of 3597 proteins with 589, 550, and 1570 proteins unique to the normal cervix, SIL, and SCC groups, respectively, while 332 proteins were similar across all three groups. The predominant protein found was histone. Interestingly, the quantification results showed an upward trend for the up-regulated proteins and a downward trend for the down-regulated proteins in the progression from normal to SIL and SCC. The main molecular function was the binding process, and the top biological processes were chromatin silencing for SIL compared to the normal cervix and nucleosome assembly for the SCC compared to SIL group. The key pathways involved were viral carcinogenesis and necroptosis, reflecting their role in cell proliferation, migration, and metastasis.

Conclusion

The identification of proteins and their associated pathways provides a deeper understanding of the underlying molecular mechanisms involved in HPV-associated cervical cancer.

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Funding

The work was funded by the Fundamental Research Grant Scheme (203.CIPPM.6711722) from the Ministry of Education, Malaysia.

O-2

The expression of apoB and 4HNE in overweight-and obese-related colorectal carcinoma tissues

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Obesity has been related to an increase in the incidence and progression of colorectal carcinoma (CRC) recently [1]. But how exactly obesity relates to CRC is still unclear. Low-density lipoprotein (LDL) is known for causing abnormal lipid metabolism and is usually measured in the blood [2]. Two LDL-related biomarkers, apoB and 4HNE, were chosen to investigate their expression in overweight-and obese-related CRC tissues, and their association with the clinicopathological data was also determined.

Methodology

Human ethical approval with a series code of USM/JEPeM 19060354 was obtained. This retrospective study involved overweight-and obese-related CRCs diagnosed in HUSM from January 2015 to December 2021. The classification of BMI followed the Asia Pacific BMI classification [3]. Clinicopathological data was retrieved from Laboratory Information System (LIS) and medical records at Pathology laboratory, HUSM. The CRC archival tissue blocks were then collected for Immunohistochemistry (IHC) staining. The data was then analysed using SPSS Version 26.

Results and Discussion

A total of 69 overweighted-and obese-related CRCs were retrieved within seven years. The patients had median age of 61 years old with interquartile range of 21, common in age of more than 50 years old (73.9%) and in males (52.2%). Most of the patients were found with CRCs localised in sigmoid colon (30.4%) and rectosigmoid colon (24.6%), larger than 2cm of tumour (97.1%), present of bowel wall invasion (94.2%), and absent of lymph nodes involvement (58.0%). The tumours were frequently classified as adenocarcinoma (91.3%) with moderate differentiation grade (82.6%) and under Modified Duke B class (55.1%). The IHC-stained CRC tissue slides showed high apoB expression (91.3%), whereas low 4HNE expression (82.6%). Significant associations were observed between apoB expression with age (p value=0.036), tumour site (p value=0.048), bowel wall invasion (p value=0.035), Duke classification (p value=0.002), while 4HNE expression with tumour size (p value=0.029). Majority CRC tumours of more than 2cm showed low 4HNE expression. This explained by the protective effect of the low 4HNE level that prevents the damage of the cancer cells [4].

Conclusion

In conclusion, the contrast tissue expression of apoB and 4HNE, and the significant associations obtained had shed light on the role of these LDL-related biomarkers in overweight-and obese-related CRC tissues. Exploration on the role of these biomarkers is recommended for *in vitro* study.

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O-3

Quantitative proteomics analysis of insecticide resistant *Ae. aegypti*

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Synthetic insecticides are the main vector control method globally. However, the widespread use of insecticide is causing resistance in mosquitoes. Understanding the proteomics basis of insecticide resistance may provide novel opportunities to control mosquito vectors. Hence, this study aimed to elucidate proteins associated with permethrin resistant *Ae. aegypti* using quantitative proteomics.

Methodology

The study evaluated the resistance pattern of *Ae. aegypti* from dengue hotspot and non-hotspot areas of Penang Island. Permethrin 0.75% insecticide-impregnated papers were used to determine the resistance status in adult female *Ae. aegypti*. The mosquito proteins were analysed using LC–ESI–MS/MS for protein identification and quantification via label-free quantitative (LFQ) analysis. In this study, differential protein expression (DEP) analysis was carried out using Perseus 1.6.14.0 statistical software to perform ANOVA and student's t-test. Protein-protein interaction (PPI) and functional ontology enrichment analyses were performed using STRING software.

Results and Discussion

The bioassay results showed 28% and 53% mortalities in mosquitoes exposed to permethrin from the hotspot and non-hotspot areas. Permethrin has been used extensively in Malaysia's dengue vector control program. The mortality percentage has shown a high permethrin resistance rate in the field strain *Ae. aegypti* mosquito, including the strains from the dengue hotspot and non-hotspot areas. These results suggested high resistance status in the dengue hotspot and non-hotspot areas. The high resistant patterns identified in the field strain *Ae. aegypti* could be because of the widespread and indiscriminate permethrin use in the investigated areas, especially if a site were labeled as a dengue hotspot. The resistance in the non-hotspot area could be because the area was once a dengue hotspot. These areas received extra attention from vector control management and private contractors lead to insecticide selection pressure and produce offspring carrying insecticide-resistant genes. The LFQ analyses revealed 501 (q-value <0.05) DEPs. The t-test showed 114 upregulated and 74 downregulated proteins in resistant versus laboratory strain exposed to permethrin. The significant functional ontology enrichment of the DEPs indicated drug-metabolic process, small molecule metabolic process, hydrolase activity acting on ester bonds, and catalytic activity. The PPI of DEPs showed a p-value at $<1.0 \times 10^{-}$ in permethrin-resistant *Ae. aegypti*. Significantly enriched pathways in DEPs revealed metabolic pathways, oxidative phosphorylation, carbon metabolism, biosynthesis of amino acids, glycolysis, and citrate cycle.

Conclusion

This study has revealed several DEPs and highlighted upregulated and downregulated proteins associated with insecticide resistance in *Ae. aegypti*.

O-4

Clarithromycin resistance in *Helicobacter Pylori* is associated with genetic polymorphism of virulence factors

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

For a long time, the macrolide antibiotic clarithromycin was used to treat *Helicobacter pylori* (*H. pylori*) infections. The efficiency of traditional triple therapy for the eradication of *H. pylori* has recently been dismally reduced in many nations [1]. Clarithromycin resistance is spreading over the world and is the leading cause of *H. pylori* treatment failure [2]. We employed whole-genome sequencing (WGS) technology to identify genomic alterations related with the development of antibiotic resistance in *H. pylori* with induced resistance to Clarithromycin.

Methodology

The clarithromycin-sensitive *H. pylori* strains were induced to become resistant against clarithromycin. To induce resistance, the strains were exposed to gradually increased concentration of clarithromycin in vitro for 3-5 days on a chocolate agar plate (CAP). The identity between resistant strains and their corresponding parental sensitive strains before induction were verified by random amplification of polymorphic DNA polymerase chain reaction (RAPD-PCR). WGS for bacterial DNA was performed, using Illumina platform.

Results and Discussion

There were 113 distinct single nucleotide variants (SNVs) and 286 unique insertions and deletions (InDels) changes discovered among the induced-resistant *H. pylori* strains. Interestingly, among the induced-resistant *H. pylori* strains, mutations in *cag1*, *cag4*, *fliR*, *obgE*, *tlpA*, and *vacA* were detected, which are known virulence genes that may help in bacterial survival, indicating that those mutations may be associated with the emergence of resistant *H. pylori* [3]. Moreover, we also found that it is not possible to infer a Clarithromycin resistance phenotype based on the existence of distinct point mutations in A2143G and/or A2142G mutations in 23S rRNA and may not be sufficient for predicting Clarithromycin resistance in *H. pylori*.

Conclusion

As a result, we hypothesised that in these strains, alternative mechanisms unrelated to the 23S rRNA gene sequence, such as the existence of an efflux pump, may play a role in Clarithromycin resistance.

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O-5

Metabolome analysis of induced-resistance *Helicobacter pylori* against Clarithromycin

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that thrives in stomach mucus and epithelial mucosa, causing gastric ulcers which may develop into gastric cancer. One of the most prevalent causes of treatment failure is the emergence of antibiotic-resistant of *H. pylori* infection [1]. The goal of this work is to use a metabolomic method to discover the metabolites associated with Clarithromycin-resistance in *H. pylori*.

Methodology

Clarithromycin-sensitive *H. pylori* strains were induced to become resistant against Clarithromycin. To induce resistance, the strains were treated to 0.1x, 0.25x, 0.5x, 1X, 2x, 4x, 8x, 16x, and 32x MICs of Clarithromycin in vitro for 3-5 days. Bacterial metabolites were isolated using the Bligh and Dyer technique and analysed using liquid chromatography-mass spectrometry (LCMS) [2]. The MassHunter Qualitative Analysis and Mass Profiler Professional tools were used to process and analyse the data. Sensitive, pre-resistant, and induced-resistant are three separate categories based on global metabolomic patterns.

Results and Discussion

Using one-way ANOVA, a total of 982 molecular features were identified to be significantly different (p-value < 0.005) between sensitive, pre-resistant, and resistant *H. pylori* strains. Additionally, 432 molecular features matched the metabolites in the Agilent METLIN Accurate Mass-Personal Metabolite Database and Library (AM-PCDL) database based on accurate mass, isotope ratios, abundances and spacing. In contrast to sensitive strains, induced-resistant strains generated more metabolites (585 features). Further investigation was carried out in order to find metabolites that varied substantially (p-value <0.05) between sensitive, pre-resistant, and induced-resistant *H. pylori* strains. These metabolites include lipids and metabolites involved in the metabolism of fructose and mannose. Our data imply that D-Mannitol, L-Leucine, and Pyridoxine, which correlate with bacterial survival, may constitute a potential antibiotic mechanism [3].

Conclusion

Understanding the underlying metabolic variations between Clarithromycin-sensitive *H. pylori* strains and Clarithromycin-resistance *H. pylori* strains may be a promising technique for developing novel antibiotic candidates.

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O-6

The antimicrobial effect of vinegar on *Escherichia coli* O157:H7 isolated from lettuce

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Lettuce *(Lactuca sativa)* is ready-to-eat (RTE) vegetables which is popular among consumers due to its convenience and high nutritional value. Unfortunately, lettuce is frequently associated with *Escherichia coli* O157:H7 outbreaks which cause serious illnesses such as haemolytic uremic syndrome and haemorrhagic colitis [1]. Thus, a proper cleaning of the products is crucial to eliminate the risk of foodborne pathogens contamination. Acetic acid, such as vinegar, has shown its antimicrobial effect on inhibiting the growth of *E. coli* O157:H7 [2]. In addition, it is commonly used in the household settings for cooking and washing. Hence, the objectives for this study were identifying and verifying the presence of *E. coli* O157:H7 on lettuces purchased from retail market in Kampar Perak, using multiplex polymerase chain reaction (mPCR); followed by evaluating the antimicrobial activity of vinegar based on the analysis of log reduction in growth of *E. coli* O157:H7.

Methodology

A total of 20 samples of lettuce was purchased from a retail market in Kampar Perak. Homogenization was performed on 10 g of shredded lettuce, followed by incubation at 37°C for 24 h in Tryptic Soy Broth (TSB) to enrich the bacteria. Serial dilution was performed prior to plating on selective agar for *E. coli* O157:H7. Verification of colonies was done using multiplex polymerase chain reaction (PCR) with primer pairs targeting genes of *rfb* (256 bp) and *flic* (625 bp). Both encodes for somatic and flagellar antigen, respectively. On the other hand, 1.0 McFarland ATCC 700728 *E. coli* O157:H7 was prepared and inoculated on lettuce at 5°C for 1 h. 5% of white vinegar was immersed into the 'contaminated' lettuce at 4 time points:10, 20, 30 and 40 min. Antibacterial effect was evaluated by enumerating the reduction of bacteria colonies in log CFU/g. The significance of log reduction of inoculated bacteria was analysed quantitatively using One-Way ANOVA test (Graph Pad Prism 9.0) with 95 % confidence level.

Results & Discussion

None of the samples collected were found contaminated with *E. coli* O157:H7. This might be due to the safety measures taken by the Food Safety and Quality Division (FoSIM) in maintaining the quality of products in the retail market. However, in this study, 5 samples of lettuce (25%) showed presence of *flic* gene but not *rfb* virulence gene. This might postulate the presence of other *E. coli* serotypes such as O55:H7 which shares similarity in *flic* H7 gene. On the other hand, significant reduction of *E. coli* O157:H7 was observed after 40 min treatment with 5% vinegar. Organic acids exert their antimicrobial effect by altering the pH level of bacteria's cytoplasm which then leads to cell lysis due to the disruption of enzymes' and proteins' structures [3]. Hence, acetic acid is the best alternative to chlorinated water because of its antimicrobial and consumer-friendly properties.

Conclusion

Lettuces purchased from Kampar are safe to be consumed. Vinegar is effective to reduce the growth of *E. coli O157:H7* on RTE vegetables after a period. Nevertheless, various parameters including pH of vinegar shall be studied to determine its optimum effectiveness towards eliminating bacteria.

Acknowledgement to the Fundamental Research Grant Scheme: FRGS/1/2022/SKK06/UTAR/02/1 (8160/0013)

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O-7

Raman-based spectroscopic techniques for *Leptospira* DNA detection

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Raman spectroscopy (RS) and surface enhanced Raman spectroscopy (SERS) are both promising techniques for biomolecule detection. In this paper, we review our preliminary finding in the utilization of these techniques for *Leptospira* deoxyribonucleic acid (DNA) detection. Functional layer formed via chemical organized monolayer (SAM) was used to immobilized DNA probe (pDNA) onto the metallic Raman layer/substrate. Successful hybridization between pDNA to the complementary DNA (cDNA) sequence was verified via vibrational features in Raman spectrum. The finding shows the potential used of Raman-based techniques as alternative diagnostic tools for leptospirosis.

Methodology

Two genes of *Leptospira*, i.e., *secY* and *lipL32*, were used. The following are the oligonucleotide sequences:

- *SecY* cDNA: 5'-TTT GAA GGG CAG GAA CAA G-3'
- *LipL32* cDNA: 5'-TTG TTT CCA TCG ACT AAA CCG TC-3'
- *SecY* pDNA: 5'-/5AmMC6/CTT GTT CCT GCC CTT CAAA-3'
- *LipL32* pDNA: 5'-/5AmMC6/GAC GGT TTA GTC GAT GGA AAC AA-3'

SecY gene is a housekeeping gene whereas *lipL32* gene is a marker for pathogenic gene. The pDNAs were terminated with amine for rapid attachment to metallic Raman layer/substrate. A 50 nm gold (Au) thin-film and 80 nm in size bi-metallic gold- silver nanoparticles (Au-Ag NPs) were the metallic Raman layer/substrate for RS and SERS measurements, respectively. The Au thin-film was deposited onto a fused silicate layer by plasma sputtering, while the colloidal Au-Ag NPs was embedded onto the polymer-based photonic crystal substrate via polyethylenimine (PEI) adhesive. The metallic surfaces were SAM-modified; (1) for Au thin-film, 3-mercaptopropionic acid (MPA) and 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride/N-hydroxyl succinimide (EDC/NHS) were used to achieve thiolate- and aminate-modified surfaces, (2) for Au- Ag NPs, the 4-aminothiophenol (4-ATP) was used to obtain the amine tail on the surface. After the functionalization process, the metallic Raman layer/substrate was incubated with 1 µM pDNA and cDNA in sequence. Raman spectrometer with 633 nm laser was used to investigate the vibration spectra from the samples.

Results and Discussion

Vibrational Raman peaks which correspond to chemical interactions and hybridization between pDNA and cDNA were observed from both RS and SERS measurements. A clear difference in Raman respond can be seen on different surfaces. For Au thin-film, Raman peaks of phosphate backbone (PO) and adenine- (A), guanine- (G), thymine- (T) and cytosine- (C) nucleobases were observed to verify the hybridization of 1 nM *secY* cDNA. For Au-Ag NPs, the signal-to-noise ratio of SERS peaks after 1 µM *lipL32* cDNA hybridization is more prominent than the RS peaks. The characteristic of SERS intensity decreases with the presence of cDNA after hybridization.

Conclusion

The vibrational Raman spectra from *Leptospira* DNA was successfully detected via Raman spectrometer via RS and SERS techniques. SERS exhibit more intense DNA hybridization peaks in comparison to RS, thus offering better detection sensitivity.

Acknowledgment

Authors wish to thanks UTM and UMT for supporting this work. This work was supported by the Ministry of Higher Education under Fundamental Research Grant Scheme (FRGS/1/2021/STG07/UTM/02/4) and Fundamental Research Grant Scheme for Research Acculturation of Early Career Researchers (RACER/1/2019/TK05/UMT//1).

O-8

Autophagy modulation as the potential target in the combination treatment of BZD9L1 and regorafenib in colorectal cancer

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

BZD9L1 is a sirtuin inhibitor with reported cytotoxic activities as a single agent or in synergy with 5-fluorouracil [1]. Regorafenib is a third-line therapy for metastatic colorectal cancer (CRC). The development of insensitivity to Regorafenib among patients may be due to the modulation of autophagy which is also a hallmark targeted by another sirtuin inhibitor (Tenovin-6) [2]. We hypothesise that BZD9L1 could synergistically work with Regorafenib to enhance cell death through autophagy. This study aims to elucidate the mechanism of action of BZD9L1-Regorafenib treatment through autophagy modulation in CRC cells.

Methodology

HCT116 and HT29 CRC cells were treated with BZD9L1 and/or regorafenib to evaluate the synergism in cytotoxicity and survival through MTT and clonogenic assay respectively. The autophagic response to BZD9L1-regorafenib treatment was assessed by qPCR to evaluate the regulation of ER stress markers which induce autophagy.

Results and Discussion

BZD9L1 and regorafenib combination treatment reduced the cell viability and survival of HCT116 and HT29 cells at 72 h. The reduction of HT29 cell viability and cell survival may be attributed to the cleavage of PARP, indicative of apoptosis. However, this was not observed in HCT116. Autophagy flux via pharmacological inhibition with Bafilomycin A1 demonstrated an increased expression of LC3II in the combination treatment group compared to sole treatment at 24 h, suggesting autophagy in HCT116 cells.

Conclusion

These findings suggest that BZD9L1 and regorafenib combination may be a novel strategy suitable to sensitise CRC to regorafenib via the perspective of autophagy modulation to reduce cell viability and survival.

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O-9

Determination of solubility and dissolution profile of BZD9L1 sirtuin inhibitor

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

BZD9L1 is a benzimidazole analogue with reported anti-cancer activities through Sirtuin inhibition. It was non-toxic in acute and repeated-dose toxicity studies. However, its solubility and dissolution profiles are unknown. The study aims to determine the pH-dependent solubility of BZD9L1 and its drug release rate in dissolution media that mimics the pH of the gastrointestinal tract.

Methodology

BZD9L1 solubility was determined using the traditional shake flask method in different pH buffer solutions (pH 1.2 0.1 N HCl, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer solutions) at 37 °C for 72 hours. The *in-vitro* dissolution of BZD9L1 was studied by using the USP Type I basket method in 900 mL of each pH buffer solution. Tween 20 and Tween 80 at 0.1% were used to determine the combined effect of surfactant and pH in the dissolution study of BZD9L1. The concentration of the dissolved BZD9L1 in each buffer solution was determined by measuring the absorbance and correlating it with the standard curve of BZD9L1 using UV-Vis spectrophotometer.

Results and Discussion

The solubility of BZD9L1 increased in 0.1N HCl solution at pH of 1.2 (195.15 ± 0.35 µg/mL). However, it exhibited low solubility in acetate buffer at pH 4.5 (10.15 ± 0.002 µg/mL) and phosphate buffer at pH of 6.8 (9.09± 0.001 µg/mL). BZD9L1 is a basic drug, largely due to the piperidinyl group (strong electron-donating group) on the molecule. A basic drug dissolves better in an acidic environment. The dissolution rate of BZD9L1 was elevated at pH 1.2 as compared to pH 4.5 and 6.8 buffer solutions. Non-ionic surfactants (Tween 20 and Tween 80) were employed to determine the combined effect on the dissolution rate in each pH buffer solution. The dissolution of BZD9L1 increased with the presence of the surfactants with Tween 80 exhibiting a greater effect compared to Tween 20. This was likely due to the presence of long hydrocarbon chains in a homologous series which enhanced the solubilizing potentialof the surfactant.

Conclusion

BZD9L1 is a basic drug that shows a typical pH-dependent solubility profile, where it exhibits high solubility in acidic dissolution media. This finding suggests that BZD9L1 can potentially be dissolved in gastric pH and best absorbed in small intestine.

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O-10

Performance evaluation of nested polymerase chain reaction (nPCR), light microscopy and *Plasmodium falciparum* histidine rich protein 2 rapid diagnostic test (*pfhrp*-2 RDT) in the detection of falciparum malaria in Akure, Nigeria

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Malaria remains a serious public health problem worldwide. In order to ensure early and accurate malaria diagnosis, the World Health Organization recommended confirmatory parasitological diagnosis of malaria by microscopy and malaria Rapid diagnostic test (RDT) prior antimalarial administration and treatment. This study was designed to evaluate the performance of nested polymerase chain reaction (nPCR), light microscopy and *Plasmodium falciparum* histidine rich protein 2 rapid diagnostic test (*pfhrp*-2 RDT) in the detection of falciparum malaria in Akure, Nigeria.

Methodology

A cross-sectional and hospital-based study involving 601 febrile volunteered participants was conducted in Akure, Nigeria. Approximately 2-3 mL venous blood sample was obtained from each study participant for standard parasitological confirmation by microscopy and RDT. Thick and thin films were prepared and viewed under the light microscope for parasite density quantification and species identification, respectively. Dry blood spot (DBS) samples were prepared on 3MM Whatman filter paper for molecular analysis through nested polymerase chain reaction (nPCR).

Results and discussion

The overall prevalence by microscopy, RDT and nPCR were 64.89% (390/601), 65.7% (395/601) and 67.39% (405/601), respectively. Obviously, the performance efficacy of microscopy was significantly higher than RDT considering PCR as the gold standard. The estimates of sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), accuracy, Youden's j index of microscopy and RDT were 96.30, 100.00, 100.00, 92.89, 97.50, 0.963 and 95.06, 94.90, 97.47, 90.29, 95.01, 0.899, respectively. Similarly, RDT recorded higher false negativity compared microscopy (4.94% vs 3.70%). A near perfect agreement was reported between microscopy and PCR, and between RDT and PCR with Cohen's kappa value (k) of 0.94 and 0.88, respectively.

Conclusions

This study revealed that RDT and microscopy continues to remain highly efficacious, though lower than PCR. Thus, while RDT continues to complement microscopy as the gold standard in high malaria-endemic settings, the application of PCR for constant evaluation and monitoring of RDT and microscopy is highly imperative to inform appropriate policy on malaria diagnosis and interventions.

O-11

Association of Single Nucleotide Polymorphisms on Iron Regulating Genes with Iron Metabolising Parameter

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Anaemia is a condition with haemoglobin concentration below the established cut-off level of 12 g/dL and insufficient to meet individual's physiological demands of oxygen. This condition had become a worldwide public health problem. Other than gender, family history, physiological condition, dietary intake, genetic factors contribute to development of anaemia a significant extent. In Malaysia, the prevalence of anaemia was up to 13.8% and more than half of the cases were due to iron deficient. Thus, this study is to elucidate the underlying factors in leading to anaemia including the association of SNPs on iron-regulating genes to iron-metabolising parameters and its predisposition to anaemia.

Methodology

A total of 183 subjects aged between 18 to 35 were recruited at Universiti Tunku Abdul Rahman with informed consent. Anthropometric measurement and haemoglobin (Hb) level were measured. Demographic data, family history and current physiological condition were self-declared by respondents through questionnaire. A volume of 6 mL of venous blood was taken for DNA extraction while the isolated plasma and serum were used for detection of hepcidin and serum iron concentration. Eight genetic variants from 4 genes (rs10414846, rs10421768, rs855791, rs4820268, rs1799852, rs12769, rs1799945 and rs1800562) were genotyped using tetra primer- ARMS PCR. Statistical analysis was done using SPSS version 22.

Results and Discussion

The anaemia prevalence rate in this study population was found at 14.75% (27/183). Women was found to have lower haemoglobin, hepcidin and serum iron level compared to men. Individuals currently on menstruation and having the experience of menorrhagia presented with lower haemoglobin, hepcidin as well as serum iron. Hepcidin level was found decreased to increase iron absorption to replenish iron loss through menstruation. Higher body mass index (BMI) associated significantly with higher haemoglobin level, probably due tohigher dietary iron intake among overweight and obese individuals. Individuals with family history of anaemia presented lower haemoglobin level. Genetic variant of *TF* gene (rs12769) was associated with higher hepcidin and serum iron level while homozygous A allele in rs10421768 associated with higher serum iron level. Other genetic variants were found to have no association with the iron-metabolising parameters that being analysed.

Conclusion

Anaemia is a multifactorial disease which could be due to gender, dietary intake, physiological condition as well as genetic factors. SNP in *TF* and *HAMP* gene modulating the hepcidin concentration as well as serum iron level. Findings can be further concluded in a larger sample size.

O-12

Stable expression of anti-BmR1 IgG4 antibody

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Lymphatic filariasis is a parasitic disease and one of the most disabling in the world. Thus, WHO called for elimination of this disease as a public-health problem in 1997. Diagnostic kits were made and distributed worldwide to address this problem, and one of the kits is the Brugia Rapid test (Reszon Diagnostic International Sdn. Bhd., Malaysia) that uses recombinant Brugia malayi antigen BmR1 which is highly specific and sensitive for antifilarial igG4 antibodies in patients with brugian filariasis. In the manufacturing of rapid tests, quality control (QC) is necessary to ascertain the reactivity and specificity of the tests [1]. An anti-BmR1-IgG4 antibody which was synthesized and verified as a QC reagent was produced through transient expression [2]. However, transient expression has its downsides of being laborious, having low productivity and high cost in the long term as compared to stable expression which requires much lower amount of plasmid DNA and act as a renewable source of expressing cells. Thus, this study investigates the conversion of transient to stable expression of the antibody.

Methodology

Stable cell lines were generated using Flp-In system and Flp-In 293 cell line (Thermo Fisher). DNA sequence of anti-BmR1 IgG4 was cloned into pcDNA5/FRT, which was then co- transfected with pOg44 into Flp-In 293 cell line using Lipofectamine 3000 (Thermo Fisher). True transfectants were selected based on Hygromycin B resistance and expanded to make cell stocks and working cells for antibody expression.

Protein A affinity chromatography was used to purify the supernatant, which contained the protein, after the cells had been harvested. The respective recombinant antibodies were analysed qualitatively using Western Immunoblot, as well as quantitively at 280nm using a nanodrop spectrophotometer.

Results and Discussion

Western immunoblot using anti-BmR1 IgG4 recombinant antibodies showed bands at the approximate molecular masses at 69.3kDa [2]. In 10 days, 1cm^2 of cell culture surface area (approximately 1.5 x 10^5 of cells) expresses up to 0.776µg of antibody. Transient or stable expression systems should be chosen based on throughput, the number and quality of resources required, and turnaround time.[3]. Stable cells are favoured for large-scale protein production because they offer high yields and reliable quality of no batch to batch variation. Site-specific recombination has the ability to consistently produce stable cells with high yields. The total surface area of a planar vessel can be increased to easily increase the stable cell pool.

Conclusion

Although transient system may be apt for initial studies, mass production of antibody would require a stable expression system. The stably expressed antibody functions similarly to its transiently expressed counterpart. The Flp-In system is able to stably express the anti-BmR1 IgG4 antibody consistently and sustainably in an infinite manner, saving cost long-term.

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O-13

Tissue-based proteomics: insight into molecular mechanisms in cervical carcinogenesis

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Reversible phosphorylation is one of the critical post-translational cell events, controlled by protein kinases and protein phosphatases. Protein kinases are involved in many human diseases including cancers. Protein phosphatases regulate cellular events by reversing protein kinases activity. The serine/threonine protein phosphatase 4 (PP4) is an essential protein for nucleation, growth, and stabilization of microtubules in centrosomes/spindle bodies during cell division and survival of T cells. It consists of catalytic subunit (PP4c) interacting with four different regulatory subunits (PP4R1, PP4R2, PP4R3 and PP4R4). Previous studies showed that PP4c has an important tumour suppressor function and plays crucial role in the control of cell fate in leukemic T-cells and untransformed human peripheral blood T-cells. The present study investigates the role of the protein phosphatase 4 regulatory subunit 1 (PP4R1) in leukaemia.

Methodology

Two different leukemic T cell lines Jurkat and CEM-C7 were transfected with two different PP4R1 specific siRNAs to study the effects of PP4R1 down-regulation on cell survival or with a plasmid encoding PP4R1 (pcDNA3.1-PP4R1) to investigate the effects of PP4R1 up-regulation.

Results and Discussion

Western blot analysis confirmed both down-regulation and over-expression of PP4R1. Reduction of PP4R1 protein levels was associated with an increase in the number of both total and viable cells. Increased levels of PP4R1 led to a significant decrease in the total and viable cell number, increase in basal apoptosis and cell cycle arrest in G1.

Conclusion

Overall, the results show that together with PP4c, PP4R1 regulates cell survival and growth of Jurkat and CEM-C7 leukemic T cells and propose that it could play an important role in maintaining the balance between cancer cell survival and death and might clarify the distinct pathological mechanism of leukaemia.

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O-14

X-chromosome wide association in Thai SLE patients

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Previous study has shown that the genetic components especially in X chromosome contributed to female-biased found in Systematic Lupus Erythematosus (SLE) patients [1]. A copy of X chromosome is relatively correlated with SLE development [2]. Due to the global hypomethylation or defective in X-Chromosome Inactivation (XCI), [2] the studies found that 23% of genes can be able to escape from this mechanism leading to X-linked gene over-expression found in SLE [3]. As genetic variation is deviated in the different population influenced the incidence and mortality rate. Therefore, our study aims to investigate a global variant on X chromosome in Thai population. This might help to address the high female to male incidence ratio found among SLE patients.

Methodology

All procedures are approved by ethnical committee at Faculty of Medicine, Chulalongkorn University (EC. 590223). Using Asian Screening Array results from previous study [4], We conducted genome-wide association study (GWAS) on X chromosome from 892 SLE samples compared with healthy controls data (n = 1,683). Low quality samples and bad SNP markers were excluded by quality control processes (QC). Inflation factors after QC process are 1.008. Next, haplotype estimating was completed by SHAPEIT to pre-phasing the SNPs before imputation step by IMPUTE v2.3.2 software using -chrX function. SNPs association analysis were analyzed using plink v1.9 with logistic correlation approach.

Results and Discussion

From our analysis, we characterized several known independent susceptibility SNPs in Thai SLE patients which are including rs1059702 (G>A) (OR = 0.63 , p-value = 9.30x10^{-}) located at *IRAK1- MECP2-TMEM187* region. Interestingly, the risk loci rs777448097 in *TLR7* (p-value 1.36 x 10^{-}, OR = 0.77) were found in Thai population while it was absent in Hon Kong and Central Chinese background [1]. Furthermore, we found novel suggestive signal (rs14229594, C>T) on *GAB3-CTAG1A loci* (OR = 0.71, P = 2.14x10). This variant showed higher effect size in female SLE patients and located within the enhancer region of CD14+ Monocytes (ENCODE database).

Conclusion

In the present study, we report a number of known SLE susceptible loci, specifically in Thai population. Although the suggestive novel risk loci on *GAB3-CTAG1A* regions have been identified, further study are required to validate this result. Our finding might help to address the female biased phenomenon associated with SLE in Thai populations.

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O-15

The Effect of Mesenchymal Stem Cells-Mediated Macrophages Activation on Breast Cancer Progression

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Breast cancer (BC) is the second most leading cause of cancer- related death among women worldwide and represent about 11.7% of all new diagnosed cancer cases in 2020 [1]. High population of tumor-associated macrophages (TAM) in tumor microenvironment (TME) which display M2-type macrophages promotes BC metastasis and correlates with poor clinical prognosis in BC patients. Reprogramming TAM into M1-type macrophages that capable of killing tumor cells has emerged as a favorable therapeutic target in BC. Mesenchymal stem cells (MSC) can stimulate immunomodulatory changes and able to skew naïve macrophages (M0) to M1-type macrophages [2]. Therefore, this study is attempted to evaluate MSC-macrophages crosstalk on BC progression.

Methodology

THP-1 cell was incubated with PMA for 48 hours to differentiate into M0. M0 were co-cultured with MSC for 30 hours. Conditioned medium (CM) were collected to evaluate M1- and M2-type macrophage related cytokine while total RNA were extracted from co-cultured cells and then synthesised into cDNA to determine IRF-4 and IRF-5 mRNA gene expression using RT- PCR. Finally, MDA-MB-231 cells were cultured with CM of respective treatments for 24, 48 and 72 h to evaluate their cell viability using MTT assay.

Results and Discussion

Results showed that UC-MSC significantly increased IRF-4 expression in M0 and M0+LPS compared to M0 alone but no effect on IRF-5. In addition, UC-MSC did not influence the secretion of IL-10 and TNF-a in the supernatant of M0 and M0+LPS. Moreover, CM from M0, M0+UC-MSC, M0+LPS and M0+LPS+UC-MSC significantly reduced the proliferation of MDA-MB-231 cells at 72 h compared with 24 h treatment respectively. The results indicated that UC-MSC induced the polarisation of M0 into M2-type phenotype as IRF-4 regulates M2-type macrophage polarisation. M0 secretory products suppressed ER- breast cancer cells (MDA-MB-231) as observed in previous study (3). UC-MSC and LPS also may stimulate the M0 to secrete secretome that compose of anti-cancer properties that hindered the proliferation of triple negative BC cells. This finding is consistent with prior study that showed umbilical cord matrix derived MSC-CM inhibited the cell viability of BC cells.

Conclusion

Our findings demonstrate that UC-MSC may polarise naïve macrophages into M2-type macrophages. UC-MSC and LPS work in synergy in stimulating M0 macrophages to secrete anti- cancer products that suppress the growth of triple negative breast cancer cells.

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O-16

Therapeutic properties of Malaysian stingless bee pollen and its protective effect against DNA damage

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Reactive Oxygen Species (ROS) can disturb cellular metabolism and damage cellular biomolecules, which could lead to DNA damage. Natural compounds including bee-collected pollen contain nutrient antioxidants that have therapeutic properties and protective effects against ROS. Bee pollen is known as a complete food since the food energy produced is relatively high, thus it serves as a source of nutrients for adult bees and larvae [1]. Studies demonstrated that 70% of bee pollen compositions are biologically active and exhibit numerous benefits including nutrition, cardioprotection, hepatoprotection, immunostimulant, antioxidation, anticarcinogen, antibacteria, antiosteoporosis, antiprostatitis, anti-anaemia, anti-ageing, and anti-inflammatory [2][3]. This study aims to investigate the protective effect of stingless bee pollen against DNA damage.

Methodology

Bee pollen ethanolic extracts (BPEs) were prepared from three stingless bee species commonly domesticated in Malaysia: i.e., *Tetrigona apicalis, Heterotrigona itama*, and *Geniotrigona thoracica.* The methodologies used in this study were spectrophotometric method for total phenolic/flavonoid content and antioxidant activities, HPLC and GC-MS chromatographic techniques for phenolic compounds identification, trypan blue exclusion assay for antiproliferation test, and comet assay for DNA damage activities. MCF-7, MCF-10A and HT29 cell lines were used in this study.

Results and Discussion

In antioxidant assay, the result showed that BPE from Malaysian stingless bee species contained different phenolic and flavonoid contents, with each extract possessing different amounts of antioxidant activity. *G. thoracica* BPE possessed the highest capacity to neutralize DPPH radicals compared to *T. apicalis* and *H. itama*. HPLC and GC-MS analysis detected various polyphenol compounds and chemical groups in each species. The chemical compounds found in BPE have lots of biological activities with the antioxidative potential to be explored as health supplements and medical treatment. In antiproliferation assay, *G. thoracica* exhibited the highest therapeutic index (TI=3.12), with the $EC_{}$ of 0.5 mg/mL in HT-29 cells. With the therapeutic potential, stingless bee pollen may provide a preventive function against ROS and the development of many lifestyle diseases.

Analysis of DNA damage activity at 24 h of *G. thoracica* treatment showed a significant decrease in $HO_{}$-induced DNA damage compared to the untreated cells (63.82% $\pm$ 2.46 vs. 90.86% $\pm$ 0.68) ($p<0.01$). Similarly, a significant reduction was also seen with the supplementation of caffeic acid (49.05% $\pm$ 4.23) and quercetin (43.98% $\pm$ 3.77) ($p<0.01$). More significant reduction of DNA damage was observed at 72 h of *G. thoracica BPE* (20.49% $\pm$ 0.73), caffeic acid (**5.65%** $\pm$ 0.35), and quercetin treatment (**7.58%** $\pm$ 0.32) ($p<0.01$) on $HO_{}$-exposed HT-29 cells. Due to its strong antioxidant activity, *G. thoracica* BPE gave a protective effect against $HO_{}$-induced DNA damage.

Conclusion

Results of the present study discovered that Malaysian stingless bees, particularly *G. thoracica* species have a genoprotective effect that can protect cells from $HO_{}$-induced oxidative DNA damage. Even though the exact mechanism concerning the protective effects of BPE is still unconfirmed, the bioactive phytochemicals and antioxidant capacity are believed to be responsible for the suppression of oxidative DNA damage and may confer protection from genetic instability.

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O-17

Novel thermoresponsive Chitosan/PEG based hydrogels and their prospective biomedical applications

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

To date there is no commercial sensor based on hydrogels, especially for point-of-care tests where utilizing hydrogels could play a significant role as they offer the possibility to integrate the visualization agent into the sensing network. The Covid-19 pandemic clearly demonstrated the importance of point-of-care tests, especially those that can be performed by unskilled personnel. The objective of this study is to present a novel approach for the synthesis of hydrogel composed of Chitosan and Poly(ethylene glycol) (PEG) and understand its temperature responsive behavior. It attempts to prove the basic temperature sensing ability of Chitosan-PEG based hydrogel composites and define their sensing span.

Methodology

The first part of the study was focused on synthesis of Chitosan and PEG based hydrogel samples by first performing dissolution of both constituents respectively and then adding Glutaraldehyde as the cross-linking agent. It further included proposed hydrogel's swelling and dynamic behavior testing, which was followed by material characterization of the hydrogel composites by FTIR and SEM. The last section emphasized on use of the proposed hydrogel composite as a Temperature Sensor.

Results and Discussion

A thermally responsive hydrogel comprising of Chitosan and PEG is synthesized using a novel synthesis protocol. A detailed analysis of its physico-chemical properties by means of hydrogel swelling test, material characterization and conductometric testing has been carried out. This study particularly focuses on the potential of using Chitosan/PEG composite as a temperature responsive hydrogel based sensor. The hydrogel-based temperature sensor testing leads us to conclude that most compositions seem to degrade after being exposed to a temperature as high as 70˚C and show mild degradation on temperatures as low as 10˚C. The safe range for their thermoresponsive operation is 30˚C to 60˚C which is a wide temperature-detection range.

This sensor is proven to possess both high water content and high electrical conduction properties. The recommended utilization of the proposed hydrogel based sensor is as a conducting material for thermoresponsive biosensors and a Interestingly, the quantification results showed an upward trend for the up-regulated proteins and a downward trend for the field-friendly in vitro diagnostic device for molecular diagnostics. Based on the developments made in this research, further studies on the robust use of this sensor as part of diagnostic interfaces in medical devices may be carried out.

Conclusion

Detailed experimentation shows that the synthesized novel Chitosan/Poly(ethylene glycol) hydrogel composites present a thermally responsive behavior. They have potential to be used in temperature responsive hydrogel-based sensor systems which could be used in medical applications. Hydrogel based biosensor can be a platform technology that is not restricted only to the diagnostics but may be integrated with other areas for the provision of real-time data that will be of use to medical care professionals for better health care and management.

O-18

Acute and sub-chronic toxicological evaluation of probiotic strain *Lactobacillus rhamnosus* GG in Sprague Dawley rats

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Lactic acid bacteria are one of the major groups of gastrointestinal bacteria in healthy humans. A strong history of commensal status and long-term usage without any obvious adverse effects has classified them as 'Generally Recognized as Safe'. In the recent years, bacteriotherapy using probiotics as therapeutic agents have shown to be promising emerging method for evasion of infectious diseases. *Lactobacillus* spp. as probiotics has been questioned for its safety due to recently reported unexpected reactions. These include *Lactobacillus*– associated systemic infections, metabolic reactions, immune disorders and gene transfer *etc.*]. Among the probiotics, opinions proposed by several organizations between countries remain still contradictory. Although *Lactobacillus* spp. as probiotics have been classified safe, *Lactobacillus rhamnosus* strain GG requires further surveillance with additional studies.

Methodology

Acute and sub-chronic toxicity studies of probiotic strain *Lactobacillus rhamnosus* GG in Sprague-Dawley rats (180 ± 20 g) as per OECD TG 423 and 407, respectively. Acute oral toxicity study was conducted by the administration of 1 × $10^{}$, 1 × $10^{}$ and 1 × $10^{}$ cfu/ ml of *L. rhamnosus* (n = 3/ group). The animals were observed for their behavioral, neurological and autonomic functions continuously for 24 h and monitored 14 days thereafter for mortality. Sub-chronic toxicity study was conducted by the administration of 1 × $10^{}$, 1 × $10^{}$ and 1 × $10^{}$ cfu/ ml of *L. rhamnosus* once daily for a 28 day period (n = 12/ group). During the study period, the animals were monitored for changes in body weight and behavioral functions. The blood sample was collected on day 14 and 28, used for biochemical and hematological parameters analysis. histopathological analysis.

Results and Discussion

In acute toxicity studies, *L. rhamnosus* did not show any changes behavioral, neurological and autonomic functions, and mortality. In sub-chronic study, *L. rhamnosus* did not show any significant changes in body weight gain, organ weight analysis and behavioral functions. *L. rhamnosus* at 1 × $10^{}$ cfu/ml did not show any significant changes in biochemical and hematological parameters when compared with the control. Whereas *L. rhamnosus* at 1 × $10^{}$ and 1 × $10^{}$ cfu/ml showed dose-depended changes in elevated aminotransferase levels when compared with the control. In histopathological analysis, mild degeneration of hepatic cells and nephrons were observed in *L. rhamnosus* at 1 × $10^{}$ cfu/ml administered group when compared with the control.

Conclusion

The probiotic *L. rhamnosus* GG did not show any significant toxic effect at 1 × $10^{}$ cfu/ml and exhibited mild-to-moderate toxic effects at the dese levels of 1 × $10^{}$ cfu/ml and 1 × $10^{}$ cfu/ml. Pre-clinical toxicity testing helps to calculate "No Observed Adverse Effect Level [NOAEL]".

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O-19

In-Depth Investigation of microRNA Methylome Signature in Colorectal Cancer

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Colorectal cancer (CRC) is one of the top causes of cancer-related deaths worldwide. Despite substantial breakthroughs in diagnostic services and patient care, various gaps remain to be filled, ranging from early detection to the identification of prognostic indicators, effective therapy for metastatic illness, and the implementation of personalised treatment methods. MicroRNAs, which are small non-coding RNAs, are unregulated in CRC and play an important role in its onset and progression. Despite this, microRNA research has traditionally relied on expression levels to assess biological importance [1]. The precise mechanism underlying microRNA dysregulation in cancer has yet to be determined, however multiple studies have shown that epigenetic mechanisms, notably DNA methylation, play essential roles in the regulation of microRNA production. Thus, we intend to investigate the miRNA methylome patterns and explore their roles CRC.

Methodology

Fifty-four pairs (n=108) of CRC and the respective adjacent normal tissues were collected from the UKM Medical Center, Malaysia. Methylation profiling was performed using the Infinium Human Methylation 450K beadchip, which covers 485,577 CpG dinucleotide sites distributed over the whole genome. The raw IDAT files were exported from the scanner, and quality control was performed using Genome Studio and were further analysed using the ChAMP R package.

Results and Discussion

We obtained 933 miRNA probes for the downstream analysis. These probes were further classified as hypermethylated or hypomethylated based on the absolute average β value difference (Δβ) at ≥0.2 between CRC and normal adjacent tissues. A total of 230 probes were identified. Distribution of hypomethylated miRNAs with respect to genomic regions revealed more than half of the miRNA probes were located in the TSS1500 (n = 130; 58%), followed by TSS200 (n=55; 24%) and body (n=40; 18%). On the other hand, the distribution of hypermethylated miRNA probes are almost equal in TSS200 (n=3; 60%) and TSS1500 (n=2; 40%). Meanwhile, categorization based on CpG islands (CGIs), 225 probes were hypomethylated and five probes were hypermethylated. The five hypermethylated miRNA probes were MIR1180_cg24553547, MIR124-3_cg02650317, MIR34B_cg22879515, MIR124-3_cg15699267 and MIR137_cg22333214. Among these, MIR1180_cg24553547 showed the most significant differentially hypermethylated delta β-value. Although atypical methylation status has been documented in tumour samples, the methylation profile of miR-1180, which is located on chromosome 17, has yet to be thoroughly explained in CRC. Many prior research demonstrated a good association between hypermethylation of the miR-1180 gene and downregulation of its expression in tumour tissue when looking at its expression level in malignancies. Unlike other miRNAs, miR-1180 functions as both an oncogene and a tumour suppressor. Tan et al. [2] and Zhou et al. [3] previously demonstrated the carcinogenic activity of this miRNA in hepatocellular carcinoma by directly targeting both OTUD7B and TNIP2 to drive cell proliferation and apoptosis resistance.

Conclusion

Our findings imply the existence of new pathways involving methylation alterations impacting miRNA genes that control CRC development. The new knowledge from this study can be utilized for personalized health diagnostics, disease prediction, and monitoring of treatment.

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Acknowledgements: This research was funded by Ministry of Higher Education Malaysia, grant number FRGS/1/2020/SKK0/UKM/02/11.

O-20

Identification of LRRC17 as colonic fibroblast activation marker and its potential role in colorectal cancer progression

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Stromal fibroblast is linked with poor prognosis of colorectal cancer (CRC) [1]. Crosstalk between cancer-associated fibroblasts (CAFs) and cancer cells facilitated by growth factors drives the malignant progression. CAFs can be identified using conventional markers such as alpha-smooth muscle actin (α-SMA) although their expression can be heterogenous. Leucine Rich Repeat Containing 17 (LRRC17) is proposed to be an emerging biomarker to specifically identify activated fibroblasts and CAFs. This study was aimed to verify LRRC17 expression in different colonic fibroblasts and further characterize LRRC17 in CRC. This may provide insights on the underlying mechanisms and verify its potential as specific marker for future prognostic purposes [2].

Methodology

LRRC17 and α-SMA expressions were analyzed using immunofluorescence staining on CCD-112CoN (MF cell line) and primary fibroblast lines derived from colorectal tumor and normal adjacent colon tissues (denoted as normal fibroblasts). SW620 (CRC epithelial cell line) was used as control. The cells were cultured under different conditions; a) serum-free medium (DMEM alone), b) DMEM + 10% fetal bovine serum (complete medium), c) conditioned medium (CM) of SW620, and d) DMEM + transforming growth factor β1 (TGF-β1). Fluorescence intensity was assessed via ImageJ 1.53k. Treated cells proliferation was assessed via MTT assay. All experiments were conducted in duplicates.

Results and Discussion

Positive LRRC17 and α-SMA expressions were observed in all fibroblast groups and CCD-112CoN except SW620. However, different co-localization pattern was shown between the proteins in treated cells. Both proteins expression was greater in complete medium and TGF-β1 as compared to serum free group, indicating more activated state of treated fibroblasts mimicking CAF property. Interestingly, contradictory to fibroblasts from CRC, LRRC17 expression was found decreased in normal fibroblasts in complete medium and TGF-β1. Higher LRCC17 expression whereas lower α-SMA expression in CM SW620 for primary cell lines. This may highlight the cancer secretome effects which trans-differentiate fibroblasts or change their molecular property. Different expression pattern seen in the present study may indicate different pathways involved in the regulation of these markers, suggesting further study. From MTT assay, highest proliferation was found in fibroblasts treated with complete medium. It was proposed that growth factors in serum support the cell growth [3].

Conclusion

LRRC17 can potentially serve as biomarker to identify and characterize CAFs of CRC.

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O-21

Effect of polyphenolic-rich fraction of cornsilk (*Stigma maydis*) in streptozotocin-induced diabetic rats

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background
Diabetes is a chronic condition that arises when the pancreas does not create enough insulin or when the insulin produced is not used efficiently by the body. Hyperglycemia, or elevated blood sugar, is a frequent complication of untreated diabetes and can cause catastrophic harm to a number of the body's systems, including the neurons and blood vessels. Current available drug such as metformin which is widely used to treat hyperglycemia have some side effects such as diarrhea, vomiting and lactic acidosis. Currently, there is a keen interest on studying the benefits of food waste, hence researchers started to study the benefits of natural product food waste in treating many pathological conditions. Therefore, the aim of this research is to study the effects of polyphenolic-rich fraction of cornsilk in reducing the fasting blood glucose level in diabetic-induced rats.

Methodology
The effect of polyphenolic-rich fraction (PRF) of corn silk (CS) extract were studied on the normal cell line Human Umbilical Vein Endothelial Cells (HUVEC) and Streptozotocin-induced diabetic rats **Streptozotocin-induced diabetic rats.**

Results and Discussion
The proliferation assay result of PRF of CS obtained shown significant percentage of cell viability when compared to metformin ($p<0.05$). The effects of PRF of CS extracts in fasting blood glucose level shown no significant different between treated and untreated group ($p>0.05$), might be due to the 14-day duration of this study. The effects of PRF of CS in body weight of rats also shown no significant different when compared between treated and untreated group ($p>0.05$).

Conclusion
However, the histopathological study shows promising effect of PRF of CS in treating diabetes when compared to untreated diabetic group by assessing the morphology of the glomerulus, tubules and Bowman space in kidney and the morphology of centrilobular area, hepatocytes and sinusoidal space in the liver. The long-term impact of PRF on CS in diabetic models has to be studied further by repeating the research study over a longer time period.

O-22

Phytochemical, anti-microbial activity and anti-proliferation test against human cancer-origin cell lines using water extracts of *Momordica Cochinchinensis* (Gac fruit)

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Momordica cochinchinensis (Gac fruit) is a seasonal tropical fruit that is widely used in Southeast Asian countries [1]. However, it's not fully exploited in Malaysia. The major component of this fruit that been mainly focussed in is the aril, however the pulp, peel and seed are often discarded. Therefore, this study aims to scientifically validate the presence of phytochemicals composition, determine the antioxidant, antimicrobial, anti-proliferative activities, and wound healing properties of different parts (aril, pulp and seed) of Gac fruit grown in Malaysia.

Methodology

This fruit (aril,pulp and seed) was macerated and extracted using water following the traditional preparation approach. Toxicity test using brine shrimp (BSLT), phytochemical tests, antioxidant, antimicrobial, antifungal, cytotoxicity cell-based assays were conducted.

Results and Discussion

The crude extract showed 50% mortality in brine shrimps after 24 hours and its LC_{50}-value was considered moderate toxic. Qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, volatile oils, glycosides and tannins. The highest scavenging activity of water extract from the pulp with 30.1 mg GAE/g FW and 0.012 mg GAE/g FW in both DPPH and FRAP respectively. The pulp water showed high levels of total phenolic and flavonoid content of 0.0215 mg GAE/g FW and 0.083 mg QE/g FW, respectively. Moreover, pulp water extract displayed strong antimicrobial activity with MIC values ranging from 5-20 mg/ml and MBC values of 10-20 mg/ml on certain microbial strains. Cancer origin cells MCF7, HepG2, A549, HCT116 and HT29 were found to be more susceptible with the treatment of aril and pulp water extracts with the LC_{50} at 15.41, 5.87, 22.89, 1.10 and 0.03 µg/ml while pulp at higher values such as 269.40, 328.46, 34.90, 68.07 and 21.44 µg/ml at 72 hours post-treatment compared to other tested cell lines.

Conclusion

The results concluded that Gac fruit from crude extracts revealed chemical constituents (alkaloids, flavonoids, saponins, volatile oils, glycosides and tannins) contributing to their antioxidant and antimicrobial activity against *E.coli, P.euroginosa, B.cereus and S.flexneri.* Further study on the mechanism pathway needs to be explored since Gac fruit showed antiproliferative activity against breast, liver, lung and two colon cancer cell lines.

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O-23

Conjugates between P. *marcocarpa* aqueous extract and TiO_2 exhibited a synergistic antimicrobial effect

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Phaleria marcocarpa (P. *marcocarpa*), also known as Mahkota Dewa is a flowering plant of Thymelaeceae family. Originated from Indonesia, it thrives in tropical climate countries such as Malaysia. It has been reported to possess anti-diabetic and anti-bacterial properties and is often brewed as tea for consumption. However, the antimicrobial activities of P. *marcocarpa* is proven to be weak and limited to limited strains of microbes. Titanium dioxide nanoparticles (TiO_2 NPs) is a semiconducting transition metal with strong antimicrobial activities. P. *marcocarpa* aqueous extract was conjugated with TiO_2 NPs in this study to determine the synergistic antimicrobial effect of the resultant conjugate against *Escherichia coli* (E. *coli*) and *Staphylococcus aureus* (S. *aureus*).

Methodology

The aqueous P. *marcocarpa* fruit extract was extracted through maceration for 24 hours. The extract was filtered through Whatman® filter paper No 1 and freeze dried. TiO_2 NPs were synthesized through the sol-gel method. The conjugation between the aqueous extract and TiO_2 NPs were carried out at various temperature (30°C, 45°C and 60°C). The resultant conjugate was characterized through FTIR. The antimicrobial activity test was carried out through MTT assay against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 6538). The assay was carried out using 96-well sterile microtiter plate. The concentration of extract, TiO_2 NPs and resultant conjugate used were from 125 to 15.6 µg/mL.

Results and Discussion

The FTIR results showed difference between the TiO_2 NPs and resultant conjugate around the fingerprint region. Bands at 3305, 2129, 1637, 1538 and 1370 cm^{-1} have been shown by pure TiO_2 NPs, while bands at 3305, 2144, 1637 cm^{-1} have been shown by the extract. Additional band at 1045 cm^{-1} was shown by the resultant conjugate while additional band at 792 cm^{-1} was observed for the conjugate prepared at 60°C. The 1045 cm^{-1} band represented anhydride and alcohol stretching while the 792 cm^{-1} band represented the bending of alkene. From the antimicrobial test against E. *coli* and S. *aureus*, the conjugates prepared at 30°C, 45°C, and 60°C has minimum inhibitory concentration (MIC) of 15.6 µg/mL. However, the conjugate at 45°C showed lowest CFU/mL of 3.5×10^{1} for E. *coli* and 9.6×10^{1} for S. *aureus* when compared to TiO_2 NPs and other conjugates (30°C and 60°C) at the same concentration. Thus, it was proven that 45°C is the optimum temperature for TiO_2 extract conjugation in view of growth inhibition for E. *coli* and S. *aureus* as it showed lower CFU/mL as compared to the pure TiO_2 NPs.

Conclusion

In conclusion, the MTT assay was effectively used at evaluating the antimicrobial activity. The resultant conjugate showed the most remarkable synergistic antimicrobial effect of MIC at 15.6 µg/mL and 45°C for both E. *coli* and S. *aureus*.

O-24

Cytotoxicity, Proliferation and Migration Assessment of BHMC, the Curcuminoid Analogue on Human Liver Cancer Cells, HepG2

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Over the years, natural bioactive compounds were acknowledged for their cytotoxic and anticancer capabilities. Curcumin is a bioactive compound derived from the rhizomes of *Curcuma longa* known to possess various pharmacological properties e.g., inhibiting cancer growth, exerting cytotoxic and inducing apoptosis [1]. Nonetheless, curcumin has some limitations that prevent it from reaching its full potential. Due to poor bioavailability, 2,6-bis(4-hydroxy-3-methoxybenzylidene)cyclohexanone (BHMC), a curcuminoid analogue was synthesised via chemical structure modification by eliminating the unstable β-diketone moiety and converting it into a conjugated double bond while retaining the phenolic hydroxyl group to overcome the limitation by exerting greater cytotoxic, growth suppressive effects with more selective on cancer cells [2]. Therefore, it would be beneficial to look at the cytotoxicity along with the anti-proliferation and -migration effect of BHMC on HepG2 cells.

Methodology

The HepG2 and Hs27 cells were treated with various concentrations ranging from 0.781-50µM for 24 and 48hrs via MTT assay. Based on the IC_{50} value obtained, several concentrations were selected to be used in Trypan Blue Exclusion (TBE) assay, Migration assay and Hoechst/Propidium Iodide (PI) staining. Data were analysed using GraphPad Prism software.

Results and Discussion

BHMC was observed to be approximately 3-5 times more toxic to HepG2 compared to curcumin with IC_{50} values of 16.73µM and 46.03µM at 24hrs while 4.77µM and 26.00µM at 48 hrs, respectively. However, BHMC was less toxic towards Hs27 cells with the IC_{50} value of more than 30µM at 24 and 48 hrs. BHMC significantly reduced HepG2 by 30-60% at 24hrs and 70-80% after 48hrs at lower concentration than curcumin. In contrast, BHMC only reduced 20-30% of Hs27 at 24hrs and 40-60% at 48hrs. Curcumin reduced 40-60% of HepG2 for 24 and 48 hrs but only reduced 30% of Hs27 cells at similar concentrations and timepoint. Although BHMC and curcumin reduced the percentage of HepG2 cell viability in a concentration- and time-dependent manner, the anti-proliferative effect exerted by BHMC was greater compared to curcumin. BHMC also showed a cytotoxic selective effect on Hs27 especially at 48 hrs with less cell death at lower concentrations compared to HepG2. It is suggested that BHMC was cytotoxic selective towards Hs27 like its parental compound, curcumin. BHMC and curcumin suppress the migration of HepG2 as compared to controls. However, below the IC_{50} value, BHMC exhibits a higher effect in suppressing HepG2 cell migration compared to curcumin. At lower concentrations, characteristics of apoptosis can be observed including cell shrinkage, membrane blebbing, chromatin condensation, DNA fragmentation and apoptotic bodies.

Conclusion

BHMC mediates a greater cytotoxic effect in HepG2 by reducing the cell viability, modulating a higher percentage of cell death at lower concentrations compared to curcumin, suppressing migration, and inducing apoptosis compared to Hs27 while cytotoxic selective towards Hs27 cells at similar concentrations.

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O-25

Unraveling the tumour-regulatory role of miR-3934 in human breast cancer

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

MicroRNAs (miRNAs) are short, single-stranded, and non-coding RNAs that could regulate the expressions of multiple downstream cellular targets post-transcriptionally. By altering the expressions of tumour-promoting and tumour-suppressing targets, miRNAs could act as tumour-modulatory miRNAs in different human cancer. The preliminary findings from our previous work suggested that miR-3934 may be responsible in promoting tumour growth and migration in human breast cancer cell lines [1]. Therefore, this mechanistic study aimed to elucidate the tumour-regulatory role of miR-3934 in human breast cancer cells using *in vitro* cellular assays.

Methodology

Triple-negative human breast cancer cell, MDA-MB-231, was stably transfected with miRNA expression plasmids to produce clones that overexpressed miR-3934 mimic, miR-3934 inhibitor, or their respective scrambled control sequences. All transfected and un-transfected cancer cell types were subjected to cell viability, invasion, and wound-healing assays to evaluate the tumour-regulatory role of miR-3934 in breast cancer cells.

Results and Discussion

Overexpression of miR-3934 significantly ($p<0.05$) promoted breast cancer cell proliferation and resistance to chemotherapeutic agent, cisplatin. Besides, miR-3934 also enhanced ($p<0.05$) the invasion and migration abilities of the MDA-MB-231 cell line as compared to un-transfected control and clones that expressed scrambled control sequences. In contrast to clones that overexpressed miR-3934, clones that overexpressed miR-3934 inhibitor were shown ($p<0.05$) to have lower proliferation rate, higher sensitivity to killing by cisplatin, and have decreased invasion and migration abilities *in vitro* as compared to all other cell groups. There were no statistically significant differences in the cell proliferation, chemosensitivity to cisplatin, invasion, and migration rates between the untransfected control and clones that expressed the scrambled control sequence. The tumour-promoting role of miR-3934 observed in the current study is consistent with the findings observed in two other miR-3934 studies that involved lung cancer [2] and neuroblastoma [3], in which miR-3934 was reported to downregulate the expression of tumour suppressor protein such as TP53INP1 [2,3]. However, to date, the tumour-modulatory role and downstream target of miR-3934 in breast cancer are still unclear and under-reported. The current study findings suggested that miR-3934 has the potential to be utilised as a prognostic breast cancer biomarker and the introduction of a miR-3934 inhibitor may potentially inhibit breast cancer development. To further confirm the tumour-promoting role of miR-3934, transcriptomics and *in vivo* studies will be conducted to unveil the molecular mechanism of how miR-3934 promotes breast cancer development.

Conclusion

Overexpression of miR-3934 was shown to promote the proliferation, chemoresistance, invasion, and migration in the triple-negative MDA-MB-231 cell line while the miR-3934 inhibitor may potentially halt the tumourigenesis process in breast cancer.

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O-26

Whole-genome-scale identification of novel non-protein-coding RNAs controlling cell proliferation and survival through a functional forward genetics strategy

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Non-protein-coding sequences in the genome play crucial functional roles in a range of different cellular processes. Although the analysis of this vast number of transcripts is still at an early stage, it is evident that many lncRNAs play crucial roles in molecular cell biology. In the healthy state, lncRNA expression is often tightly restricted to specific tissues at specific times. It is not surprising therefore that deregulation of lncRNAs has been implicated in pathology. Although demonstrations of the functional importance of RNAs are impressive, the vast majority of lncRNAs are still uncharacterised; some of these currently uncharacterised lncRNAs likely play critical roles in important control processes that have yet to be revealed. This provides motivation for the development of high-throughput strategies for the identification of key lncRNAs by targeting their functional activity. Here we present a human genome-scale forward-genetics approach for the identification of lncRNAs based on function. This approach can identify genes that play a causal role and distinguish them from those that are differentially expressed but do not affect cell function. Our genome-scale library plus next-generation-sequencing and bioinformatic approach, radically upscales the breadth and rate of functional ncRNA discovery.

Methodology

Whole human genomic DNA was digested with the restriction enzymes Dra1 and Aan1. The digests were combined at equimolar concentration to produce our initial "Genomic Digest" (restriction fragments with blunt termini). The Genomic Digest was cloned into the Sma1 site of lentiviral expression vector pCDH-CMV4 (a third-generation lentiviral vector); producing the expression library with inserts in both sense and antisense orientation. The library was used to transduce human Jurkat T-leukaemic cells. Cell populations were selected using continuous culture ± anti-FAS IgM, and custom sequencing and bioinformatic approaches developed to identify sequences controlling cell fate (i.e. survival following previously lethal stimuli) and/or changes in proliferation rate.

Results and Discussion

Our sequencing and bioinformatic approach demonstrated that > 85% of the human genome was represented in our lentiviral library and confirmed that we were able to transduce up to 88% of these sequences into Jurkat JKM1 cells. We thus confirm that our CL3c library is highly diverse, and that we were able to transduce Jurkat JKM1 cells with many sequences simultaneously. In comparing naive cells, cells following transduction, and cells following selection, we demonstrate that (a) specific transduced cells are able to evade previously lethal stimuli, and (b) the population of inserts identified is markedly reduced. These data support our hypothesis that selection reduces the population of sequences to those with demonstrable function i.e. the ability to evade otherwise lethal stimuli. Our strategy identified thousands of new sequences based solely on their function, including many ncRNAs previously identified as modulating cell survival or key cancer regulators.

Conclusion

Herein we report a genome-scale screening approach that overcomes the limitations imposed by the highly tissue-specific expression of lncRNAs by interrogating sequences from the whole genome. Combining our established forward genetics approach with next generation sequencing and bioinformatics, we identify many thousands of lncRNAs from across the breadth of the genome, based entirely upon their function.

O-27

Selection of T-cell receptor (TCR) like antibody against Human Leukocyte Antigen A-2 (HLA-A2) for cervical cancer diagnostics

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Cervical cancer is a leading cause of death among women. Persistent human papillomavirus (HPV) contributes to the carcinogenesis of cervical cancer, in which 70% of all cervical cancer cases are linked to HPV 16 and 18. The available HPV vaccines effectively prevent cervical cancer but do not treat existing cervical cancer. Besides, cervical malignancy detection at early phases remains a challenge. Consequently, treatment begins at the late stages of the disease. Hence, new diagnostics and therapeutics methods are required to treat cervical cancer. In this study, a novel antibody class, known as T cell receptor (TCR)-like antibodies, specific to HPV 16 and 18 E7 oncopeptides, has been hypothesised to enhance cervical cancer diagnostic potentials. The dual functionality of the antibody in mimicking the function of T cell receptor (internal immunosurveillance) and simultaneously executes its antibody effector mechanisms to eliminate a disease effectively opens a new and promising pathway to target cervical cancer.

Methodology

Bioinformatics were used to select the target peptides specific to HLA-A2 of HPV 16 and 18 E7. Peptide-MHC (pMHC) complex was formed by refolding HPV peptides with HLA-A2 heavy chain and β2m light chain. Potential TCR-like antibodies were generated by panning the pMHC complexes against an antibody phage display library. The monoclones were analysed with monoclonal ELISA and comparative ELISA before sequence analysis.

Results and Discussion

The successful refolding of pMHC was analysed via ELISA with refolded β2m as positive control. A domain antibody library was used to select potential TCR-like antibodies specific to HPV 16 and 18 E7 pMHC by biopanning. Three rounds of biopanning were performed to get highly enriched antibody phages. Antibody phages from round three polyclonal panning were selected for monoclonal analysis because they have highest enrichment. For HPV 16 E7, 20 out of 92 clones were selected for monoclonal analysis and finally 9 clones were sent for sequencing. Meanwhile, for HPV 18 E7, 44 out of 92 clones were selected for monoclonal analysis and finally 17 clones were sent for sequencing. It was difficult to generate a novel group of TCR-like antibody until latest development in technologies like phage display techniques made it possible. The coat protein of a bacteriophage is fused with a protein/peptide and presented on a virion's surface in phage display technique. Antibody phage display technology has emerged as a famous approach for production of antibodies against various targets including cancer. TCR-like antibody is development based on distinctive roles of T (T cell receptor) and B cells (antibody). Generally, T cell receptor recognises the antigenic peptide presented by MHC complex on all nucleated cell while antibodies generated by B cells can differentiate soluble or membrane bounded antigens which will result in the elimination of illness due to its wider effector functions. Hence, dual functionality of TCR-like antibody in combining both humoral and cell mediated immunity in a single approach makes it an appropriate candidate for cervical cancer diagnosis.

Conclusion

2 out of 9 clones and 7 out of 17 clones for HPV 16 E7 and HPV 18 E7, respectively had proper sequence which were analysed by VBASE2 and IMGT/V databases. In the downstream process the proteins of positive antibody clones will be expressed and purified. The purified antibodies will be tested for their binding ability using ELISA and western blot. Finally, the antibodies will be tested in cervical cancer cell lines.

O-28

Development of T-cell Receptor-like Antibody Against Human Leukocyte Antigen-A11 (HLA-A11) Human Papillomavirus (HPV) Type 16 & 18 Oncoprotein E7 for the Diagnosis of Cervical Cancer

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background
Cervical cancer is the fourth most common cancer in women worldwide. Human papillomavirus (HPV) is the causative agent of cervical cancers with HPV 16 and 18 being the predominant types causing more than 70% of all cervical cancer cases. Cervical cancer can be prevented through HPV vaccination. However, diagnosing cervical cancer at an early stage remains a challenge. Therefore, a new diagnostic tool is required. In this study, TCR-like antibodies which are a novel class of antibodies that is specific to E7 oncoproteins of HPV 16 and 18 have shown to have the ability to enhance the diagnosis of cervical cancer. The ability of TCR-like antibodies in sandwiching the humoral and cell-mediated immunity in a single approach has brought light to a new promising pathway for targeting cervical cancer.

Methodology
Target peptides which are specific to Human Leukocyte Antigen A-11 (HLA-A11) HPV 16 and 18 E7 oncoproteins were identified and selected by bioinformatics. Peptide-MHC (pMHC) complex was formed by refolding the target peptides with heavy chain (HLA-A11) and light chain (β2m). Refolded pMHC were used to develop TCR-like antibodies via biopanning against the antibody phage display library. Positive monoclones obtained were sent for sequencing before being analyzed using VBASE2 and IMGTV databases.

Results and Discussion
The successful refolding of pMHC complexes for both HPV 16 E7 and HPV 18 E7 were analysed via ELISA using refolded β2m as the positive control. The refolded pMHC complex was subjected to three rounds of biopanning against antibody phage display library for selection. Phages from the third round of biopanning were selected for both HPV 16 E7 and HPV 18 E7 based on the polyclonal ELISA results as they showed the highest enrichment compared to the first and second rounds. 94 single colonies for each HPV 16 E7 and 18 E7 were randomly selected from the phages' titration for monoclonal biopanning. 10 out of 94 potential monoclones for HPV 16 E7 and 37 out of 94 potential monoclones for HPV 18 E7 were subjected to comparative biopanning based on the monoclonal ELISA results with a value after normalization of ≥0.3. A total of seven out of 10 potential monoclones for HPV 16 and 26 out of 37 potential monoclones for HPV 18 were sent for sequencing based on the monoclonal ELISA results with a value after normalization of ≥0.2. TCR-like antibodies are based on the distinct roles of T cell receptors that are involved in immunosurveillance and direct killing, and the B cell is involved in antibody production. TCR- like antibodies function by differentiating the antigenic peptide which is presented on the MHC molecules and establishing primary defence mechanisms of the antibody. Therefore, the duality of TCR-like antibodies' function makes it a great candidate for cervical cancer diagnosis.

Conclusion
In conclusion, one out of seven clones for HPV 16 and three out of 26 clones for HPV 18, respectively had proper sequences. In the downstream process, the proteins of the positive clones will be expressed and purified. Later, the purified protein will be evaluated for its binding capabilities using Western blot and ELISA. Finally, the antibodies will be evaluated on the cervical cancer cell lines.

O-29

High throughput molecular profiling of bacterial diversity in Johor mangroves

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Biomedical research heavily relies on the discoveries of natural compounds and thus it is very important to safeguard nature to build resilience in biomedical research. Mangrove is the coastal ecosystem that has proven to harbour several beneficial microbial communities with a wide range of therapeutic molecules such as antimicrobial agents, anticancer compounds and enzymes. Mangrove provides habitat for several organisms including bacteria that are adapted to the microclimate with high salinity and tidal variation [1]. Some of these bacteria include actinobacteria which form the major source of antibiotics. It is important to identify and discover new antibiotics as there is an increasing threat of resistance to currently available antibiotics and limited discovery of new antibiotics [2]. Mangrove biodiversity in rapidly developing parts of the globe is severely threatened due to the destruction of habitat and thus there is a high threat to the bacterial community. One of the major hurdles in studying mangrove soil bacteria is that it is tedious to culture them and hence difficult to get a complete profile of bacterial diversity by conventional methods. Thus, it is important to adopt high throughput molecular techniques to rapidly identify bacteria to accelerate conservation efforts [3].

Methodology

DNA was isolated from mangrove soils sediments from eight mangrove sites around Johor, Malaysia that is grouped based on their vulnerability status. The library was prepared using the multiplexing method with 24 barcodes to distinguish samples and sequenced for the 16S gene using Oxford Nanopore Technologies MinION sequencing. Base-calling of sequencing reads was performed by the Guppy programme and analysed using EPI2ME software. Quantitative PCR was performed to verify linear amplification.

Results and Discussion

We have successfully sequenced the bacterial 16S gene in mangrove soil samples from Pulav Kukup, Sungai Pulai, Sungai Melayu, Sungai Danga, Sungai Skudai, Sungai Johor, Sungai Sedili Besar and Sungai Sedili Kechil. We show that the barcoding would enable parallel sequencing of samples from all sites in the same run and amplicons showed linear amplification. We achieved over 6 million high-quality reads per run in less than 24 hours. We report a widespread difference in bacterial diversity between protected mangroves compared to mangroves that have been exposed to anthropogenic activities. This is the first study to show the bacterial community profile of Johor mangroves.

Conclusion

MinION sequencing is a quick and efficient way to evaluate bacterial diversity in environmental samples. The findings of this study would enable to develop a map of the bacterial community as a repository to help in conservation/rehabilitation as well as monitor the impact of mangrove loss on bacterial diversity.

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O-30

Ranking of the *Mycobacterium tuberculosis* T-cell epitopes using tabulated immuno-properties for potential Tuberculosis diagnosis and vaccine candidates

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Mycobacterium tuberculosis (*Mtb*) is a causative agent for Tuberculosis (TB) with estimated a quarter of the world population has been infected and has the highest cumulative mortality rate among other infectious diseases with 1.3 million death in 2020 alone (WHO, 2021). The report also highlighted TB as the leading cause of mortality by single infectious agent. Ten percent of Latent TB Infection (LTBI) will develop into active infection. Existing diagnostic methods e.g. Tuberculin Skin Test and are not accurate. Newer technique i.e. Interferon Gamma Release Assay is expensive, thus hardly affordable especially in low to middle income countries with high TB incident rate. Currently, there are lack of cheap, sensitive, and specific diagnostic methods to diagnose individuals with LTBI and the current BCG vaccine has limited coverage against adult TB infection.

Methodology

Reversed vaccinology technique was applied to shortlist potential *Mtb* T-cell epitopes (TCEs) for diagnosis and vaccine candidates. Six immuno-criteria were selected to rank the TCEs i.e. matching to *Mtb* H37Rv antigens, conservation across 273 *Mtb* strains, association with highly expressed *Mtb* genes and their experiment types, promiscuous epitopes, and population coverage. Each criterion dataset was obtained from a different database and individually pre-processed, analysed using a selected prediction method, and collated using an in-house script. Each factor was equally weighted and all of the factor results were integrated into a relational table to be used as an interactive scoring platform. At the end, each TCE has six scores from the six factors, and they are combined to produce the final score.

Results and Discussion

Looking from the perspective of individual criterion: 1) almost all TCEs have 1-to-1 matching to *Mtb* H37Rv antigens. 2) They also have a high matching coverage across all 273 completed strains tested. 3) majority of them matched to gene used in human-related experiment literature. 4) Almost all of the TCEs bind to at least a locus, with the combination of locus A, B, C has the highest match. 5) They were tested with their coverage in world, Asian, Southeast Asia and Malaysia populations; where more than half of TCEs cover more than 90% of the world population. 6) All alleles matched to TCEs showed very strong correlations to the tested populations covered. Looking from the perspective of top scoring TCEs, top thirty TCEs were further analysed for their associations to cellular functional groups, sequence motifs, common genes used for diagnosis and vaccine and *Mtb* B-cell epitopes. Most of the shortlisted epitopes were found to be parts of potential genes (encoded for proteins) that are widely studied for their cellular and humeral body response i.e. ESAT-6 family (e.g. esxJ, esxK, esxM, esxP, esxW, espJ, and espK), Ag85B, Ag85C, and HSP16.3.

Conclusion

These genes have been showed to have potential in TB diagnosis (e.g. Interferon Gamma Release Assay technique) and vaccine candidates.

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O-31

Desiccation tolerance mediates ST239-SCC*mec* type III-SCC*mercury* to ST22-SCC*mec* type IV MRSA Clonal Replacement in Hospital Settings

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

ST239-SCC*mec* type III-SCC*mercury* (ST239-III) to ST22-SCC*mec* type IV (ST22-IV) clonal replacement in methicillin-resistant *Staphylococcus aureus* (MRSA) has been reported globally in many hospitals during the first decade of the new millennium, including in Hospital Canselor Tuanku Muhriz (HCTM), Kuala Lumpur, Malaysia. It was hypothesized that the clonal replacement occurred due to faster growth rate of ST22-IV [1]. To confirm this, we studied and compared the competitive fitness of representative strains from the two clones, ST239-III and ST22-IV isolated from HCTM via broth co-culture and desiccation experiments.

Methodology

The strains used in this study were M57/2005 and M222/2009 (both ST239-III), M181/2017 and M080/2017 (both ST22-IV). Chloramphenicol (20ug/ml) susceptibility was used to differentiate the STs of tested strains; ST239 strains were resistant, ST22-IV strains were susceptible. In broth co-culture experiments, pairs of tested strains from different STs were cultured together for 48 hours in brain heart infusion (BHI) broth and platted on drug-free and chloramphenicol-supplemented BHI agar. In desiccation experiments, pairs of tested strains from different STs were exposed to desiccation for 48 hours on petri plates prior rehydration of the cultures with normal saline, followed by drug-free and chloramphenicol-supplemented BHI agar platting and incubation at 37°C overnight. Numbers of colonies on agar plates for both STs in both experiments were enumerated. SCC*mec* typing of representative colonies was also performed for ST confirmation.

Results and Discussion

For broth co-culture experiments, mean of 194.5, (135-272) ST239-III colonies and 31.5, (4-58) ST22-IV colonies were observed at 10^{-5} serial dilution. On the other hand, ST22-IV produced higher number of surviving colonies on desiccation assays (29, 5-58) compared to ST239-III (2.5, 0-5) at 10^{-5} serial dilution. While ST239-III outcompeted ST22-IV in all tested pairs for the broth co-culture studies, the latter clone had better tolerance to desiccation. As MRSA is a nosocomial pathogen with clonal dissemination in hospitals [2], higher desiccation tolerance could have provided ST22-IV an edge to survive and disseminate, ultimately replacing ST239-III in the hospital environment.

Conclusion

ST22-IV was found to have higher desiccation tolerance compared to ST239-III. This edge in bacterial fitness might be the cause of ST239-III to ST22-IV clonal replacement in nosocomial MRSAs; further studies will be required to confirm this.

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O-32

IL-21 gene silencing suppressed the proliferative activity of HCT116 and HT-29 colorectal cancer cells

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Interleukin-21 (IL-21) is a class I cytokine produced mainly by natural killer T (NKT) cells and CD4+ T cells. IL-21 has been implicated in possessing pleiotropic functions in cancer immunity associated with infectious diseases [1]. However, the functional role of IL-21 in the solid tumor of colorectal cancer has not been fully elucidated. Sustaining proliferative signalling is one of the important hallmarks of cancer [2]. Therefore, the present study aimed to investigate the effect of IL-21 gene silencing on the proliferative activity of colorectal cancer cells.

Methodology

HCT116 and HT-29 cells were first transduced with lentiviral shRNA to silence IL-21 gene expression. A clonogenic or colony formation assay was then performed to determine the clonogenic activity of IL-21-silenced cells. The percentage of colony formation in a fixed area relative to control after one week of incubation was determined. Besides, western blotting was used to detect proliferation cell nuclear antigen (PCNA) in IL-21-silenced cells, and its expression level was later determined via densitometry analysis using Image J software.

Results and Discussion

Results in the clonogenic assay demonstrated that IL-21-silenced cells experienced a decrease in cell proliferation rate when the IL-21 gene was silenced. IL-21-silenced HCT116 cells established only 65.19% (P<0.01; shIL-21a), 69.31% (P<0.01; shIL-21b), and 71.87% (P<0.05; shIL-21c) of colony formation relative to control. IL-21-silenced HT-29 cells displayed percentages of 87.48% (P<0.01; shIL-21a), 88.22% (P<0.01; shIL-21b), and 85.67% (P<0.001; shIL-21c) of colony formation relative to control. Besides, the expression level of PCNA was shown to be downregulated by 0.26-fold (shIL-21a), 0.32-fold (P<0.05; shIL-21b), and 0.32-fold (P<0.05; shIL-21c) in IL-21-silenced HCT116 relative to control. Downregulation in PCNA expression of 0.42-fold (P<0.01; shIL-21a), 0.45-fold (P<0.01; shIL-21b), and 0.50-fold (P<0.001; shIL-21c) was also observed in IL-21-silenced HT-29 cells compared to control. In parallel to these findings, a previous study suggested that silencing IL-21 receptor (IL-21R) decreased the cell proliferative activity in breast cancer cells [3].

Conclusion

IL-21 gene silencing resulted in the suppression of proliferative activity in HCT116 and HT-29 cells. The downregulation of PCNA in IL-21-silenced HCT116 and HT-29 cells also supported the findings of the clonogenic assay. Thus, IL-21 may be developed as a useful tool to treat colorectal cancer.

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O-33

Ligand-based pharmacophore modeling, molecular docking and molecular dynamics study targeting prolyl oligopeptidase enzyme for effective treatment for Parkinson's disease: computational approach

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Prolyl oligopeptidase (POP) are serine protease enzymes implicated in the pathogenesis of Parkinson's disease (PD) through an increase aggregation of α-synuclein protein in the brain. The current treatment options for PD are only symptom-targeted while effective therapeutic strategy remains a challenge. The study aimed at a identifying potent anti-PD drugs with inhibitory potentials against POP using ligand-based receptor modelling, Glide XP docking, molecualr dynamics and pk-CSM pharmacokinetics ADMETprediction parameters. Results indicated that the ligand-based (LB) model generated pharmacophore with 6 features, having 1 hydrophobic, 1 positive ionazable group, 2 aromatic rings, and 2 hydrogen bond acceptors. A total of 23 hits with Gunner-Henry (GH) score of 0.7 and enrichment factor (EF) of 30.24 were obtained as validation protocols, making it an ideal model. The LB model retrieved 177 hit compounds from 69,543 natural Interbioscreen database virtually. Interensendly, ligands 1, 2, 3, 4 and 5 orderly demostrated higher binding affirnity to the enzyme with Glide XP docking of -9.004, -8.818, -8.749, -8.708, -8.707 kcal/mol than GSK552 and ZPP with -8.187, and -6.835 respectively. Similarly, their binding free enrgies were -48.424, -51.879, -51.684, -45.191, and -49.567 kcal/mol respectively. Molecular dynamics indicated that the ligands 1, 2, & 4 demostrated a better stability than GSK552. Pharmacokinetic profiles of the ligands indicated their druggability and and low toxicity profile. The ligands are recommended as adjuvant /single candidate as ant-PD candidates for translational study.

Keywords: Parkinson's disease, phytochemicals, polyloligopeptidase, Ligand-based drug design, pharmacophore modelling, molecular dynamics.

O-34

Inhibitory effects of andrographolide in PC-3 cell line and the induction of apoptosis via the involvement of caspases activity

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Andrographolide is a labdane diterpenoid isolated from the plant Andrographis paniculata. This substance has numerous medicinal uses, notably anticancer effects. A previous study has revealed that andrographolide inhibits the growth of lung, brain, colon, and breast cancer cells (Mishra, 2015). Due to a lack of research, however, it is thought that the knowledge of andrographolide's anti-cancer effects on prostate cancer cells is relatively poor. In the current study, andrographolide was assessed on PC-3 cells, an aggressive androgen-independent prostate cancer cell line.

Methodology

Cytotoxicity analysis is vital in drug discovery research for assessing the biocompatibility of the drug being used on cancer cells. This work used the WST-1 assay to determine the cell survival of PC-3 cancer cells and Hs27 normal cells exposed to varying doses of andrographolide (0-200 µM). Metastasis is essential for the disease to progress; hence, the scratch assay and the transwell invasion assay were used to test andrographolide on PC-3 cells. In order to study the cell death mechanism after the PC-3 cells being treated with andrographolide, DNA damage and apoptosis assay has been done. Finally, the caspase mediated pathway is analysed by carrying out caspase 3, 8 and 9 assay upon the treatment of andrographolide.

Results and Discussion

The results indicate that andrographolide dose-dependently suppresses the viability of PC-3 cells but not Hs27 cells. The NCI considers the LC 50 value of 26.42 µM (after 48 hours of incubation) acceptable. In this study, three different concentrations of andrographolide were used: control, half LC 50, and LC 50 (0, 13.21, and 26.42 µM) in all subsequent analyses. The results showed that andrographolide inhibits both migration and invasion compared to the control.

The presence of a comet tail has revealed that a 26.42 µM andrographolide treatment produces the maximum DNA damage to single cells, followed by 13.21 µM and 0 M. In addition, the maximum cell death activity was seen at 26.42 M andrographolide concentration, followed by 13.21 µM and the control. The activity of caspases 3 (executor caspase), 8 (intrinsic pathway), and 9 (extrinsic pathway) in mediating apoptosis increased significantly. The half LC 50 (13.21 µM) demonstrates significantly more activity in these caspases than the LC 50 (26.21 µM), in accordance with the new finding related to the caspase storm scenario (Tao, 2007). This enables us to identify 13.21 µM as the andrographolide dosage that approaches the ideal range for caspase activity activation.

Conclusion

The ability of andrographolide to prevent the progression of cancer in PC-3 cancer cells has been proven through the regulation of caspase-mediated apoptosis, suppression of metastasis, and induction of DNA damage.

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O-35

Lentiviral modification of hard-to-transduce NK-92MI cells

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Natural killer (NK) cells are lymphocytes that play important role in both our innate and humoral immunities. NK cell functions are dictated by the myriad of receptors, regulating both activating and inhibitory signals. Studies found that through interactions with the CD16 receptor alone, NK cells can be activated for antibody-dependent cellular cytotoxicity (ADCC) to occur. The ADCC mechanism can be used to home NK cell killing towards designated targets through detection by target-specific antibodies. Unfortunately, NK cell studies are hampered by the tedious sourcing of primary NK cells. NK cell lines such as NK-92 and NK-92MI are now available but they lacked certain features compared to primary cells. Furthermore, NK cells are less susceptible to genetic modifications such as electroporation and viral-based methods. Recently, lentiviral vectors were successfully used in the clinical setting for cell modifications. In this study, we used a third-generation lentiviral vector, termed RRL-lentivirus, to modify the NK-92MI cells for surface expression of CD16 receptors and possibly restore their ability for ADCC.

Methodology

High binding affinity 158V-CD16 and GFP gene sequences were molecularly cloned into RRL-lentiviral transfer vector. The transfer vector plasmid containing the gene of interest and three types of helper plasmids were simultaneously transfected into HEK-293T packaging cells by calcium phosphate transfection. After 48 hours, the supernatant containing the lentiviral particles was collected and filtered through 0.45um CA filter. This was then concentrated using PEG-6000 precipitation method and stored in serum free media at -80°C. Using K-562 cells as positive controls, NK-92MI cells were transduced using GFP-RRL lentivirus or CD16-RRL lentivirus. Transduction replicates were done with or without the addition of polybrene polycation. Expression of reporter GFP was monitored using fluorescence microscopy and the levels of transgene expressions were further validated through flow cytometry using FITC-conjugated anti-human CD16 antibodies.

Results and Discussion

After GFP-lentivirus transduction was performed, the control K-562 samples showed GFP fluorescence on day 3, while the NK-92MI samples displayed GFP on day 5. The difference between the samples transduced with and without polybrene was compared through flow cytometry. At MOI of 1, GFP-transduced K-562 cells resulted in a GFP-positive population of 95.6% without polybrene and this improved to 99.5% when polybrene was added. At the same MOI, CD16-transduced K-562 did not resulted in the formation of two distinct CD16 populations. However, all transduced samples showed a significant positive shift in the detected FITC compared to negative controls. At the MOI of 10, GFP-transduced NK-92MI cells resulted in GFP-positive population of 3.5% without polybrene, the population increased to 7.2% with polybrene added. CD16-transduced NK-92MI cells resulted in a CD16-positive population at 1.0%, while the addition of polybrene increased this expression to 3.1%.

Conclusion

This study demonstrated the feasibility of using a third generation RRL-lentiviral vector for NK-92MI cell transduction, but further optimisations will be required before *in vitro* assays such as natural cytotoxicity and ADCC assays can be performed using these modified cells.

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O-36

Higher wheal sizes of *Dermatophagoides farinae* sensitization exhibit worse nasal symptoms in allergic rhinitis patients

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Allergic rhinitis (AR) is a global health burden and it manifests in both nasal and non-nasal symptoms. Skin prick test (SPT) is a routine procedure to diagnose AR sensitized to common allergens including house dust mites (HDMs). The degree of sensitivity of a patient toward allergens is determined by the size of the wheal formed by SPT procedure. SPT wheal sizes are influenced by recent anti-histamine usage or treatments, however it remains unclear if SPT wheal sizes could predict symptoms severity of AR patients or if they are influenced by other factors. In this study, we set out to investigate the association between SPT wheal sizes with the demographical, clinical and environmental characteristics, as well as nasal and non-nasal symptoms severity scores, of AR patients sensitized to common HDMs.

Methodology

30 AR patients were recruited from Otorhinolaryngology, Head and Neck Surgery (ORL-HNS) clinic of Hospital Universiti Sains Malaysia (HUSM). SPT was conducted to examine their sensitization towards HDMs (*D. farinae*, *D. pteronyssinus*, or *B. tropicalis*) allergens. The wheal sizes was recorded. The patients were assessed with AR nasal and non-nasal symptoms severity scores. The nasal symptoms assessed were sneezing, runny or itchy nose, congestion (stuffiness) and postnasal drip. Non-nasal symptoms assessed were eye, throat or ear symptoms, chronic cough, headache and mental functions. We also assessed the global assessment of nasal and non-nasal symptoms severity and the quality of life (QOL) assessment of rhinitis severity. The 7-point visual analogue scale (VAS) was used in these assessments. All clinico-demographic data from each participant was obtained through a *Pro Forma* questionnaire.

Results and Discussion

We showed that SPT wheal sizes of HDM allergens were not associated with clinical, demographical and environmental characteristics examined. Nonetheless, significant correlations were observed between SPT wheal sizes of *D. farinae* sensitization with worse severity scores of all five nasal symptoms examined (i.e., sneezing, runny nose, itchy nose, congestion and postnasal drip) and four of the six non-nasal symptoms examined (i.e., throat symptoms, ear symptoms, headache and mental function). Such relationships were not observed in SPT wheal sizes of *D. pteronyssinus* and *B. tropicalis* sensitization. We suggest that increased SPT wheal sizes for *D. farinae* sensitization may predict the likelihood of more severe nasal and, to a lesser extent, non-nasal manifestations in AR patients.

Conclusion

We demonstrated the significant correlation between wheal sizes of *D. farinae* sensitization with all nasal and majority of non-nasal symptoms severity scores. Such relationships were not observed in *D. pteronyssinus* and *B. tropicalis*. We suggest that increased SPT wheal sizes for *D. farinae* sensitization may predict the likelihood of more severe nasal severity in AR patients, and that these patients should be routinely monitored or managed to avoid the potential onset of severe nasal manifestations.

O-37

Immunoinformatics Analysis on Human Coronavirus Spike Protein for Universal Immunogen Discovery

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Coronaviruses are well-known to possess high mutation rate. The recent pandemic has demonstrated this fact that multiple SARS-CoV-2 variants have emerged since the first occurrence in 2019. Variants such as Beta, Delta and Omicron variant, even showed the ability of immune evasion in convalescent and vaccinated individuals [1], raising global concern about the efficacies of existing vaccines. Immunoinformatics approach is gaining traction in vaccine development due to significant time and cost reduction in immunogenicity studies and improved reliability [2]. Viral genome can be analysed for the mapping of potential T-cell and B-cell epitopes. Structural proteins, particularly spike protein (S) have been studied extensively as promising vaccine candidates. One rationale is that the RBD of SARS-CoV-2 attaches to the ACE2 receptor on the host cells to initiate infection. Taken together, a vaccine that offers protection over a wide spectrum of coronaviruses is crucially demanded.

Methodology

The sequences of S proteins of the SARS-CoV-2 variants as well as SARS-CoV and MERS-CoV were retrieved from NCBI database. Multiple sequence alignment (MSA) was performed to identify the conserved regions among S proteins from different human coronaviruses. All conserved regions were analysed for the antigenicity through VaxiJen. The conserved regions that passed the threshold value of 0.5 were then analysed for T-cell and B-cell epitope predictions using NetMHCpan EL4.1, IEDB recommended 2.22 and BepiPred 2.0, respectively. The antigenic conserved regions were also used to construct 3D model using SWISS-MODEL followed by structural refinement using GalaxyRefine2. All refined models were validated using ERRAT and PROCHECK. Lastly, the docking of 3D models against TLR-3 was performed via PatchDock and FireDock.

Results and Discussion

Based on the MSA result, S protein contains 12 conserved regions, which were then subjected to further analyses. Altogether 9 conserved regions were above the antigenicity threshold value and therefore selected. In terms of epitope prediction, the antigenic conserved regions were predicted to contain a total of 69 MHC Class-I epitopes, 45 MHC Class-II epitopes and 5 linear B-cell epitopes. Furthermore, all antigenic conserved regions were sent for 3D model building followed by structural refinement. The best refined model for each conserved region was chosen based on the Galaxy energy. These refined models were validated and the results showed that the models were of good quality. Following that, the refined model for each conserved region was docked against TLR-3. The global energies of complexes formed between the conserved regions and TLR-3 fell within the range of -11.74 to -7.36 kcal/mol. This implies that the conserved regions have good binding affinities with TLR-3.

Conclusion

The identified conserved regions of S protein were predicted to have a significant number of epitopes and showed promising docking results. This study provides some insights about the interaction of conserved S peptides with TLR-3, contributing to the vaccine design. Still, further analyses such as molecular dynamics and immune simulation are required to polish the results. *In vitro* and *in vivo* validation are also essential to evaluate the immunological roles of designed universal coronavirus vaccine.

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O-38

Andrographolide induced apoptosis by enhancing c-Myc/p53 in human glioblastoma DBTRG-05MG cell line

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Human glioblastoma multiforme (GBM) is one of the most malignant brain tumors. The conventional GBM treatment faces a problem due to presence of blood brain barrier (BBB) and post-treatments effect. Then, andrographolide has attracted many researchers because it can cross the BBB and easily distribute into different brain regions [1]. Next, the targeted therapy also is one of the treatments that can reduce the post treatment effect because it can specifically target cancer cells without affecting normal cells. Recently, the small molecule targeted therapy by exploring on the molecular targets of oncogenes or tumor suppressor genes such as c-Myc and p53 in regulating the apoptosis signaling pathway still remains debatable [2]. Therefore, this study aimed to elucidate mechanism action of andrographolide towards c-Myc/p53 induced apoptosis in DBTRG-05MG cell line.

Methodology

The cytotoxic effect of andrographolide was assessed by WST-1 assay in DBTRG-05MG and SVGp12 cell lines. Both cell lines were treated for 24, 48, and 72 hours with varying concentrations of andrographolide (0.781 – 200 µM). The WST-1 reagent was added in each well. Then, plates were incubated, and the absorbance (Abs.) was measured with ELISA reader (Multiscan Spectrum) at wavelength 450 nm and the reference reading of 630 nm. The LC_x values were computed and will further used as active doses and time in the subsequent experiments. Next, the apoptosis assay was assessed using annexin V-FITC / PI double staining. Cells pellet of DBTRG-05MG were collected after treatment with andrographolide and resuspended with V-FITC, and PI staining. The cells were incubated in the dark at room temperature for 30 minutes before analyzing the stained cells using a BD FACS CantoTM Flow Cytometer. The gene and protein expression level c-Myc and p53 signaling pathway was then assessed using qRT-PCR and western blot. The protein-protein interaction between c-Myc and p53 was determined by a reciprocal experiment of the co-immunoprecipitation (co-IP) using DBTRG-05MG total cell lysate.

Results and Discussion

Andrographolide has significantly reduced the viability of DBTRG-05MG cell lines in a concentration- and time-dependent manner. The recorded LC_x values for DBTRG-05MG cell lines for 24, 48 and 72 hours treatment were 42.82 µM, 27.21 µM and 13.93 µM respectively. While in non-cancerous cell line, SVGp12 cannot be determined because the percentage cell viability for normal cell is remained ~80% after being treated with the highest concentration (200 µM) of andrographolide for 24h results. Andrographolide also induced apoptosis in the DBTRG-05MG cell line, by inducing c-Myc and p53 expression at the gene and protein level. The percentage of apoptotic cells in control cells was 0.01% and after the cell lines exposed with 13.95 µM and 27.9 µM of andrographolide for 72 hours, the percentage of the apoptotic cell were increased to 5.2% and 16.5% respectively. Western blot results demonstrated that c-Myc overexpression also increased the production of the anti-apoptotic protein p53. Our findings revealed that c-Myc and p53 positively interact in triggering the apoptotic signaling pathway when the lane immunoprecipitated (IP) result for p53 and probed with anti-c-Myc in western blot analysis, which showed the presence of the band for c-Myc protein (62kDA).

Conclusion

This study successfully discovered the involvement of c-Myc and p53 in the inhibition of DBTRG-05MG cell line via by apoptosis following andrographolide treatment. However, further study needs to be extended to evaluate the cytotoxicity of andrographolide *in vivo* and *in vitro*. The function of other regulatory genes that may interact with the c-Myc signaling pathway must also be investigated intensively.

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O-39

In vitro uptake and activation of human dendritic cells by liposomes derived from total lipid of *Mycobacterium smegmatis*

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background
Liposomes are self-assembled lipids that have attracted scientific attention as efficient drug delivery vehicles and adjuvants. They possess a unique vesicular structure and can be derived from natural and synthetic substances. Liposomes can mimic the biological membrane of drugs, thus extending the half-life and minimizing the toxicity levels while delivering them to the target organs. The adjuvant mechanism of liposomes has been intimately associated with the stimulation of desired immune responses upon the exposure of antigen and immune cell targeting. This current study mainly targets to investigate the uptake and activation of human dendritic cells (DCs) by liposomes derived from the total lipid of *Mycobacterium smegmatis.*

Methodology
Liposomes derived from *Mycobacterium smegmatis* were synthesized and characterized by using Ziehl-Neelsen (ZN stain) and field emission scanning electron microscopy (FESEM). Fresh whole blood has been collected from three distinctive groups: TST-negative individuals, TST-positive individuals, and active pulmonary TB patients. Peripheral blood mononuclear cells (PBMCs) separation by Lymphoprep density centrifugation was performed and cultured with lipopolysaccharide (LPS) and liposomes. The activation of DCs by *M. smegmatis* liposomes has been analyzed through the expression level of DCs surface markers (HLA-DR, CD11c, CD123, CD86) in flow cytometry and the secretion of cytokines (IL-4, IL12p70, and IFN-γ) via ELISA.

Results and Discussion
The characterization of liposomes under FESEM demonstrated a size ranging from 20 nm-135 nm with spherical structures. The results showed levels of HLA-DR and CD86 expression reduced in the group of DCs of active pulmonary TB with liposomes compared to the group of TST-negative and TST-positive individuals. However, the interaction of DCs from active pulmonary TB patients with liposomes exhibited an increased level of HLA-DR with low expression of CD86 compared to other groups. IL-12p70 was highly significant increase in active pulmonary TB group in comparison to the group of TST-negative and TST-positive individuals. The group of active pulmonary TB patients also showed the significant increase of IL-4 and IFN-γ than the group of TST-positive individual. Meanwhile, the secretion of all cytokines was the lowest in the group of TST-positive individuals compared to the other groups. These events emphasized the malfunction of DCs in a TB condition and highlighted the capability of liposomes to support the antigen presentation by human DCs in TB infection.

Conclusion
Liposomes synthesized from *Mycobacterium smegmatis* had been successfully produced and classified as small unilamellar vesicles (SUVs). The exposure of DCs with liposomes particularly improved the antigen presentation activity with low activation of DCs in active pulmonary TB patients compared to the other study cohort. The results from this current study supported liposomes as potent vaccines and adjuvants for immunotherapy.

O-40

Identification of Medicinal Fungi by Molecular Analysis

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Mushrooms or higher fungi are heterotrophic eukaryotic organisms on Earth. Fungi are also having its great medical and environmental importance. Hence, the precise and rapid identification of fungi species from specimens is important for the field applications in, such as, sustainable use and conservation of biodiversity, prevention and control of fungal pathogens, human health and ecological monitoring [1]. DNA barcoding is a great tool for specimen identification that can be used for identifying and recognizing various types of fungi. The internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA gene cluster were utilized as a universal DNA barcode marker for fungi specimens which was suggested by Schoch and his team [2]. In this study, we identified 11 randomized fungi specimens by using ITS primers.

Methodology

The fungi specimen was grown on petri plates containing PDA medium was obtained from the lab. A small quantity of mycelia of fungi specimen was picked using a sterile forceps from the fully-grown culture and was transferred into a 1.5 mL microcentrifuge tube consisting of 600 µL of sterile distilled water. The mycelia were homogenized by using pipette tips. The mixture was vortexed thoroughly and centrifuged at 10,000 x g for 1 minute. The supernatant was discarded and continued by using the EasyPure Plant Genomic DNA kit according to the manufacturer's instructions to extract fungi DNA. MyTaq HS Red Mix, 2x, universal ITS primer pairs, extracted fungi DNA sample template and sterile distilled water were used for performing PCR amplification using Agilent SureCycler 8800 with 35 cycles of amplification. The PCR products were undergone electrophoresis to separate DNA. The gel was observed and captured using Syngene Bioimaging system. The EasyPure PCR purification kit was used to purify PCR products to remove primers and nucleotides according to manufacturer's instruction. The purified PCR products were sent for sequencing. The FinchTV software, BLASTn algorithm at the NCBI website and DNA Bold program were used to analyze the sequences data.

Results and Discussion

Eleven fungi specimens were determined its size by using the Syngene Bioimaging System. The highest base pair of fungi specimen was Sample 10, which had 850 base pair. The lowest base pair of fungi samples were Sample 1, 4, 5 & 6, which had 600 base pair. The range of base pair of fungi specimens were from 600 bp to 850 bp. Therefore, 11 fungi specimens were identified by using the FinchTV software, BLASTn algorithm at the NCBI website and DNA Bold program through analyzing their sequences data. The outcomes of analysis revealed these specimens were *Hypoxylon sp. LA01*, *Lignosus rhinocerotis*, *Ganoderma lucidum*, *Cordyceps militaris*, *Hemistropharia albocrenulata*, *Inonotus obliquus*, and *Hericum erinaceus*.

Conclusion

The DNA extracted from randomized fungi specimens were identified successfully using molecular analysis. The works presented here reveals this method will potentially aid to the authentication of medicinal fungi from unknown specimens.

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O-41

Effect of Andrographolide on proliferation, migration, and invasion of MDA-MB-231 and MCF-7 cell lines

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Breast cancer is the commonly diagnosed and one of the leading causes of death among women worldwide [1]. Current standard treatment includes chemotherapy, surgery, and radiotherapy. Some patient discontinued their treatment due to the side effects of the treatments which leads to the cancer to relapse. Therefore, alternative medicines from natural plants with fewer side effects now widely studied. Andrographolide is a diterpene lactone derived from *Andrographis Paniculata.* Andrographolide had previously demonstrated to have anti-inflammatory, anti-cancer, anti-fungal, and anti-viral properties. This study is done to determine anti-cancer effects of andrographolide in breast cancer cells by evaluating the cell viability, migration, and invasion in two different cancer cell lines which are MDA-MB-231 (a triple negative cell line) and MCF-7 (ER positive cell line).

Methodology

MDA-MB-231 and MCF-7 cells were treated with a different concentration of andrographolide (0.781µM - 200 µM) for 24, 48 and 72 hours. The cell viability was observed using the WST-1 reagent. The LC_{∞} and ½ LC_{∞} values obtained from WST-1 were then used for scratch and transwell invasion assays to determine the rate of migration and invasion for both cell lines. The cells were both treated with LC_{∞} and ½ LC_{∞} concentration of andrographolide for a different timeline, MDA-MB-231 was treated for 72H and MCF-7 was treated for 48H before proceeding to scratch and invasion assay.

Results and Discussion

Andrographolide had shown a significant effect on MDA-MB-231 and MCF-7 cells by suppressing the cell viability in a concentration dependent manner. Our findings are consistent with the previous study that showed andrographolide reduced the cell viability in cancer cell but have a minimum effect on normal cells in a concentration dependent manner. Andrographolide inhibit the migration of MDA-MB-231 and MCF-7 cell lines at 72 and 48 hours, respectively

The wound gap area increased in both cells treated with andrographolide at two different concentrations compared to both untreated cells. Invasion assay showed that andrographolide inhibit the invasion of MDA-MB-231 and MCF-7 cell lines at a low concentration which aligned with the previous research that showed andrographolide inhibit the invasion of MDA-MB-231 [2].

Conclusion

Andrographolide showed the migration and invasion inhibition properties in a concentration dependent manner in MDA-MB-231 and MCF-7 cells. Therefore, andrographolide is shown to have a promising future to be develop as a potential treatment for breast cancer. Further research needs to be done to elucidate the mechanism of cell death and whether it involved in either cell cycle or apoptosis pathway.

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O-42

Culture and biochemical testing versus 16S rRNA next- generation sequencing for bacterial identification from clinical samples: Practicability, cost and turn-around time in a Malaysian laboratory

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background
Bacterial pathogens are mostly identified via culture and biochemical testing (CBtest) in Malaysian clinical microbiology laboratories. In this study, we assessed the practicability, cost and turn-around-time (TAT) of utilising 16S rRNA next- generation sequencing (16SNGS) versus CBtest for bacteria identification from clinical samples collected at the Department of Diagnostic Laboratory Services, UKM Medical Centre, Kuala Lumpur.

Methodology
Twenty-four clinical samples (eight each from pus, respiratory and urine specimens) were included into the study. CBtest was carried out via microscopy and staining the samples, bacterial culture and isolation followed by biochemical tests for species identification. 16SNGS was performed via amplification of V3- V4 regions of 16S rRNA gene from total DNA extracted from each sample and sequenced using the MiSeq system; raw sequencing data was analyzed using the B.E Patho software. TAT was calculated from the initiation of sample processing to test report generation. Cost analysis included cost for reagents and disposable procurement as well as hands-on labour charges.

Results and Discussion (No figures or tables)
Via CBtest, 18 samples were reported as positive for single- bacterial infection (n = 5, 20.8%), mixed growth (n = 8, 33.3%) and normal flora (n = 5, 20.8%), respectively; remaining samples were culture-negative. 16SNGS detected the relative abundance of various bacteria genera in all tested samples. TAT of 16SNGS workflow (six days) was shorter compared to CBtest for identification of slow-growing, fastidious bacteria (72 days). Utilisation of the B.E. Patho "plug-and-play" bioinformatics suite allowed 16SNGS analysis to be performed by personnel without bioinformatics training. Nonetheless, identification cost per sample via 16SNGS (RM 1704.70) was very much higher than CBtest (RM 2.80 – RM 42.90) for all samples.

Conclusion
16SNGS allowed identification of various bacterial genera in clinical samples with a faster TAT for slow-growing and fastidious bacteria. Lowering of sequencing costs will facilitate future adoption of the platform in clinical microbiology laboratories.

O-43

Isolation and production of recombinant monoclonal antibody proteins against a *Toxocara canis* antigen using phage display technology

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Toxocariasis is a neglected zoonotic parasitic disease which mainly caused by *Toxocara canis*, an intestinal parasitic roundworm of dog. Human toxocariasis has a global distribution, with people from socioeconomically deprived populations more adversely affected. The limitations of diagnosing human toxocariasis is the signs and symptoms of the disease are non-specific and similar to other helminth infection [1]. This may lead to misdiagnosis thus underestimating the actual global impact of the disease. Existing serology-based detection method mainly rely on IgG antibody-based assays that suffers from the difficulty in differentiating past and active infections. The assays also lack of high diagnostic specificity due to cross-reactivity with antibodies to other helminths [2]. In the present study, novel recombinant monoclonal antibodies were isolated and the binding of the proteins were verified.

Methodology

Monoclonal antibody against recombinant *Toxocara* excretory-secretory 26 antigen (rTES-26) was isolated utilizing previously reported helminth phage display library via biopanning. Polyclonal and monoclonal phage ELISA were carried out at the end of selection. Positive clones were sent for sequencing and the scFv antibody clones verified using IMGT/V-QUEST bioinformatics tool. Selected positive monoclonal antibody clones with complete scFv gene were subcloned into pET-51b(+) and transformed into SHuffle® T7 Express *Escherichia coli* host cell. Antigen-antibody binding assays were performed using rTES-26 and native *T. canis* antigens.

Results and Discussion

The significant increase in absorbance reading of polyclonal ELISA (OD_{∞} ranging from 0.321 to 3.020) indicating rTES-26 specific phage antibodies successfully enriched. In total 384 individual clones were screened during monoclonal ELISA and five positive binders were identified with absorbance values ranging from 0.45 to 3.91. Three clones from distinct gene families (Ab 48: IgHV3-LV1; Ab 49: IgHV3-LV3; Ab 50: IgHV6-LV3) were selected, however only two clones: Ab 48 and Ab 49 demonstrated complete insertion of the full-length scFv antibody sequence following sub-cloning. Both clones were successfully expressed and purified at satisfactory levels in terms of purity and yield. The antigen-antibody binding analyses showed that both Ab 48 and Ab 49 able to bind the recombinant and native form of *Toxocara* protein. However, in total Ab 49 showed better performance in protein yield and binding affinity compared to Ab 48. Hence, the enriched monoclonal antibodies from the immune libraries shows to have varying binding affinities and specificities [3].

Conclusion

In summary, both antibody clones showed their diagnostic potential and the binding evaluation suggest that Ab 49 has the potential to be used as an efficient tool for human toxocariasis diagnosis.

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O-44

Optimization of Transient Expression of Recombinant IgG Binding Protein, FcγRIIa

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

1. Background

IgG Fc binding proteins (FcγRs) are immunoglobulin receptors that particularly bind to the human IgG (hIgG) molecules through its Fc portion. Among the members of FcγRs, FcγRIIA is slowly gaining interest from scientific community due to its different isotype specificities and the significant binding affinity towards IgG2. Current technology has presented the production of recombinant proteins in mammalian cell lines to introduce appropriate protein folding and post-translational modifications [1]. However, the production of recombinant proteins using mammalian expression systems is laborious, time-consuming, and costly [1]. Therefore, this study provided a simple protocol for optimized transient transfection and expression of recombinant IgG binding proteins to achieve a high yield in a short span of time.

2. Methodology

FreeStyle™ 293-F cells in the prewarmed FreeStyle™ 293 Expression Medium were incubated in a 37°C incubator containing a humidified atmosphere of 5% CO_2 in air on an orbital shaker platform rotating at 120 rpm. On the day of transfection, the cells were suspended in a volume of prewarmed medium to obtain a concentration of 1.0×10^6 cells/mL. For each mL of transfection volume, 1µg of plasmid DNA and 2µL of 293fectin diluted in Opti-MEM were needed. After adding the DNA/293fectin mixture, the suspension cells were incubated for 96 hours. The cell pellets were harvested by spinning down the cells for 5 mins at 300 ×g and the intracellular protein was harvested by using lysis buffer. After the protein has been purified using immobilized metal affinity chromatography (IMAC), the protein was subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western Blot analyses for the visual evaluation of expression. The protein concentration was measured by using Nanodrop Spectrophotometer.

produced in less than one week. In general, the recombinant proteins with expression levels up to 100 mg/L can be obtained. Since the expression level may differ depending on the nature of the recombinant proteins [2], the transfection method had been refined by varying the incubation time post-transfection, ending up with an ideal harvest time of 96 hours. The harvested protein was purified by using IMAC for downstream analysis. In SDS-PAGE analysis, an intense protein band at approximately 38 kDa had been observed, indicating the expression of the recombinant proteins. Meanwhile, in Western blot analysis, anti-histidine antibody was used to detect the hexa-histidine tagged recombinant protein.

3. Conclusion

An optimized protocol that serves as a general guide for the transient transfection and expression of recombinant IgG binding protein, FcγRIIA had been described. The simplicity of this protocol had exhibited an efficient transfection and a fast yet high-yield expression of proteins.

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O-45

Cloning of IgM Fc Receptor for Mammalian Expression System

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Mammalian expression system has become routine in the production of pharmaceutical protein as it offers post-translational modifications (PTMs) which allows production of complex proteins and glycosylated enzymes where their native structures and activities are preserved. In order to express the recombinant protein in mammalian cell lines efficiently, selection of an appropriate expression vector that carry the interested recombinant gene plays an important role. Polymeric immunoglobulin receptor (pIgR), an IgM Fc receptor is chosen to be expressed due to its various clinical potentials such as the possibility to serve as predictive biomarker target owing to its correlation with various cancer malignancies, potential role, and underlying mechanisms in body immunity [1,2].

Methodology

The plasmids that carried *PIGR* gene were purified from the bacterial overnight culture, followed by polymerase chain reaction (PCR) to amplify the interested DNA segment. The PCR products and intermediary vector (pET-28a+) were then subjected to restriction digestion and ligation process prior to bacteria transformation. Subsequently, the targeted gene sequences from the recombinant plasmid were cloned to the mammalian expression vector (pcDNA5/FRT). The final product was sent for sequencing before the mammalian cells transfection.

Results and Discussion

The presence of bacterial colonies in ampicillin-containing agar plate indicating the success of bacteria transformation. The cloning of the gene of interest into the mammalian expression vector (pcDNA5/FRT) was then verified through restriction digestion, showing two bands where both represented the vector backbone (5002bp) and *PIGR* gene sequence (2490bp) respectively. The sequence was checked for any point mutation through comparison of each nucleotide with the reference sequence. Flp-In System involves the introduction of Flp Recombination Target (FRT) site into genome of both expression vector and mammalian cell line of choice. The expression vector that carrying the gene of interest will integrate into the host cell genome through Flp recombinase-mediated DNA recombination at the FRT site. This system allows stable expression cell lines to be created, providing advantages such as rapid and efficient protein expression by the subsequent generation of Flp-In cell lines [3].

Conclusion

Gene cloning is an important step for the protein expression by living cells, especially for mammalian cells. The quality and sequences of the recombinant plasmid will impact the efficiency of protein expression. In future, the constructed recombinant plasmid (pcDNA5/FRT-PIGR) will be transfected into chosen mammalian cell line to express the recombinant protein as the sequence were checked accordingly. Antibiotic selection will be performed to select cells where the plasmids are integrated into genome successfully. Single cells that are able to survive upon transfection and selective growth will be expanded to generate a clonal population. Stable clones are selected after screening and ready for protein production.

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O-46

New β-carboline compound as a promising anticancer agent in chronic myelogenous leukemia (CML)

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Cancer is known as one of the serious causes of deaths among the world population and it is believed that the number of cases to rise over the years. Chemotherapy drugs are considered as the most reliable and common treatment for cancer. However, these drugs have limitations in terms of effectiveness and severe side effects. In addition, drug resistance is another factor that hinders successful treatment rate among patients. Therefore, finding a lead compound for cancer which addresses these issues are of utmost important. In the constant search for new anticancer agent alternatives, β-carboline has been identified as a good candidate due to its various reported health indications, particularly in anticancer. Therefore, the aims of the study are to investigate the potential of a newly synthesized β-carboline compound in eliciting anticancer activity in chronic myelogenous leukemia (CML) and to determine the apoptosis mechanism induced by the compound.

Methodology

β-carboline compound (M25) was synthesized and confirmed using gas chromatography-mass spectrometry (GC-MS) analysis. MTT assay was conducted to evaluate cytotoxicity effect of M25 against CML cells (K562 cell line). Toxicity of M25 was determined by calculating its selectivity index (SI), through human fibroblasts (Hs27 cell line) and mouse fibroblasts (BALB/c3T3). Mode of cell death induced by M25 was further evaluated through the apoptosis assay using flow cytometry analysis with Annexin V-FITC and Propidium iodide (PI). Lastly, *in silico* analysis was conducted to study the binding affinity between M25 and identified proteins involved in the apoptosis pathway using Discovery Studio software.

Results and Discussion

β-carboline compound M25 was synthesised with high purity and high yield. M25 induced cytotoxicity in K562 cell line with a low IC_{50} value of 0.8 µM. This indicated high potency of M25 in killing human CML cells. Based on IC_{50} values for non-cancer cells Hs27 and BALB/c3T3, the calculated SI were 16 and 25 respectively. The high selectivity showed that M25 is highly specific in killing CML cells, thus reflecting good safety profile for the compound. Flow cytometric analysis also showed that significant number cell death induced by M25 compound was due to apoptosis. In understanding proteins involved in the apoptosis pathway, molecular docking study investigated the binding affinity of M25 as ligand with apoptosis-related proteins such as apoptosis inducing factor (AIF), endonucleases G (Endo G), caspase 3 (CAS 3), caspase 9 (CAS 9) and caspase 8 (CAS 8). Interestingly, the highest binding affinity was recorded for AIF and Endo G, which suggested a unique caspase-independent pathway of apoptosis.

Conclusion

The newly synthesized M25 has excellent potential as an anti-leukemic agent as it has shown high cytotoxicity activity and selectivity for K562, thus indicating good effectiveness and safety profile. In addition, in silico study suggested that M25 induced caspase-independent apoptosis, with the possible involvement of AIF and Endo G. This unique pathway may address drug resistance issue which has become a hindrance for most conventional chemotherapeutics.

O-47

Discovery of potent small molecule inhibitor for dengue through *in silico* and *in vitro* approaches

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background
Dengue is a catastrophic arboviral disease, globally and locally. At present there are no effective antiviral drugs and only a limited approved vaccine available. The much-needed treatment for dengue has pushed studies to look into strategizing and developing methods to search for prospective inhibitor candidates. Due to the pivotal role of dengue NS2B/NS3 viral protease in the viral replication, the inhibition of this enzyme is considered to be the key strategy for the development of new dengue antiviral drugs. In this present study, we sought to identify and evaluate small molecules by adapting in silico and in vitro approaches in search of potent inhibitor against the dengue NS2B/NS3 protease.

Methodology
To that aim, total of 80 small molecules from the Riken NPDepo Authentic library were screened. Based on the highest percentage of inhibition exhibited, the hit compounds were chosen and molecular docking was performed to predict the binding model of the compounds to dengue NS2B/NS3 protease. Then, a chromogenic-based protease inhibition assay was carried out to evaluate whether the selected candidates inhibited dengue protease activity in vitro.

Results and Discussion
Based on *in silico* evaluation, computational simulation revealed the free energy of the compound I, II and III are comparable to that of quercetin, a known NS2B/NS3 protease inhibitor. Through the docking evaluation also showed that all three compounds bound to the DENV NS2B/NS3pro near the active site, hence confirming the possible interaction between the compounds and dengue NS2B/NS3pro. Interestingly, our results in the protease inhibition assay carried out indicated that compound I, II and III have the ability to inhibit dengue NS2B/NS3 proteolytic activity.

Conclusion
The *in vitro* inhibition results of the three compounds that we obtained from this study, agrees well with the docking outcome, hence, portrays a potential inhibition activity against dengue virus.

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O-48

Systematic review on preclinical reports: Titania Nanotube Arrays technology for medical orthopaedic screw implant application

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background
Titania Nanotube Arrays (TNA) technology is a promising surface technology for medical implants, especially for orthopedic applications. The long-term stability of a surface technology on a medical implant is frequently associated with osseointegration. This review provides an overview on TNA technology for medical orthopaedic screw implant applications from preclinical reported studies.
Keywords: Titania Nanotube Arrays, Medical Implant, Orthopaedic Screw Implant, Surface Technology

Methodology
The review was conducted based on PRISMA-P protocol and pre-determined keywords such as "orthopedics implants screw", "bone implant", "Orthopaedic Screw Implant" "TNA surface", "*In vitro", "Ex vivo" "In vivo*" "Titania Nanotube Array", "titanium dioxide nanotube arrays", "TiO_2 nanotube arrays", "preclinical studies", "preclinical testing" and "osseointegration". Search engine database such as PubMed, Springer Link, Science Direct and Google Scholar were used. The pre-clinical studies published in English language within the past 5 years (2016 till 2021) were included. To broaden the search results, the terms were combined interchangeably by using the Boolena operators 'and' or 'or.' The titles and abstracts of each article were are initially pre-screened. Studies involving those in predatory or blacklisted journals were excluded. To ensure transparency, replicability, and reanalysis feasibility, every step of this approach was meticulously recorded. To prevent double duplication, Endnote software version X7 was used as a reference manager to combine the results of all extracted research

Results and Discussion
The search has generally generated 778 articles. In further analysis, 30 articles were finalized according to inclusion and exclusion criteria. Data were further grouped into *in vitro, ex vivo, in vivo* studies. Most preclinical reports discussed the ideal TNA physiochemical properties that enhance cell proliferation, differentiation and surface properties that could contribute to long-term osseointegration activity.

Conclusion
The systematic reviews reported on TNA's ideal physiochemical properties are crucial for long-term bone-implant interaction. Knowledge from this study may contribute to the cutting-edge development of biomedical implant technology.

Acknowledgement
The authors would like to thank Universiti Sains Malaysia (RUI EKSESAIS 2019 (No:1001/CIPPT/8012338) for sponsoring this work.

O-49

Clinicopathological Association of Chronic Rhinosinusitis with Nasal Polyp (CRSwNP) and Periostin Expression

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

In the recent years, periostin (POSTN), a gene encoding an extracellular matrix protein with similarity to fasciclin family has emerged as a potential biomarker for various types of cancers. Besides that, POSTN also plays a role in stimulating eosinophil migration and activation in chronic inflammation and immune pathways. Chronic rhinosinusitis (CRS) with nasal polyp (NP) (CRSwNP) is mediated by Th2-/eosinophilic inflammation type of immune response [1]. POSTN has been proposed to be found in NP tissues and contribute to formation of NP in CRS patients. In this study, we investigated the POSTN protein expression in NP tissue and then we determined its association with the clinicopathological features of CRSwNP patients.

Methodology

Tissue samples were collected from 24 CRSwNP patients and their clinicopathological features such as demographic data (age, gender, race and smoking history), clinical (history of bronchial asthma and/or atopy, SNOT-22 scores, Lund-Kennedy scores, Lund-Mackay scores), haematological (percentage of serum eosinophils and total IgE) and pathological features (tissue eosinophil count, degree of inflammation, mucosal ulceration, squamous metaplasia, and fibrosis) were evaluated. POSTN protein expression was assessed by immunohistochemical analysis. A single linear regression analysis was performed to find the association of POSTN protein expression with the clinicopathological features.

Results and Discussion

Expression of POSTN protein was detected in all 24 NP samples. The mean IHC score POSTN is 7.31. There was no significant association of POSTN protein expression with clinicopathological features (gender, smoking history, history of bronchial asthma and/or atopy, SNOT-22 scores, Lund-Kennedy scores, Lund-Mackay scores, percentage of serum eosinophils, total IgE, tissue eosinophil count and other histopathological features (p value of 0.66, 0.11, 0.64, 0.57, 0.76, 0.17, 0.09, 0.21, 0.66, and 0.57 respectively) except for age (p=0.045). Our findings of POSTN expressed in all NP tissue staining the area of subepithelial tissue and infiltrating inflammatory cells was consistent with other study [1-2]. POSTN is produced in fibroblasts, endothelial and epithelial cells by interleukin (IL)-4 and IL-13 stimulation. Fibroblasts is found to be abundant in NP tissue. Tissue eosinophilia (eosinophil count >10 per high power field) is known to be a hallmark for CRSwNP and a predictor for NP recurrence. Ninomiya et al. 2018 found that protein expression associated with severity of CRSwNP but not in our study. Even though we excluded those patients taking steroids which can suppress the periostin production, we should have exclude those who was asthmatic and on any immunomodulator. Lebrikizumab was shown to be effective in controlling exacerbation of asthmatic patients with high POSTN level in their serum [1]. A larger sample size study at multi-centre setting would be more representative of CRSwNP population.

Conclusion

Although presence of POSTN is detected within all nasal polyp tissues, however, POSTN protein expression was not significantly associated with clinicopathological features of CRSwNP. Therefore, tissue POSTN appears to have no role in evaluation of patients with CRSwNP.

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O-50

Detection of herpes simplex virus-1 by direct immunofluorescence and viral isolation from cerebrospinal fluid

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Herpes simplex virus (HSV) is the human herpesvirus that leads to herpes simplex encephalitis or meningoencephalitis and is frequently lethal if not treated properly. Here, we described a case of a 21-year-old man who presented with acute confusion and abnormal behaviour and was later diagnosed with HSV-1 meningoencephalitis based on immunofluorescence and viral isolation from cerebrospinal fluid.

Methodology

A lumbar puncture was performed immediately during admission to the ward. Cerebrospinal fluid was also sent for viral culture. The culture was inoculated into human cells in culture (HEp-2) cell monolayers and observed for cytopathogenic effect (CPE). Then the slide was prepared for direct immunofluorescence staining using fluorescein isothiocynate-conjugated HSV type 1 and HSV type 2 antisera. Positive findings would demonstrate cells with fluorescent staining, whereas negative specimens would demonstrate cells with a reddish-brown counterstain.

Results and Discussion

Brain MRI was performed for further evaluation, which showed a focal area of gyral thickening at the left frontoparietal lobes with leptomeningeal enhancement at the left Sylvian fissure, suggestive of meningoencephalitis, and no hydrocephalus was noted. The CSF results revealed 0 polymorphs cells/mm and lymphocyte count, with 0 pus cells. CSF biochemistry showed glucose of 1.67 mg/dl and a very high total protein of 1596 mg/dl. The results of viral culture were obtained on admission day ten. After 10 days of culture with daily CPE observation, CPE evidence of HSV was detected. The prepared slide was observed under the ultraviolet microscope and revealed positive for HSV-1 and negative for HSV-2. HSV-1 has accounted for more than 90% of all herpes simplex encephalitis cases in adults and children. It spreads by oral contact and primarily results in cold sores, while HSV-2 is sexually transmitted and causes genital herpes.

Conclusion

It has been proven that immunofluorescence antigen detection is a quick, accurate, and sensitive method for distinguishing HSV-1 and HSV-2 antigen in the cerebrospinal fluid of those infected individuals.

O-51

Development of cisplatin-resistant urothelial cancer cells using pulse-shock treatment

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Cisplatin has been used in chemotherapy of advanced urothelial carcinoma (UC) that is no longer amenable to local treatment such as surgery and radiotherapy. However, almost 30% of UC patients develop resistance to chemotherapy and recurrence within 2 years post- therapy. The understanding of the mechanism of cisplatin resistance in UC patients is scarce. In pulse-shock chemotherapy, cancer cells are subjected to chemodrug treatment in a short period of time.

Methodology

In this study, cisplatin-resistant UC model was developed and analysed for morphological and transcriptomic changes. Firstly, cisplatin IC50 dosage of a urothelial cancer cell line, 5637 cells was determined. Cells were then subjected to repeated pulse-shock treatment with the respective IC50 dosage and morphological changes will be recorded. At the end of the repeated treatment cycles, IC50 of the cisplatin-resistant cell was compared to its parental counterpart and transcriptomic changes in drug resistance genes were analysed.

Results and discussion

When pulse-shock treated for 2 hours, cisplatin IC50 dosage of 5637 cells was 11uM. After sixth treatment cycle, IC50 was significantly increased to 21uM, which was 2 times higher compared to parental cells. During the course of treatment, morphological changes vary from polyploid giant cells, MSC-like cell, parental cells and bizarre shaped cells. ABCA1, ABCG2, Bax and BIRC5 genes were found to be significantly upregulated in cisplatin-resistant 5637 compared to parental cells.

Stress induction through pulse-shock cisplatin treatment promotes cancer plasticity through transformation into polyploid giant cells. These polyploid giant cells has been reported to drive cancer progression through activation of EMT, drug resistance, apoptotic inhibition and many more. Molecular mechanism and regulation of polyploid giant cells are not fully understood, hence, an exciting niche awaiting exploration.

Conclusion

The model has a potential to be used for evaluation of existing and novel targets for recurrent therapeutic regimes, thus making UC management more efficient, convenience and increased patients' quality of life.

Acknowledgement

This study is funded by Ministry of Higher Education Malaysia through Fundamental Research Grant Scheme FRGS/1/2019/SKK15/UITM/03/1, RMC file number 600- IRMI/FRGS 5/3 (286/2019).

O-52

Biochemical and biophysical characterization of leishmanial histidyl tRNA synthetase

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Visceral leishmaniasis, caused by the protozoan parasite *Leishmania donovani* affects the human population worldwide and has no proper treatment till date. Aminoacyl tRNA synthetases (aaRSs) are responsible for the transfer of amino acids to their cognate tRNAs during protein translation [I]. Since aminoacyl tRNA synthetases have been considered as promising drug targets in bacteria, fungi etc., it would be interesting to validate these macromolecules for the development of antileishmanials. Previous knockout studies on *Trypanosoma brucei* histidyl tRNA synthetase have shown that this protein is indispensable for the parasite's survival [II]. Realizing the relevance of this protein in the survival of trypanosomatids, our study is focused on the characterization and inhibition of the leishmanial histidyl tRNA synthetase (HisRS).

Methodology

The full length ORF of *Leishmania donovani* histidyl tRNA synthetase (*Ld*HisRS) was amplified from the genomic DNA of *Leishmania donovani* and cloned into pET28a expression vector. The confirmed clone was transformed into *E.coli* BL21(DE3) cells and the protein was purified using sequential chromatographic techniques. Biophysical characterization of the protein was carried out by varying physiological conditions and analysed through circular dichroism and fluorescence spectroscopy. Similarly, the protein's activity was validated by its aminoacylation efficiency using the malachite green phosphate detection method with modified buffers [III]. In order to compute the half maximal inhibitory concentration (IC_{50}), heterocyclic compounds were added in the range of 1-50 µM.

Results and Discussion

The histidyl tRNA synthetase from *Leishmania donovani* (*Ld*HisRS) was cloned, purified and analyzed by various biophysical and biochemical approaches. The recombinant protein was retrieved in its trimeric state through gel filtration chromatography. The folding parameters investigated by circular dichroism studies revealed that the protein comprises mostly of α-helices when compared to β-sheets as observed in the case of leishmanial aspartyl tRNA synthetase [III]. The melting temperature of the protein in apo form was observed to be 41°C and its stability successively increased on the addition of ligands. Furthermore, denaturants such as guanidine hydrochloride and urea were found to affect the structural integrity of the protein. On subjecting to different pH conditions, the secondary structural content was found to be altered. Similarly, the intrinsic fluorescence was also affected upon varying the pH. The quenching studies of *Ld*HisRS with acrylamide and potassium iodide hinted towards the hydrophobic localization of tryptophan residues. *Ld*HisRS depicted more affinity towards ATP than L-histidine and its highest activity was found to be at pH 7.5 and 40°C. A heterocyclic compound was found to compete with ATP that was also capable of inhibiting the protein at a concentration almost half as required for its human counterpart.

Conclusion

In the current study *Ld*HisRS was purified to homogeneity and then characterized at various dimensions. Various physiological conditions affected the protein's folding and its activity was observed to be inhibited by a heterocyclic compound at a much lesser concentration as compared to human counterpart. Furthermore, attempts are going on to explore more potent inhibitor molecules which could be helpful to combat leishmaniasis.

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O-53

Isolation and characterization of ssDNA aptamers against HlyE of *Salmonella* Typhi

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background
Aptamers have emerged as a popular ligand in clinical diagnostics as it offers several advantages compared to antibodies, such as shorter generation time, lower costs of manufacturing, minimal batch-to-batch variability, higher modifiability and better thermal stability. Thus, the presents study aims to isolate and characterize ssDNA aptamers against HlyE of *Salmonella* Typhi for future typhoid surveillance.

Methodology
In this study, we demonstrated the isolation of ssDNA aptamers against HlyE of *Salmonella* Typhi via Systematic Evolution of Ligands by EXponential Enrichment (SELEX). Meanwhile, binding affinity of the aptamers was determined using direct Enzyme-Linked Oligonucleotide Assay (ELONA) and the dissociation constants (K_d) were calculated using non-linear regression analysis by GraphPad Prism 8.

Results and Discussion
Enriched pool of ssDNA from 12 SELEX cycle was cloned and a total of 100 positive transformants were sequenced. Phylogenetic analysis of the positive transformants revealed five clusters of 11 distinct aptamers. Of these, three most probable aptamers were subjected for characterization of their binding affinity. For all tested aptamers, K_d -values were obtained in the nanomolar range. The highest binding affinity with K_d of 83.6 nM was determined for AptHlyE97, followed by AptHlyE11 with K_d of 102.2 nM. Significant lower binding affinity was shown for the AptHlyE45 with a K_d of 119.3 nM.

Conclusion
This novel AptHlyE97 could be of potential application as a diagnostic ligand for future development of point-of-care testing towards typhoid surveillance.

O-54

Development of DNA Aptamers for the Detection of the *Burkholderia pseudomallei* towards the Diagnosis of Melioidosis

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Melioidosis is caused by *Burkholderia pseudomallei,* a Gram-negative bacterium which can be found in soil and water [1]. Diagnosis of melioidosis is challenging due to the similarity in symptoms with other diseases such as tuberculosis and amebiasis [2,3]. Other than that, difficulties in detection of the bacteria using laboratory diagnosis such as culture and serology methods has delay treatment to patient. Therefore, there is a need for rapid, sensitive, and specific assay for the diagnosis of melioidosis. With a recent finding on *Burkholderia* invasion protein D (BipD) has enlighten future development of antigen-based diagnostic of melioidosis. Hence, this study was designed to develop highly specific and sensitive DNA aptamers against BipD to detect the *B. pseudomallei*.

Methodology

Agarose bead-based Systematic Evolution of Ligands by EXponential enrichment (SELEX) was performed to isolate BipD-specific DNA aptamers and products from enriched cycle of SELEX were sequenced. The sequences were analyzed using Mega X and mfold software for cluster and secondary structure determination respectively. Two sequences (aptamers) were chosen based on Gibbs free energy. Then, the affinity and specificity of aptamers were evaluated using Enzyme-Linked Oligonucleotide Assay (ELONA).

Results and Discussion

The SELEX cycle 8 was identified having enriched oligonucleotides and the sequence analysis showed one main sequence cluster was isolated. Secondary structure analysis showed a present of single-stranded hairpin loop structure and double-stranded hairpin stem which postulated that interaction of aptamers and BipD might occur in this region. Two DNA aptamers with minimum Gibbs free energy structure were selected because these structures retain greater folding stability. Affinity assay for DNA aptamers (Apt 1 and 2) revealed the dissociation constant (K) value was 5 and 3 micromolar (uM) respectively. The more the K value, the greater the affinity, the lesser the amount of aptamer is needed in identification of *B. pseudomallei*; thus, it can reduce the costs required for the diagnosis of melioidosis. The specificity assay showed both aptamers cross-reacted with other Gram-negative bacteria (*Salmonella* Paratyphi A and *Shigella sonnei*).

Conclusion

The specificity of aptamer is crucial in the diagnosis of melioidosis to avoid false positive results. Thus, this study will further focus on counter-selection in the SELEX process with pathogenic Gram-negative bacteria such as *Escherichia coli*, *S. sonnei* and *Salmonella* Paratyphi A to eliminate the cross-reaction of selected aptamers.

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O-55

Epigenetic oncogenesis, biomarkers and emerging chemotherapeutics for breast cancer

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Breast cancer (BC) remains one of the leading causes of cancer-related deaths globally and the most prominent among females, yet with limited effective therapeutic options [1]. Most of the current medications are challenged by various factors including low efficacy, incessant resistance, immune evasion and frequent recurrence of the disease. Further understanding of the prognosis and identification of plausible therapeutic channels thus requires multimodal approaches. Such include epigenetic pathways in which modifications affect genetic oncogenic and oncotherapeutic factors without change in genomic sequences.

Methodology

In this review, epigenetics pathways to BC oncogenesis *via* the inducement of oncogenic changes on relevant biomarkers have been overviewed. Similarly, the counter-epigenetic mechanisms to reverse such changes as effective therapeutic strategies were surveyed. Series of chemotherapeutic agents with potentials for inhibition and modifications of relevant epigenetic biomarkers amenable for BC treatment were curated from recent reports. Perspectives and challenges were outlined for further studies.

Results and Discussion

Epigenetic oncogeneses occur through several pathways, notably, deoxyribonucleic acid (DNA) methyltransferase (DNMT)-mediated hypermethylation of DNA, dysregulated expression for estrogen receptor alpha, receptor tyrosine-protein kinase 2 and progesterone receptor, and overexpression of transcription factors including the cyclin-dependent kinase 9-cyclin T1 complex, sphingosine kinase 2, Y-box binding protein 1, histone deacetylases, mesothelin and inhibitor of differentiation protein 4. Others include the suppression of tumour suppressor genes including cadherin 10, procadherin β15, DENN protein, suppressor of tumourigenicity 5, lysine-specific demethylase 1 and leukaemia inhibitory factor receptor. Upregulation of some tumour promoter factors such as the bromodomain-containing protein 4, Pellino protein, sex-determining region Y-box 2 protein, octamer binding transcription factor 4, NANOG and mitochondrial dynamic proteins (MiD49 and MiD51) are also strongly implicated as tumourigenic and metastatic pathways to BC [2]. Scientifically, the regulatory reversal of the mechanisms involving similar biomarkers constitutes effective epigenetic approaches for mitigating BC initiation, progression and metastasis. These were induced at various experimental levels by various chemotherapeutic agents including some repurposable drugs, endocrine inhibitors, monoclonal antibodies and miRNAs, natural products, metal complexes and nanoparticles with dozens currently in clinical trials while others are still at preclinical stages showing anti-BC potentials [2]. The oncotherapeutic pathways implicate similar biomarkers interestingly for network pharmacology, amenable for overcoming treatment resistance associated with BC. However, most of the studies were carried out *in vitro* experimental models where physiological aftereffects could not be succinctly observed. More rigorous assessments through *in vivo* animal models are required to further observe the safety profiles of the interesting candidates. Again, the oncogenic processes could possibly overlap with the counter therapeutic pathways. As such, adequate scientific efforts are required to discriminatively focus the targeted chemotherapeutic effects toward the oncogenic suppressor mechanisms without activating the oncogenic promoters.

Conclusion

The review represents a model for a wider understanding of epigenetic oncogenic and oncotherapeutic pathways to BC and advances the scientific amelioration of the global burden of BC upon further translational studies.

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O-56

Selection of ssDNA aptamers against Programmed Death -Ligand 1 (PD-L1)

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Recently, serum PDL1 expression has been demonstrated to be a potential biomarker for cancer patient's treatment response and outcome or can even be used for early cancer detection. The most common biomarker detection approach utilizes antibody-based, but the use of antibody face numbers of drawback. Consequently, another potential alternative to antibody known as aptamers have become increasing popular. Aptamers exhibit characteristic that can overcome the drawbacks of antibody such as lower production cost, minimal batch-to-batch variation, possess better thermal stability and offers variety of chemical modification. Thus, this study aims to isolate ssDNA aptamers against recombinant human PD-L1 for future diagnostic purpose.

Methodology

In this study, we demonstrated the isolation of ssDNA aptamers against rhPD-L1 via agarose bead-based Systematic Evolution of Ligands by Exponential Enrichment (SELEX). The SELEX enrichment monitoring was determined by direct Enzyme-Linked Oligonucleotide Assay (ELONA). The enriched SELEX cycle was then cloned, followed by sanger sequencing to identify the sequence of potential aptamers. The sequences were analysed using MegaX software, online mFold software and online MEME (Multiple Em for Motif Elicitation).

Results and Discussion

Total of 9 SELEX cycle was performed and SELEX cycle 6 was selected as an enriched cycle. Pool of ssDNA from
SELEX cycle six was cloned and a total of 36 positive colonies were sequenced. Phylogenetic analysis of the positive colonies yields 9 clusters distinct aptamers. Further analysis of Gibbs free energy, frequency of a sequence and significant motif of all the sequences revealed 3 potential aptamer sequence.

Conclusion

These 3 potential aptamer binding affinities should be characterized as it could be potential biorecognition element targeting the PD-L1 for the diagnostic application.

e-POSTERS

JUDGES :	1. Associate Prof Dr Lim Theam Soon
	2. Dr Mohd Nor Azim Bin Ab Patar

	e-POSTER PRESENTATION 1	
P-1	**Suppression of microRNA-9 promotes anti-tumour activity in KMS-28BM human multiple myeloma cells** *Ivyna Pau Ni Bong, Nor Soleha Mohd Dali, Norodiyah Othman, Aliza Mohd Yacob and Ezalia Esa*	Link e-poster Link audio
P-2	**Association of oxidative damage measured by 8-Hydroxyguanosine formation with altered risks to hepatocellular carcinoma in Malaysian study population** *Zalinah Ahmad and Suzana Makpol*	Link e-poster Link audio
P-3	**Mechanisms of optical light properties using optical spectroscopy for various honey detection** *Nurul Syazana Ramlee, Nur Izzah Atirah, Irneza Ismail, Fatin Hamimi Mustafa, Wan Zakiah Wan Ismail and Juliza Jamaludin*	Link e-poster Link audio
P-4	**The effects of small molecules chaperone on cell viability in primary neonatal fibroblast cell line: Toxicity indicator for pharmacological chaperone therapy** *Affandi Omar, Muhammad Nor Farhan Saat, Fatimah Diana Amin Nordin, Salina Abdul Rahman, Balqis Kamarudin, Nur Jannaim Muhamad, Mohd Shihabuddin Ahmad Noorden and Julaina Abdul Jalil*	Link e-poster Link audio
P-5	**Decrease in mitochondrial dynamics in a patient with energy deficiency disorder** *Fatimah Diana Amin Nordin, Pung Yuh Fen, Affandi Omar, Nur Azian Aziz, Izyan Mohd Idris, Rosnani Mohamed, Balqis Kamarudin, Nur Jannaim Muhamad and Julaina Abd Jalil*	Link e-poster Link audio
P-6	**Genome profiling of genetic aberrations in adult acute lymphoblastic leukemia** *Lam Kah Yuen, Ezalia Esa, Aliza Mohd Yacob and Zubaidah Zakaria*	Link e-poster Link audio
P-7	**Selectivity and storage stability of the aptamer-modified multi-walled carbon nanotubes screen-printed carbon electrode for direct detection of 25-hydroxyvitamin D2** *Balqis Kamarudin, Nur Dalila Rizuan, Mohd Azerulazree Jamilan and Mohd Fairulnizal Md Noh*	Link e-poster Link audio
P-8	**Spectrum of F9 mutations in Malaysian Haemophilia B patients** *Abdullah Aziz, Nursaedah, Lam, K Y, Lukman, Izzatul Nabila, Md Afandi, Faridah, Abdul Karim, Faraizah, Mohd Fathullah, Tun Maizura, Mat Yusoff, Yuslina and Esa, Ezalia*	Link e-poster Link audio

JUDGES :	1. Dr Nur Haslindawaty Binti Abd Rashid
	2. Dr Ruzilawati Abu Bakar

	e-POSTER PRESENTATION 2	
P-9	**Small RNA signatures from isoniazid-resistant *Mycobacterium tuberculosis*** *Mohd Iskandar Jumat, Jaeyres Jani and Kai Ling Chin*	Link e-poster Link audio
P-10	**Serum urea/albumin ratio; COVID-19 Patients** *Norsyuhadah Musa, Wan Norlina Wan Azman, Nor Amirah Mohammad Nazri, Tuan Salwani Tuan Ismail, Azian Harun, Najib Majdi Yaacob, Sarina Sulong, Sirajudeen K N S, Mahaya Che Mat and Siti Sarah Mustapa*	Link e-poster Link audio
P-11	**Determining an optimal DNA isolation method for rodent fecal samples by 16S rDNA bacterial diversity identification** *Nik Amirah Auni Binti Nik Mohd Asri, Zarina Amin and Yew Chee Wei*	Link e-poster Link audio
P-12	**The toxic effects of p-cresyl sulfate on bone metabolism** *Ahmad Faizal Bin Jelani, Gabrielle Ruth Anisah Fromming, Dayang Erna Zulaikha Binti Awang Hamsin and Mohammad Zulkarnaen Bin Ahmad Narihan*	Link e-poster Link audio

P-13	**Characterization of ssDNA aptamers against ACE2 protein as therapeutic targets for COVID-19 infection** *Zi Yuan Chang, Falah Abbas Muhamad Salih and Kai Ling Chin*	Link e-poster Link audio
P-14	**Detection of *Campylobacter jejuni* and *Campylobacter coli* from retails broiler chicken by duplex PCR** *Susilahwati Muhammad, Nur Syafikah Muhammad Nasir and Zaidah Abdul Rahman*	Link e-poster Link audio
P-15	**The efficacy and safety of PD-1/PD-L1 immunotherapy in endometrial cancer: A meta-analysis** *Mohd Nazzary Mamat@Yusof, Kah Teik Chew, Nirmala Kampan, Nor Haslinda Abd Aziz and Mohamad Nasir Shafiee*	Link e-poster Link audio
P-16	**Plasma lactate level among patients with brain tumours pre-operative: A preliminary study** *Michelle Hui Ling Tiong, Wan Norlina Wan Azman, Anani Aila Mat Zin and Zamzuri Idris*	Link e-poster Link audio
	Subashini Raman, Vikneswaran Murugaiyah and Thaigarajan Parumasivam	

JUDGES :

1. Dr Nor Syahida Ariffin (USM)
2. Dr Norzila Ismail (USM)

	e-POSTER PRESENTATION 3	
P-17	**Assessment of the NGS data using FastQC for quality control in the study of antiproliferative effects of dichloromethane *Clinacanthus nutans* fractions extracts on human breast cancer cell lines for biomarker discovery** *Zaleha Md Toha, Nor Hasyimah Haron, Kristine Sandra Pey Adum, Nik Nur Syazni Nik Mohamed Kamal, Melati Khairuddean and Hasni Arsad*	Link e-poster Link audio
P-18	**IGF-1 and IGFBP-1 expressions as the potential prognostic biomarkers in women with endometrioid endometrial cancer (EEC)** *Abdul Muzhill Hannaan Abdul Hafizz, Reena Rahayu Md Zin, Nor Haslinda Abd Aziz, Muaatamarulain Mustangin, Nirmala Chandralega Kampan, Norfilza Mohd Mokthar, Kah Teik Chew and Mohamad Nasir Shafiee*	Link e-poster Link audio
P-19	**Pharmacological consequence of inhibiting MAPK p38 using ralimetinib dimesylate on lipopolysaccharide-induced E-selectin and VCAM-1 expression in HUVEC** *Dayang Erna-Zulaikha*	Link e-poster Link audio
P-20	**AST, ALT, Bilirubin and AST/ALT ratio role; COVID-19 Patients** *Nor Amirah Mohammad Nazri, Wan Norlina Wan Azman, Norsyuhadah Musa, Tuan Salwani Tuan Ismail, Azian Harun, Najib Majdi Yaacob, Sarina Sulong, Sirajudeen K N S, Mahaya Che Mat and Hani Ajrina Zulkeflee*	Link e-poster Link audio
P-21	**MALDI-TOF mass spectrometry-based strategy in discovery reproducible N-glycans in human fibroblast** *Salina Abdul Rahman, Affandi Omar, Nur Jannaim Muhamad and Julaina Abdul Jalil*	Link e-poster Link audio
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P-23	**Cross-reactivity between Leptospira Serovars in microscopic agglutination test for Leptospirosis** *Nur Nadia Jamil, Murnihayati Hassan, Fairuz Amran, Rohaidah Hashim, Anira Abdul Hadi and Noor Hadirah Wahid*	Link e-poster Link audio
P-24	**Susceptibility of human lung-derived cell lines MRC-5 and A549 to Malaysian SARS-CoV-2 isolate** *Siti Nur Zawani Rosli, Sitti Rahmawati Dimeng, Farah Shamsuddin, Mohammad Ridhuan Mohd Ali, Nur Afrina Muhamad Hendri, Hana Farizah Zamri, Jeyanthi Suppiah, Rozainanee Mohd Zain, Ravindran Thayan and Norazah Ahmad*	Link e-poster Link audio

e-POSTER 4

JUDGES :	1. Dr Siti Norasikin Mat Nafi (USM)
	2. Dr Noor Fatmawati Mokhtar (USM)

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P-26	**Autoantibodies profile of Glioblastoma patients in Malaysia: A preliminary study** *Johannes Low Jun Wei, Soon Bee Hong and Nadiah Abu*	Link e-poster Link audio
P-27	**Effects of kaempferol on cell morphology, migration and microtubule functions in colorectal cancer cell HT-29** *Nurhuda Mohamad Ansor, Fan Xu, Nozlena Abdul Samad and Norfarazieda Hassan*	Link e-poster Link audio
P-28	**Genomic Characterization of the First Case of Extensively Drug-Resistant, Travel-Related Salmonella enterica Serovar Typhi in Malaysia** *Nor Hazrin Abd Hazis and Rohaidah Hashim*	Link e-poster Link audio
P-29	**Suggestive SNPs of Dengue Pathogenesis Towards Severity in Single-Centre Case-Control Study** *Norfarhana Khairul Fahmy, Tengku Nurainna Fatihah Tengku Abdullah, Jamiila Ismail, Ching Yee Ming, Nurhanani Md Nor, Erina Faizati Kadri, Koay Bee Tee, Muhammad Zhafri Md Zakariah, Masita Arip and Norhazlin Mustafa*	Link e-poster Link audio
P-30	**Probiotics alter biofilm formation of periodontal pathogen, *Porphyromonas gingivalis* virulence-associated genes**	Link e-poster Link audio
	Nurul Szawani Mohd Zubri, Rohazila Mohamad Hanafiah, Siti Aisyah Abdul Ghafar and Nor Zaihana Abdul-Rahman	
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	Hui Ting Lim, Boon Hui Kok, Chin Peng Lim, Chiuan Yee Leow and Chiuan Herng Leow	

JUDGES :	1. Associate Prof Dr Oon Chern Ein (USM)
	2. Prof Peter Hoffman (University of South Australia, AUSTRALIA)

	e-POSTER PRESENTATION 5	
P-33	**Selecting antibacterial aptamers against an essential outer membrane protein in Pseudomonas aeruginosa via aptamer repurposing approach** *Rupany Selvam, Michelle Yap Khai Khun and Tan Hock Siew*	Link e-poster Link audio
P-34	**Philadelphia chromosome and FISH-negative, cryptic BCR/ABL1 positive acute myeloid leukaemia: A diagnostic dilemma** *Sarah Abdul Halim, Norlelawati A. Talib and Dhamirah Nazihah Bt Mohd Nasiruddin*	Link e-poster Link audio
P-35	**Production of recombinant shark VNAR single domain antibody specific against DENV Type 2 NS1** *Boon Hui Kok, Hui Ting Lim, Chin Peng Lim, Chiuan Yee Leow and Chiuan Herng Leow*	Link e-poster Link audio
P-36	**Vasoprotective Effects of Red Yeast Rice Supplementation in Spontaneously Hypertensive Rats** *Tan Jiunn Jye, Kang Waye Hann, Lee Siew Keah, Dharmani Devi Murugan and Ling Wei Chih*	Link e-poster Link audio
P-37	**A fatal case of cerebral malaria complicated with *Gemella bergeri* bacteremia: Role of Plasmodium mitochondrial cox3 gene and MALDI-TOF mass spectrometry for species identification** *Ummu Afeera Zainulabid, Nor Diyana Dian, Jauhary Effendy Juma'at, Petrick Periyasamy, Najma Kori, Cheah Saw Kian, Asrul Abdul Wahab and Zulkarnain Md Idris*	Link e-poster Link audio

P-38	**Association of MTHFR polymorphism in stroke risk and rehabilitation outcomes** *Eric Tzyy Jiann Chong, Kok Yeow Phneh, Syahiskandar Sybil Shah, Yuen Kang Chia, Dayang Maryama Awang Daud, Elyana Jalil, Chek Siang Kelvin Cheng and Ping-Chin Lee*	Link e-poster Link audio
P-39	**RNU6B and miR-16 as stable normalisation control for relative RT-PCR of urinary microRNA in patients with colorectal polyps** *Faizah Ahmad, Nurul Atiqah Muhamad Fauzi, Mowaffaq Adam Ahmed Adam, Muhammad Radzi Abu Hassan, Nil Amri Mohamed Kamil and Muhammad Amir Yunus*	Link e-poster Link audio
P-40	***Vernonia amygdalina* leaf extract and luteolin inhibits nasopharynx cancer cells and display synergism with cisplatin** *Maelinda Daker, Udyanee Jayaweera, Marini Marzuki, Gunaletchumy Gunasegaran and Gabriel Akyirem Akowuah*	Link e-poster Link audio

JUDGES :

1.	Dr Mohd Harizal Bin Senik @ Nawi (USM)
2.	Dr. Leow Chiuan Herng (USM)

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P-41	**A novel mutation of the NOTCH3 gene in Malaysian patient with Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL)** *Ilia Nazihah Mohamad Ayob, Amelia Azman, Law Wan Chung and Yusnita Yakob*	Link e-poster Link audio
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P-43	**Interaction of UV-VIS optical properties towards enterovirus 71 RNA for detection of hand foot mouth disease using optical spectroscopy** *Ahmad Aiman Zuhaily Ahmad Munawar1, Irneza Ismail1, Fatin Hamimi Mustafa2, Wan Zakiah Wan Ismail1 and Juliza Jamaludin1*	Link e-poster Link audio
P-44	**Analysis of microsatellite instability in colorectal cancer patients from Hospital USM** *Nurfadhlina Musa1, Marahaini Musa1, Wan Faiziah Wan Abdul Rahman2, Tengku Ahmad Damitri Al-Astani3, Ahmad Aizat Abdul Aziz1, Mohamad Ros Sidek1, Rosline Hassan4, Zaidi Zakaria5 and Sarina Sulong1**	Link e-poster Link audio
P-45	**Integration of Nanotechnology and Biomedical Sciences – A Perspective Review** *Lakshme Shree1* and Rohini Karunakaran*	Link e-poster Link audio
P-46	**Antimicrobial activity of astaxanthin extracts of wild type and mutant *Xanthophyllomyces dendrorhous*** *N. Sathiabalan, S.Y. Khaw and A.L. Chew*	Link e-poster Link audio
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P-48	**COVID-19 Pandemic and Biomedical Sciences – Current and Future Application** *Rohini Karunakaran*	Link e-poster Link audio

JURIES :

1.	Dr Mohd Harizal Bin Senik @ Nawi (USM)
2.	Dr. Leow Chiuan Herng (USM)

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P-50	**Comparative Evaluation of Three Methods for Protein Extraction Efficiency from Formalin-Fixed Paraffin-Embedded Tissues for Proteomic Analysis** *Wan Muhammad Shafiq, Gaayathri Kumarasamy, Mohd Nazri Ismail and Gurjeet Kaur*	Link e-poster Link audio
P-51	**Optimization of expression and purification of soluble *E. bieneusi*** *Rahila Qureshi, Philip Raj Abraham and Sangita Mukhopadhyay*	Link e-poster

	B7XHF2 recombinant protein in *E.coli* *Muhammad Danial Hakim Muhammad Hanif, Putri Sabrina Mohamed Yusoff, Norsyahida Arifin and Emelia Osman*	Link audio
P-52	**Production of Enterocytozoon bieneusi recombinant protein and reactivity of its polyclonal antibodies toward Encephalitozoon cuniculi** *Putri Sabrina Mohamed Yusoff, Norsyahida Arifin, Petrick Periyasamy, Nor Rafeah Tumian, Fuad Ismail, Raja Zahratul Azma Raja Sabudin, Rahmah Noordin and Emelia Osman*	Link e-poster Link audio
P-53	**Differential expression of E-cadherin and ICAM-1 in triple breast cancer cell lines** *Fu Hou Wong, Palanirajan Vijayarajkumar, Edmond Siah Chye Ng, Tan Chung Keat and Farahnaz Amini*	Link e-poster Link audio
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P-1

Suppression of microRNA-9 promotes anti-tumour activity in KMS-28BM human multiple myeloma cells

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Multiple myeloma (MM) is the second most common blood cancer characterised by clonal expansion of malignant plasma cells within the bone marrow [1]. Epigenetic aberrations such as microRNAs (miRNAs) dysregulation are of great significance in the development and malignancy of MM. MiRNAs are short, single stranded non-coding RNAs (~19-25 nucleotides) that play an important role in regulating post-transcriptional expression of target genes. Up-regulation of miR-9 has been reported in various cancers including MM; however, the mechanisms underlying its aberrant expression and functional alterations in MM are still unclear [2].

Methodology

This study aims to investigate the functional role of miR-9 in MM cells *in vitro*. KMS-28BM MM cells were transfected with miRNA-9 inhibitors or miRNA inhibitors scrambled negative control. The expression levels of miR-9 vs control were determined by RT-qPCR. The effect of miRNA-9 suppression on cell proliferation was assessed by MTS assay. Flow cytometry analysis using Annexin V-FITC/PI double staining was used to measure the proportion of apoptosis cells.

Results and Discussion

The RT-qPCR results demonstrated that miR-9 inhibitors were successfully delivered and suppressed the miR-9 expression level in KMS-28BM compared to the control ($P<0.05$). Furthermore, MTS and flow cytometry analysis revealed that suppression of miR-9 expression significantly decreased cell growth and increased the number of early apoptosis cells in KMS-28BM, respectively ($P<0.05$). Our previous microarray-based miRNA expression profiling results showed that miR-9 is up-regulated in the majority of MM patients and cell lines, suggesting miR-9 plays a pivotal role, at least in part, in myelomagenesis. The present study findings further confirm that miR-9 functions as an oncogene in MM pathogenesis, and suppression or silencing of miR-9 inhibits cell proliferation and induces apoptosis in KMS-28BM MM cells.

Conclusion

Suppression of miR-9 promotes anti-tumour activity by inhibiting proliferation and inducing apoptosis in MM cells indicates that miR-9 may be a potential therapeutic target in MM.

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P-2

Association of oxidative damage measured by 8-hydroxyguanosine formation with altered risks to hepatocellular carcinoma in Malaysian study population

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Hepatocellular carcinoma (HCC) is the most devastating type of liver cancer. This disease is the third leading cause of cancer deaths with a 5-year survival rate of 7% [1]. Cancer is the pathogenesis product of DNA damage resulting from multiple factors which among others is oxidative stress. Oxidative stress can be detected by the DNA base damage, through the formation of 8-oxo-7, 8-dihydro-2'deoxyguanosine (8-oxodG) [2]. The 8-oxodG acts as oxidative stress indicator and has been essentially specified as a recognized initiator of the carcinogenic process and premutagenic injury in mammalian cells [3]. In this preliminary study, we investigated the possible association of oxidative DNA damage in a form of 8-oxodG in HCC patients in comparison with Malaysian healthy controls. We also analysed the effect of 8-oxodG in different races and gender in both groups.

Methodology

DNA of peripheral white blood cells was isolated from 91 HCC patients and 304 controls. The level of oxidative DNA damage was determined by highly sensitive ELISA kit produced from Japan Institute for Control of Ageing (JaICA), Nikken Foods. Ltd. The determination of oxidative DNA level was digested by Ravanant (1998) and Evans (1999) methods with some modifications. The results were reported as 8-oxodG per 50 mL DNA sample in ng/ml.

Results and Discussion

Quantitative measurement of 8-oxodG was higher in HCC patients at mean value of 3.30 ± 2.32 ng/ml. In controls, the average value is 1.57 ± 1.92 ng/ml. There was a significant difference in the average value of 8-oxodG level between the controls and HCC patients where $p < 0.001$. Comparison between gender showed that there was a significant difference observed in the level of 8-oxodG between male and female in controls ($p < 0.05$). The level of 8-oxodG was higher in male (1.736 ± 2.033) ng/mL than in female controls (1.087 ± 1.433) ng/mL. From previous studies, it was reported that smoking, alcohol consumption and bad sleeping pattern were the sporadic factors for male tendency to get the oxidative damage [4]. Hepatocarcinogenesis has been reported to be associated with DNA damage through the process of oxidation. Oxidation in turn, leads to the huge amount of transversion from G → T in tumor genes such as p53 gene [5]. In this study, however, Malays and no-Malays showed no significant difference in their level of 8-oxodG indicating no disparity.

Conclusion

HCC patients showed greater oxidative damage to DNA as compared to controls. This suggests that oxidative DNA damage may contribute to the pathogenesis of HCC. Since 8-oxodG was higher in males, it is indicating that the males are at a greater risk of developing HCC than the females.

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P-3

Mechanisms of optical light properties using optical spectroscopy for various honey detection

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Honey is a natural sweetener made by honeybees from plant nectar that has been consumed by humans for thousands of years [1]. There are two kinds of honey which are monofloral honey and multifloral honey. Monofloral honey is made from a single plant nectar that has been transformed into honey. It includes Acacia, Sidr, and Kelulut honey. Meanwhile, multifloral honeys like Tualang, Manuka, and Kelulut honey are made from a blend of several different plant nectar types found in deep forests or mixed by the beekeeper before being converted into honey [2]. Honey consumption in Malaysia is increasing due to its various nutrient contents that are beneficial to one's health. Different types of honey contain varying amounts of sucrose, fructose, and glucose, which contribute to the sweetness of honey.

Methodology

In this study, pure Tualang, Kelulut, Acacia, Manuka, and Sidr honey samples were used. Optical spectroscopy in the ultraviolet-visible-near infra-red (UV-VIS-NIR) range was used to observe the optical properties of all of the honey, including absorbance and transmission. The wavelength range covered during the measurement was 350 nm to 1662 nm. The Ocean View software was used to capture the spectra of all honey. To improve the accuracy of the results, the spectra was collected 15 times for each honey. The average of the results were plotted, and boxplot analysis was performed for all spectra results.

Results and Discussion

The spectra results were taken based on the wavelength of the optical properties, which were absorbance and transmittance. Based on the observed results, the peak absorbance spectra for all honey in both UV-VIS-NIR were similar. Further analyses were done using boxplot analysis to differentiate the characteristics for all type of honey. Sidr honey has the highest mean absorbance value compared to other honeys. The current study found that all Malaysian raw honey met the international standard because the sum of their fructose and glucose contents were less than 60% [3]. But since Manuka and Sidr honey originated outside of Malaysia, it was assumed that both honey not adhere to the standard and thus consisted a higher sugar content compared to other honey. According to the Beer Lambert Law equation, increasing the sugar content will increase the number of molecules in the solution, which will increase the absorbance value. Based on previous research, an increasing pattern in absorbance value was highly noticeable as sugar concentration increased. Maximum transmission spectra for all honey in both ranges were close for both UV-VIS and VIS-NIR ranges. From the transmission boxplot analysis, Sidr honey recorded the lowest mean compared to other honeys. As a result, when the concentration of the molecules increased, the transmission value decreased, giving Sidr honey the lowest mean among the others.

Conclusion

Two optical properties, which are absorbance and transmission, were studied for all of the honey using optical spectroscopy in both the UV-VIS and VIS-NIR ranges. A boxplot analysis was performed on the spectra results to compare the mean value of the peak spectra. Sidr honey recorded the highest mean absorbance value compared to other honeys since it has the highest sugar content. According to Beer Lambert's law, as the concentrations of molecules increase, the absorbance value increases but the transmission value decreases. Therefore, Sidr honey has the lowest transmission mean value compared to other honeys.

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P-4

The effects of small molecules chaperone on cell viability in primary neonatal fibroblast cell line: Toxicity indicator for pharmacological chaperone therapy

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Hunter syndrome (HS) is a lysosomal storage disorder (LSD) caused by a mutation in the gene *IDS*, which produces the defective enzyme iduronate-2-sulphatase (IDS). Due to the limitations of enzyme replacement therapy for the treatment of HS, the use of small molecule compounds as pharmacological chaperone therapy (PCT) has recently been widely investigated [1]. Usually, small molecules with higher binding affinity that can be used at low concentrations level will be chosen as pharmacological chaperone candidates [2]. As a result, *in vitro* assays such as methyl tetrazolium test (MTT) assay can be used to determine the cytotoxicity of small molecules before proceeding with *in vivo* studies. Therefore, the aim of this study is to investigate the cytotoxic effects of selected small molecules on the viability of primary neonatal fibroblast cell line.

Methodology

Selected small molecules of heparin tetrasaccharide, (H004), heparin octasaccharide (H008), heparin decaoctasaccharide (H018), heparin disaccharide standard (HD001) and chondroitin dermatan trisulphate (CD007) were diluted in minimum essential medium (MEM) with several concentrations (0.16-500 µM). The BJ CRL-2522 fibroblast cell lines were seeded into 96-well cell culture plate at a density of 1x104 cells/well before the medium was removed from the cells after 24 hours. Subsequently, the cell lines were treated with the medium containing the respective small molecules for 72 hours (5% CO_2, 37oC). The cell viability was assessed by MTT assay, and the half maximal inhibitory concentration (IC_{50}) was calculated to determine the toxicity of small molecules.

Results and Discussion

The cell viability was decreased in a dose- and time-dependent manner when treated with the respective small molecules. HD001 has significantly exhibited concentration and time- dependent inhibitory effect to the cells with the highest IC_{50} of 239 µM. H018 showed IC_{50} of 18.82 µM making it the most toxic among all small molecules tested. The IC_{50} of H004, H008 and CD007 were 83.52 µM, 68.13 µM and 72.03 µM, respectively. Despite having non-toxic outcomes, HD001's chaperone action is inadequate to serve as a pharmacological chaperone because it binds to the IDS at a high concentration (inhibition constant, K 431.4 µM). [2]. On other hand, the high binding affinity of CD007 has a greater chaperone effect (K, 21 µM) compared with HD001 with a compromised moderate toxicity level towards the cell lines.

Conclusion

This study concludes that CD007 could be considered as potential pharmacological chaperone for PCT in HS based on its safety profile.

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P-5

Decrease in mitochondrial dynamics in a patient with energy deficiency disorder

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Mitochondrial dynamics refers to the coordination of fission and fusion process in mitochondria for the maintenance of their distribution, shape and size. Mitochondrial dynamics is crucial in the regulation of cell cycle, immunity, apoptosis and mitochondrial quality control of various processes such as biogenesis and mitophagy. Defective mitochondrial dynamics due to mutations were reported to be linked with human diseases [1]. For example, patients with energy deficiency are suspected of having primary mitochondrial disorders that can be inherited through defects of either nuclear (autosomal-dominant, autosomal recessive, X-linked) or mitochondrial (mtDNA) genes [2]. In this study, we aim to characterize the mitochondrial dynamics of a patient with energy deficient disorder.

Methodology

Fibroblasts from a 2-month-old male patient with clinical symptoms of encephalopathy, seizure, cardiomyopathy, severe metabolic acidosis, pulmonary hypertension idiopathic and low level of lactate were cultured and harvested for western blot experiment. Fibroblasts from American Type Culture Collection (BJ CRL-2522™) was used as normal control. Total protein was extracted, and the concentration was measured by Bradford assay. Next, samples were probed antibodies with GTPases of the dynamin superfamily. Antibodies used were Mitofusin 1 (Mfn1), Mfn2, Optic Atrophy 1 (Opa1) and Dynamin-related Protein 1 (Drp1). Anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) monoclonal antibody was used as normalization control.

Results and Discussion

Mitochondrial fusion protein level of Mfn1; Mfn2; and Opa1 were found to be significantly decreased in this patient's cells as compared to wild type (WT) ($p<0.05$). Meanwhile, there were no differences observed in mitochondrial fission protein level of Drp1 ($p>0.05$) as compared to WT cells. From these results, the reduced fusion proteins may contribute to energy deficiency in this patient. Previous studies suggested that mitochondrial fusion is known to allow functional complementation of mitochondrial DNA, protein and metabolites, whereas mitochondrial fission facilitates mitochondrial transport, mitophagy, and apoptosis [3].

Conclusion

Our study suggested a defective mitochondrial dynamic, that appeared to affect the fusion process. Further studies such as immunofluorescent staining is highly recommended in order to gain complementary insights into mitochondrial dynamics as well as its involvement in many primary mitochondrial disorders.

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P-6

Genome profiling of genetic aberrations in adult acute lymphoblastic leukemia

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Acute lymphoblastic leukemia (ALL) is the most common type of leukemia diagnosed in young children and adults. ALL is caused by a series of acquired genetic aberration and methylations on tumour suppressor genes. The objective of this study was to identify genetic aberration and methylation in adult ALL.

Methodology

We studied 41 cases of adult ALL compared with healthy normal controls using Single Nucleotide Polymorphism (SNP) array and Methylation-specific MLPA (MS-MLPA).

Results and Discussion

Our SNP analysis revealed 935 chromosomal aberration regions. Forty three out of 935 chromosomal aberration regions showed more than 40% aberrations, of which 19 (44%) regions were amplifications and 24 (56%) regions were deletions. The most common amplifications were on chromosome regions 8p23.1 (71%) and 1q44 (66%) while the deletions were on chromosome regions 1p31.1(76%), 3q26.1(68%) and 11p11.12(63%). Eighty one out of 111 promoter regions of CpG islands on tumour suppressor genes were methylated. The most frequently methylated tumour suppressor genes were BNIP3 (73%), NTRK1 (63%), SFRP1 (63%) and CDKN2B (59%). Amplification of 8p23.1 and deletion of 1p31.1 were the most frequently found alterations in this study. These chromosome regions contain oncogene and tumour suppressor genes. Methylation on tumour suppressor genes may contribute to leukemogenesis in adult ALL.

Conclusion

This study indicated that the genetic aberration and methylation status of tumour suppressor genes in adult ALL may serve as a potential biomarker for prognosis and development of new treatment for patients with ALL.

P-7

Selectivity and storage stability of the aptamer-modified multi-walled carbon nanotubes screen-printed carbon electrode for direct detection of 25-hydroxyvitamin D_2

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Electrochemical biosensors are some of the promising platforms that are low cost, easy to use with the possibility of being integrated into portable devices for point-of-care use. Recently, aptamer-based electrochemical biosensors have received a significant attention because of their advantages such as good stability, high sensitivity and selectivity. The immobilization of the aptamer onto the working electrode is very important so that the target recognition by the aptamer can be transduced into a measurable electrical signal [1]. Here, we described the selectivity and storage stability of an aptamer-based electrochemical biosensors for direct detection of 25 (OH) vitamin D on the aptamer-modified multi-walled carbon nanotubes screen-printed carbon electrode (MWCNTs-SPCE).

Methodology

Aptamer specific to 25 (OH) vitamin D was immobilized onto the working electrode surface of the MWCNTs-SPCE. The performance of the modified electrodes was observed to determine their selectivity towards 25 (OH) vitamin D. The long-term storage stability of the aptamer-modified MWCNTs-SPCE was investigated by storing the fabricated devices for a period of up to 30 days at 4°C. Electrochemical measurements were carried out using electrochemical impedance spectroscopy (EIS) in a redox probe (0.1 M KCl containing 5.0 mM Fe $(CN)_6^{3-}$).

Results and Discussion

The immobilization of the aptamer was determined through the differences of resistance to charge transfer (Rct) values obtained. The Rct values increased when aptamer was immobilized onto the surface of MWCNTs-SPCE compared to the bare MWCNTs-SPCE. The specific reaction between aptamer and 25 (OH) vitamin D causing the Rct value to be decreased. However, there were no changes observed when the assay carried out in the absence of the aptamer. This demonstrates that the signals were due to the specific interaction and not to non-specific adsorptions [2,3]. A good linear response was observed for different 25 (OH) vitamin D concentrations in the range of 0.1 - 30 ng/ml with a detection limit of 0.72 ng/ml. For the long-term storage stability, the aptamer-modified MWCNTs-SPCE showed a consistent response for the first 14 days, then experiences a drop afterwards. Therefore, the aptamer-modified MWCNTs-SPCE was determined to be stable for 14 days at 4°C.

Conclusion

The aptamer-modified MWCNTs-SPCE was shown to be selective towards 25 (OH) vitamin D and had a reasonable shelf-life of around 2 weeks which can be further improved for a better long-term storage.

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P-8

Spectrum of *F9* mutations in Malaysian Haemophilia B Patients

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Haemophilia B (HB) is a rare X-linked bleeding disorder characterized by deficiency in coagulation factor IX (FIX) due to mutation within Factor 9 (*F9*) gene [1]. *F9* gene is located at Xq27.1-q27.2, consists of 8 exons and 7 introns, dispersed across 34 kb length [2]. Severity of HB patients can be classified into mild, moderate and severe based on the coagulation FIX level. Patients with FIX level <1% are classified as severe whereas patients with FIX level 1%-5% and >5% to <40% are classified as moderate and mild, respectively [3]. In Malaysia, there are limited studies on *F9* gene mutations. Thus, this study aimed to identify the mutations within a representative cohort of HB patients in Malaysian population.

Methodology

A total of 41 non-familial related HB patients were studied. The patients' blood samples and clinical details which contained information on factor level, disease severity and inhibitor status were obtained from National Blood Centre and hospitals all over Malaysia. Genomic DNA was extracted from the blood samples using QIAamp® DNA Blood Midi Kit and subjected to polymerase chain reaction (PCR). Eight different primer sets were used to amplify exon/intron borders and all eight exons of *F9* gene. PCR products were then sequenced using ABI 3730XL DNA analyzer. Sequencing data of patients were aligned against reference sequences, NG_007994 and NM_000133.4 using CLC Main Workbench to identify the mutation and deduce amino acid changes.

Results and Discussion

F9 gene mutations were successfully detected in all patients, which comprise 32 point mutations (26 missense, 5 nonsense and 1 silent), 6 deletions, 1 insertion, 1 insertion-deletion (indel) and 1 splice site mutation. These mutations were distributed throughout all exons, except exon 3 with most of the mutations located in exon 8 (~42%). Five of the discovered mutations in Malaysian HB patients, c.874C>T, c.523_539delinsGAA, c.318_319insGGC, c.230T>G and C.40delC were found to be novel, whereas the remaining variants have been reported by various countries in several online Haemophilia B databases. In our study, two patients developed inhibitor against FIX and the mutations detected in these patients are nonsense mutation (c.1150C>T) and deletion (c.40delC).

Conclusion

Our study identified heterogenous mutation profile in HB patients in Malaysian population. These findings are important for genetic confirmation of HB patients, genetic counselling, prediction of inhibitor development and also for carrier screening among family members.

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P-9

Small RNA signatures from isoniazid-resistant *Mycobacterium tuberculosis*

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB), remains a significant public health concern worldwide. The emergence of multidrug-resistant TB (MDR-TB) (resistance to at least isoniazid or rifampicin) has significantly jeopardized the treatment and control of TB. Isoniazid-resistant TB (Hr-TB) is much more common than rifampicin-resistant TB (RR-TB) [1]. The regulatory mechanism underlying the emergence of Hr-TB remain to be fully elucidated. Small non-coding RNAs (sRNAs) regulate bacteria essential functions such as survivability and metabolism, among others. Also, the sRNA has been implicated as one of the caused for development of drug resistance [2]. The present study investigates the expression profile of sRNAs in drug-susceptible TB (DS-TB) and Hr-TB strains using next generation sequencing (NGS) with Illumina HiSeq2500 Deep Sequencing Technology for better understanding of the mechanism of resistance to isoniazid in MTB.

Methodology

Three DS-TB (SBH49, SBH149, and SBH372) and three Hr-TB strains (SBH365, SBH438, and SBH509) isolated from pulmonary TB patients were cultured in BD BACTEC™ MGIT™ (Becton Dickinson, USA) and their drug susceptibility to first-line antibiotics, i.e., streptomycin, isoniazid, rifampin, and ethambutol were tested with BACTEC™ MGIT™ 960 SIRE kit (Becton Dickinson, USA). Resazurin microtiter assay (REMA) was performed to determine the minimum inhibition concentration (MIC) for isoniazid. Next, RNA from both susceptible and resistant strains were extracted using Masterpure™ Complete DNA and RNA purification kit (Lucigen, USA). The integrity of RNA was determined by Agilent RNA 6000 Nano kit (Agilent Technologies, USA). Small RNA libraries preparation was carried out using NEBNext® Small RNA Library Preparation kit (NEB, UK). Samples were sequenced by single-end reads of 50 base pairs generated through Illumina HiSeq 2500™ in Rapid Run mode. RNA sequences were trimmed for adapter sequence and low-quality reads were filtered out. The sequences were annotated to reference genome (MTB H37Rv) and differential analysis were carried out with CLC Bio Genomic Workbench (version 8). sRNAs target prediction was performed by using TargetRNA2.

Results and Discussion

DS-TB strains were susceptible to all first-line antibiotics, while Hr-TB strains only resistance to isoniazid with MIC of 0.125µg/mL for SBH365, and 2mg/mL for SBH 438 and SBH509. A total of 63,252,209, 63,636,812, and 61,148,224 qualified Illumina reads were obtained from DS-TB strains and 75,296,115, 58,965,389, and 67,935,496 reads from Hr-TB strains. The overall *de novo* assembly of sRNA sequence data generated 255, 255, and 255 for all DS-TB strains and 310, 359, and 403 for Hr-TB strains with sRNA length of 18-30bp. Comparative analysis revealed that 728 sRNAs were differentially expressed in the Hr-TB compared with the DS-TB, of which 422 were upregulated and 306 were downregulated. sRNA target prediction using computational method reveals these sRNAs target wide range of mRNAs which involve in toxin production, survivability, and others [3].

Conclusion

sRNAs play a major role in the regulation of gene expression and mediates the cellular processes in bacteria. This data demonstrates that sRNA may serve as an invaluable resource for revealing the molecular basis of the regulation of expression associated with the mechanism of isoniazid resistance in MTB.

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P-10

Serum urea/albumin ratio ; COVID-19 Patients

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background
The blood urea to albumin ratio is an inflammatory biomarker that has been linked to clinical outcomes in a variety of diseases [1]. In this regard, the urea/albumin ratio can be a useful biomarker that allows the clinician to identify those at higher risk of critical illness quickly. This study aims to determine the association between the urea/albumin ratio and severity among COVID-19 patients.

Methodology
This was a retrospective study on hospitalised adult COVID-19 patients aged 18 to 80 at Hospital Raja Perempuan Zainab II (HRPZ II) between February 2021 and February 2022. A total of 170 COVID-19 patients were enrolled in this study. The serum urea, albumin, and creatinine level on admission were recorded. The patients were classified into five clinical stages based on Annex 2e guidelines by Malaysia's Ministry of Health. The patients were grouped by disease severity into mild to moderate disease (Stage 1-3) and severe to critical illness (Stage 4-5). The statistical analysis was using SPSS version 27. This study was approved by (JEPeM-USM) protocol code USM/JEPeM/21100691 and Ministry of Health Malaysia NMRR-21-762-58458 (IIR).

Results and Discussion
Of the patients who were included in the study, 56 (32.9%) were mild to moderate category, and 114 (67.1%) were in the severe to the critical group. 69(40.6%) were male, and 101(59.4%) were female. The mean age was significantly higher in the severe to the critical group, 59.26 ± 13.6 years, compared to the mild to moderate group, 52.09 ± 22.2 years, p=0.010. The severe to critical group had a significantly higher median value of urea, creatinine, and urea/albumin ratio compared to the mild to moderate group. (urea: 7.0(7.20) and 3.6(3.2), p<0.001; creatinine 89.50(59.75) and 54.3(39.75), p<0.001; urea/albumin ratio 0.21(0.24) and 0.08(0.07), p<0.001). The mean albumin value in the severe to the critical group was significantly lower than in the mild to moderate group (34.95 ± 5.57 and 37.8 ± 5.58, respectively; p = 0.002). These findings suggest that markers of renal function could reliably identify the risk of COVID-19 in individuals.

Conclusion
Based on our study findings, a high urea/albumin ratio on admission was associated with severe COVID-19 infection. This biomarker could aid in risk stratification models for predicting serious and fatal outcomes of COVID-19 disease. Further studies are needed to define the optimal cut-off point for this marker and reach a consensus on its prognostic value.

Acknowledgements
This study is funded by the Ministry of Higher Education Malaysia for the Fundamental Research Grant Scheme with project code: FRGS/1/2021/SKK06/USM/03/6 and supported by External Agencies UTAS MAJU SDN BHD, 304.PPSP.6150238.U167.

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P-11

Determining an Optimal DNA Isolation Method for Rodent Fecal Samples by 16S rDNA Bacterial Diversity Identification

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

The choice of an optimum DNA isolation method from fecal samples is a constant challenge as ineffective lyses of diverse bacterial cells may lead to bias detection in the representation of a bacterial community in a sample. In addition, degraded DNA and presence of PCR inhibitors such as humic acid and polysaccharides carried over from fecal samples have been known to further reduce PCR efficiency. The purpose of this study is to determine an optimal DNA extraction method for rodent samples by assessing the general bacterial diversity based on PCR products amplified from the partial 16S rDNA gene. The PCR-DGGE technique separates the identical size of PCR amplicons by their differential mobility on gel based on sequence variations.

Methodology

In this study, five (5) fecal samples from wild rodents were collected and added into the Zymo Research DNA/RNA Shield reagent as preservative. Six DNA extraction methods i.e. QIAamp PowerFecal Pro DNA Kit (QIAGEN), the QIAamp AllPrep PowerViral DNA/RNA Kit (QIAGEN), the QIAamp UCP Pathogen Kit (QIAGEN), the ZymoBIOMICS DNA Miniprep Kit (ZYMO Research), and two conventional methods using different component in lysis buffer which were guanidium thiocyanate and CTAB were tested using between 10 to 15 mg of feces. The samples were extracted according to manufacturer's protocol and previous article [1] with some modifications, respectively. The extracted DNA were then subjected to amplification of V2 to V3 region of the bacterial 16S rDNA gene and DGGE separation of the amplicons. The DGGE banding pattern was analysed using the GelCompar II software (Applied Maths, Belgium) to compare the microbial diversity by using the Shannon-Weaver index [2].

Results and Discussion

The results showed that DNA yield varied with the extraction method; where the conventional method using guanidium thiocyanate showed a higher yield (average 324.22 ng/ul) than the other methods (average 45.33 ng/ul for the QIAamp PowerFecal Pro DNA Kit, 51.92 ng/ul for the QIAamp AllPrep PowerViral DNA/RNA Kit, 220.74 ng/ul for the QIAamp UCP Pathogen Kit, 42.4 ng/ul for ZymoBIOMICS DNA Miniprep Kit, and 165.74 ng/ul for the conventional method using CTAB). Majority of DNA extracted showed degradation when checked by gel electrophoresis. The QIAamp PowerFecal Pro DNA Kit was observed to show intact DNA. The DGGE profiles showed that the QIAamp PowerFecal Pro DNA Kit and QIAamp AllPrep PowerViral DNA/RNA Kit extracted the highest number of DNA bands (total of 168 and 167), while QIAamp UCP Pathogen Kit, ZymoBIOMICS DNA Miniprep Kit, and both conventional methods using guanidium thiocyanate and CTAB recorded 161, 160, 147, and 152 total of bands, respectively.

Conclusion

As a conclusion, QIAamp PowerFecal Pro DNA kit is the optimum DNA isolation method for rodent fecal samples as it provides better quality of DNA and microbial diversity in their DGGE profiles. Furthermore, this method is much time efficientQIAamp PowerFecal Pro DNA Kit and QIAamp AllPrep PowerViral DNA/RNA Kit produced more bands on their DGGE profiles than the other methos due to their use of bead-containing lysing matrix and vigorous homogenization.
compared to the other methods ensuring a reliable biosurveillance and metagenomic studies.

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P-12

The Toxic Effects of *p*-Cresyl Sulfate on Bone Metabolism

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

p-Cresyl Sulfate (*p*CS) is a uremic toxin that has been implicated in kidney disease, cardiovascular risks, endothelial dysfunction and neuropathies. In chronic kidney disease (CKD), *p*CS progressively accumulates in the body as the dysfunctional kidneys have a reduced ability to excrete toxins normally. *p*CS accumulated as a consequence of CKD cannot be removed from th body through dialysis, hence leading to further accumulation. This ultimately leads to bone loss which correlates with the worsening of CKD. As such, *p*CS could play a part in the development of bone loss with CKD. The objective of this review is to further understand the comprehensive effects pCS has on the bone.

Methodology

An extensive literature search was conducted in PubMed using the following keywords, '*p*-Cresyl Sulfate' OR '*p*-Cresol Sulfate' OR '*p*-Cresyl Sulphate', 'uremic toxin' OR 'uraemic toxin' AND 'bone' OR 'osteoblast' OR 'osteoclast' OR 'osteocyte'. From 2013 to 2022, 54 papers were found that contained the following keywords. Out of the 54 papers, 27 papers with significance were selected. The inclusion criteria for the study are in vivo and in vitro studies that examined the effects of *p*CS on bone. The exclusion criteria for the study is as follows: review article. After reviewing each article, 5 papers were selected for this review.

Results and Discussion

*p*CS is a prototype protein-bound uremic toxin associated with a multitude of toxic biological and biochemical effects. *p*CS is a substrate for human organic anion transporters (hOAT) 1 and 3 which is expressed in osteoblasts and other tissues. hOAT1 and hOAT3 may have a physiological role as large-capacity *p*CS transporters which could be a factor in the accumulation of *p*CS to toxic levels in osteoblasts [1]. Accumulation of *p*CS affects sclerostin production.

As an inhibitor of the Wnt-signalling pathway, sclerostin prevents osteoblast formation and osteoprotegerin (OPG) production, inhibits osteoblast-mediated bone formation, and stimulates bone resorption by stimulating RANKL expression in osteocytes [2]. *p*CS also increase apoptosis and reduce osteoblast proliferation and viability with or without increased oxidative stress. This manifests by downregulation of the parathyroid hormone receptor (PTHR) on osteoblasts and a decrease in PTH-stimulated cAMP production through activation of c-Jun N-kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) [3]. and triggers JNK phosphorylation by attenuating PTH responsiveness in the bones under CKD conditions.

Conclusion

This review evaluates the toxic effects *p*CS induces on bone. However, further studies must be conducted to understand the mechanism of action and full effects *p*CS has on bone metabolism. In the future, *p*CS could potentially become a new therapeutic target for the management of bone disorders.

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P-13

Characterization of ssDNA aptamers against ACE2 protein as therapeutic targets for COVID-19 infection

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Coronavirus disease 2019 (COVID-19), a highly contagious and rapidly spreading disease with significant fatality in the elderly population has swept across the world for the past three years since 2019. The enormity scale of this pandemic has resulted in the emergence of several SARS-CoV-2 variants with Omicron, the current main circulating variant of concern [1]. Nonetheless, all the variants identified to date share the same cell entry mechanism where the process is initiated when the spike protein of the viruses attach to the angiotensin-converting enzyme 2 (ACE2) receptors on their host cells [2]. Therefore, blocking the spike protein-ACE2 interaction using a biological binder targeting the ACE2 is a viable strategy for COVID-19 treatment. Aptamer is a short length of nucleic acid [RNA or single-stranded DNA (ssDNA)] which is selected through an *in vitro* selection called systematic evolution of ligands by exponential enrichment (SELEX). The selected aptamers have a 3D conformation that can specifically bind to their target with high affinity and pose many superior properties such as being smaller in size, thermally stable, and nearly non-immunogenic.

Methodology

Single-stranded DNA library of 76-bp oligonucleotides containing a randomized core region of 40 nucleotides, flanked by primer binding regions of 18 nucleotides on each side (5'-ATACCAGCTTATTCAATT-N40-AGATAGTAAGTGCAATCT-3') was synthesized. Recombinant His-tagged ACE2 protein (BBI Life Sciences, China) was immobilized to Ni-NTA Magnetic Beads (Gold Biotechnology, USA). Aptamers which showed high affinity towards the ACE2 were then selected from the initial library using SELEX. The isolated aptamers were cloned using the PCR Cloning Kit (NEB, USA) and the plasmids were extracted using DNA-spin Plasmid DNA Extraction Kit (iNtRON Biotechnology, Korea). The plasmids were sequenced and the resulting aptamer sequences were subjected to the UNAFOLD web server (http://www.unafold.org/) for ssDNA secondary structure prediction, followed by RNA tertiary structure prediction using RNAComposer (https://rnacomposer.cs.put.poznan.pl/), and finally, converted to equivalent ssDNA tertiary structure using Discovery Studio Visualizer v3.5. The aptamer-ACE2 interaction was predicted using the HDOCK docking program (http://hdock.phys.hust.edu.cn/).

Results and Discussion

Aptamers specifically bound to ACE2 were isolated after 13 rounds of magnetic bead-based SELEX. Here, we reported one of the isolated aptamers, Apt15, which potentially binds to the ACE2. The UNAFOLD revealed that this aptamer has a hairpin loop structure and single-stranded region with a Gibbs free energy value of -0.10. The low free energy value indicates that the ssDNA structure is thermodynamically stable [3]. *In silico* 3D molecular docking demonstrated that the single-stranded region of Apt15 binds to the ACE2 at the site recognized by the SARS-CoV-2 spike protein with a confidence score of 0.9894, thus suggesting the potential application of this aptamer as a therapeutic target for COVID-19 infection.

Conclusion

In conclusion, one ssDNA aptamer targeting ACE2 with therapeutic potential against COVID-19 was successfully identified in this study. Further investigations are necessary to determine its binding affinity to ACE2 and its ability to block the virus spike protein-ACE2 interaction. In future, this aptamer could serve as a broad-spectrum inhibitor against any existing or future emerging viruses that also use ACE2 for cell entry.

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P-14

Detection of *Campylobacter jejuni* and campylobacter coli from retails broiler chicken by duplex PCR

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Campylobacter is one of the leading causes of foodborne diarrhoea illness in the developed countries and cause a significant public health concern worldwide. This study was designed to determine the prevalence of *Campylobacter coli and C.jejuni* contamination in poultry retails meat in Kota Bharu by duplex PCR from direct samples.

Methodology

A total of 50 fresh and chilled poultry chicken meat were purchased from 13 retail markets in. The samples were put into polyethylene bag and wash with Phosphate-buffered solution, the bacterial lysate were prepared directly from chicken wash, enriched in CCDA broth for 48 hours and the presence of *C.coli* and *C.jejuni* were detected by duplex PCR.

Result and discussion

Overall results revealed 22% contamination of *Campylobacter* occur in poultry retail meat in Kota Bharu, Kelantan. Out of fifty samples of fresh (n= 16) and frozen/chilled (n=34) chicken thigh, 10/50 (20%) was positive for *C.coli* and/or *C.jejuni*. Majority (9/10, 90%) of contamination were *C.coli* whereas, the remaining (1 sample) was *C. jejuni*. Frozen/chilled samples have lower frequency of *Campylobacter* contamination,11.8% (4/34) as compared to fresh samples, 37.5% (6/16). No *C.jejuni* contamination was observed in fresh samples, and only 2.9%(1/34) in frozen/chilled samples. While *C.coli* detected at rate 6/16(37.5%) in fresh chicken samples and 4/34(11.7%) in frozen/chilled chicken samples. The prevalence of *Campylobacter* spp in retail meat Kota Bharu was 22%(11/50) of fresh and frozen chicken meat is relatively low as compared to other result reported previously in Japan and Egypt who obtained retail level rate varying from 64-68%. Meanwhile, the reported prevalence of *Campylobacter* spp in broiler chicken in Malaysia even greater up to 97.1%, other studies done in East-cost Malaysia shows 20% of contamination from total of 120 cloaca swabs taken whereas the later obtain 45%-70%. The contamination attributed by the cross-contamination of poultry carcasses during defeathering, evisceration and carcass chillers, which reflected by the contamination rate of cases and meat. The result shows higher prevalence of contamination with *C.coli* and *C.jejuni* in fresh chicken samples compared to frozen/chilled. Previous studies showed the influence of chilled meat and inclusion of skin as determining factors in the prevalence of *Campylobacter* spp. Out of 34 chilled chicken samples, 11.8% (4/34) were positive with *Campylobacter* spp, this 3-fold higher in fresh chicken samples, 37.5%(6/16). This in consistent with study done in central part of Malaysia, stated that apart from chilled temperature, packing in polyvinylidene film-overwrapped storiform trays may indirectly creating microaerophilic conditions which facilitate the survivability of *Campylobacter*.

Conclusion

The prevalence of *C.coli* and *C.jejuni* among poultry retails chicken meat in Kota Bharu, Kelantan is low as compared to limited data in other part of Malaysia. However, more studies with a larger sample sizes and involvement of retail market are required.

P-15

The efficacy and safety of pd-1/pd-l1 immunotherapy in endometrial cancer: a meta-analysis

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

The programmed cell death protein 1 (PD-1)/Programmed cell death ligand 1 (PD-L1) pathway has a crucial role in the immune escape mechanism and growth of cancer cells in various cancer types, including endometrial cancer [1]. In exploring better treatment options, immune checkpoint inhibitor studies of PD-1/PD-L1 in endometrial cancer are actively conducted in clinical trials with promising findings in other cancers. However, the efficacy of those immunotherapy remains inconclusive, and guidelines are inconsistent [2]. Therefore, this meta-analysis aims to provide an updated and robust analysis of the effectiveness and safety of PD-1/PDL1 immunotherapy in endometrial cancer on the objective response rate (ORR), disease control rate (DCR), and adverse events.

Methodology

A literature search was conducted using the database of PubMed, Scopus, and Web of Science databases by using the keywords of "endometrial cancer" OR "PD-1 inhibitor" OR "PD-L1 inhibitor" OR "immunotherapy" OR "immune checkpoint inhibitor" OR "atezolizumab" OR "avelumab" OR "dostarlimab" OR "durvalumab" OR "nivolumab" OR "pembrolizumab". Meta-analysis of proportion and association was pooled using STATA version17 and RevMan version 5.4 software, respectively. The effect of immunotherapy on outcome parameters was estimated by effect size (ES) or odds ratio (OR) with 95% confidence intervals (CIs) for each study.

Results and Discussion

Five studies between 2017 and 2022 met the inclusion criteria, with 480 endometrial cancer cases undergoing PD-1/PD-L1 immunotherapy clinical trials. The pooled proportion of ORR undergo PD-1/PDL-1 inhibitor treatment was 26.47% [95% CI = 16.68–37.52]. Subgroup meta-analysis showed the pooled ORR of the proficient mismatch repair (pMMR) group was 8.80% [95% CI = 0.20–24.39], and which was 37.03% [95% CI = 23.22–51.95] of the deficient mismatch repair (dMMR) group. Meta-analysis proportion of DCR undergo PD-1/PD-L1 inhibitor treatment was 41.44% [95% CI = 36.38–46.60]. Subgroup meta-analysis showed the pooled proportion DCR of the pMMR group was 24.97% [95% CI = 14.87–36.49] and dMMR group was 45.02% [95% CI = 39.26–50.85]. The efficacy of the PD-1/PD-L1 inhibitor showed a significantly higher ORR in dMMR than pMMR [OR = 6.30; 95% CI = 3.60–11.03]. Results were the same for DCR, a significantly higher in dMMR than pMMR [OR = 2.57; 95% CI = 1.66–3.99]. The safety pooled proportion of patients with at least one adverse event was 69.19% [95% CI = 62.56–75.46], and grade three or higher facing adverse events was 15.38% [95% CI = 12.01–19.04]. Based on the MSI status subgroup analysis of ORR and DCR, the efficacy of PD-1/PD-L1 immunotherapy in endometrial cancer was significantly better in dMMR patients.

Conclusion

PD-1/PD-L1 immunotherapy in endometrial cancer shows a promising outcome in early clinical trials, found to have better efficacy in the dMMR population. Thus, patients with dMMR are more suitable with this treatment and further studies of targeted immunotherapy approaches need to fully explore before can be applied for a better treatment outcome.

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P-16

Plasma Lactate Level among Patients with Brain Tumours pre-operative: A preliminary study

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

[1] Background

Brain tumours have histologically different subgroups with varied descriptive epidemiology, clinical features, therapies, and prognosis [1]. A previous study showed the Warburg effect or increased lactate generation in tumours through anaerobic glycolysis [2]. However, various plasma lactate results in patients with glial and non-glial tumours before brain tumour excision. Therefore, we measure baseline plasma lactate levels between glial and non-glial tumours before the surgical procedure.

[2] Methodology

This is a prospective study done at Hospital Universiti Sains Malaysia (HUSM) from 2021 to 2022. Patients who presented to the neurosurgical ward/clinic that met the criteria for CNS tumours as indicated in the 2021 WHO Classification of Tumors of the Central Nervous System were recruited in the study. The patients were divided according to glial and non-glial tumours. Plasma lactate was taken before the tumor resection and measured using the ARCHITECT c8000 biochemistry analyzer. The patient's plasma lactate was compared with the reference value of 0.50 – 2.20 mmol/L. The data were analyzed using SPSS version 27. This study was approved by the Jawatankuasa Etika Penyelidikan Manusia Universiti Sains Malaysia (JEPeM-USM) protocol code USM/JEPeM/21010049.

[3] Results and Discussion

A total of nine patients verified by histopathology were included in the study with a mean (SD) age of 37.7 (10.2). Six were glial tumours, and three were non-glial tumours. The mean (SD) of plasma lactate level among glial tumours compared to non-glial tumours before the surgical procedure was 2.54 (0.99) and 3.21(2.44), respectively. There was no significant difference in plasma lactate level between glial and non-glial tumours (p- value: 0.557). High pretreatment serum lactate level in patients with brain tumour might be produced from abnormal aerobic glycolusis in cancer celles and injured normal brain tissues.
Plasma lactate serve as nutrient and signaling agaent for cancer cells metabolic activitiy. Therefore plasma lactate can serve as a effective blood-based biomarker to trace and treat brain tumours.

[4] Conclusion

This study reinforces the role of serum lactte as a more prognostic biomarker of brain tumour. However this study had provide a different result compared to prevous published research as there are no significance difference in plasma lactate level between gial and non-glial tumours. Larger sample size would benefit to affirm a more effective result.

[5] Acknowledgement

The study is funded by Ministry of Higher Education Malaysia for Fundamental Research Grant Scheme with Project Code: FRGS/1/2021/SKK06/USM/02/13 and Universiti Sains Malaysia RU Top-down 2020 ; 1001/PPSP/8070015.

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P-17

Assessment of the NGS data using FastQC for quality control in the study of antiproliferative effects of dichloromethane *Clinacanthus nutans* fractions extracts on human breast cancer cell lines for biomarker discovery

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

In Malaysia, *Clinacanthus nutans* (CN) leaves are used traditionally in treatment for breast cancer. However, the scientific approval of the mode of action of CN extract on MCF-7 cell lines at a molecular level is still lacking. The high throughput Next-Generation Sequencing technology (NGS) for obtaining large sequence numbers that benefit genetic medicine research was used in this experiment [1, 2]. Bioinformatics tools such as FastQC and Picard were used to evaluate the sequence data for quality control as an essential method during the analytical pipeline [3] that is important for the identification of systematic bias challenge in this study. The objective of this study was to evaluate the quality of NGS data using FastQC in the early part of transcriptomic analysis pipelines of antiproliferative effects of dichloromethane CN fractions extracts on human breast cancer cell lines for biomarker discovery.

Methodology

Dichloromethane *CN* leaves extracts with 50% inhibition concentration (IC_{50}), 108µg/mL were exposed to the human breast cancer cells, MCF-7 for 72hrs chosen from a previous study. RNA was extracted from the treated and untreated cells for transcriptomic sequence, NGS technology used the Illumina HiSeq4000 platform. The output of the sequence was raw data that must be removed from the adapter sequences by the short read rimmed using FastQC (Babraham Bioinformatics, UK). This FastQC analysis used Linux operating system, Ubuntu 18.0v and the adapter was removed by a barcode tool known as Flexbar with its command line and script. All the results were presented in multiQC which aggregates results and generates a single HTML report with plots to visualize and compare various metrics between the samples involved.

Results and Discussion

The output of multiQC was filed from FastQC. In the report summary of FastQC status, important attention was based on sequence quality and sequence length distribution. After RNA sequencing using paired ends modules the samples generated GC overall percentage of 54% and length 150bp showed the no different composition of GC and no biases of complexity libraries, differences in amplification, or library specific causes. The sequence quality resulted in a pass or good result in the green area and upper than 30 phred scores for all samples. As a result, total overrepresented sequences found in each library make up more than 0.1% of the total that was passed and good quality which does not affect the biological consideration. Overall, the quality of RNA-seq data was high and good sequence quality.

Conclusion

Multi QC report used FastQC as a tool was advantageous for being relatively quick to generate and provides a really clear method for comparing samples to determine consistency and to identify problematic samples. From this study, the multiQC report contained high quality clean data that was downstream in all future transcriptomic analyses for biomarker discovery in breast cancer research.

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P-18

IGF-1 and IGFBP-1 Expressions as the Potential Prognostic Biomarkers in Women with Endometrioid Endometrial Cancer (EEC)

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

1. Background

Insulin-like growth factor-1 (IGF-1) and the IGF binding protein 1 (IGFBP-1) expressions have been shown to play a vital role in cancer biology, including endometrioid endometrial cancer (EEC). We examined the prognostic value of these locally expressed biomarkers in endometrial biopsies and correlated them with clinicopathological data of EEC.

2. Methodology

mRNA expression of *IGF-1* and *IGFBP-1* in the endometrial biopsies were analysed in patients with EEC (n=25) and control (n=25) cases using quantitative polymerase chain reaction (qPCR) method. These data were then validated using immunohistochemistry (IHC) analysis and combined with EEC cases form a separate cohort (n=71) comprised of consecutive patients who underwent hysterectomy at UKMMC, between the year of 2014 to 2019. Overall survival was evaluated using the Kaplan-Meier method, with differences compared using the log- rank test. Independent relationships between these biomarkers and clinicopathological data were assessed using multivariate logistic regression models.

3. Results and Discussion

The *IGF-1* and *IGFBP-1* mRNA expressions were not significantly different between both groups, EEC vs. control. However, IGF-1 expression in IHC analysis was noted to be highly expressed in the EEC compared to the control group, while IGFBP-1 had low expression in the EEC cases (P<0.05). IGF-1 was significantly associated with prognostic features of EEC (P<0.05), while no associations were found in the IGFBP-1 expression. In our sub-analysis, high IGF-1 and negative IGFBP-
1 expressions were significantly correlated with poor progression-free survival (PFS) in advanced stage of EEC (P<0.05). Univariate and multivariate analyses showed that IGF- 1 served a predictive biomarker in EEC survival. Therefore, we postulate the continuous high expression of local IGF1 protein in EEC cells could lead to poor outcomes. Our findings support that the circulating estrogen and IGF1 were independently associated with a higher risk of recurrence in patients with stage III and IV of EC (Merritt et al. 2021). We also propose that a shift in the local expression of IGFBP1 and IGF1 may serve as a biomarker for the prognosis of EEC development.

4. Conclusion

Local expression of IGF-1 and IGFBP-1 may serve as prognostic biomarkers for EEC.

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P-19

Pharmacological consequence of inhibiting MAPK p38 using Ralimetinib dimesylate on lipopolysaccharide-induced E-selectin and VCAM-1 expression in HUVEC

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Endothelial dysfunction plays a prominent role in the pathogenesis of sepsis and is associated with life-threatening organ dysfunction. Lipopolysaccharide (LPS), a Gram-negative bacterial component, is an important sepsis-associated mediator that induces the expression of adhesion molecules such as E-selectin and VCAM-1 upon its binding to dedicated pattern recognition receptors on endothelial cells (EC) [1]. Endothelial E-selectin and VCAM-1 expression promotes leukocyte adhesion, and high leukocyte infiltration in various organs is associated with a poor prognosis in sepsis patients. As the expression of E-selectin and VCAM-1 is partly driven by the activation of the MAPK p38 signaling pathway [2], it is unknown whether Ralimetinib dimesylate (RM), a selective p38 MAPK inhibitor, can be used as a treatment to reduce E-selectin and VCAM-1 expression once LPS-driven activation of EC has started. RM treatment was previously shown to reduce TNF-α production in LPS-induced macrophages *in vitro* [3]. In this study, I investigated the pharmacological effect of RM treatment on E-selectin and VCAM-1 expression in LPS-stimulated HUVEC.

Methodology

Ten µM of RM was added into the HUVEC medium at 0.5, 1, 1.5, and 2 hours after HUVEC was exposed to 1 µg/ml of LPS. After 2, 4, 5, and 6 hours of LPS exposure, the cells were trypsinized and subjected to flow cytometry analyses. The Mean Fluorescence Intensity (MFI) of E-selectin and VCAM-1- conjugated fluorochromes was determined in the treatment groups and statistically compared to LPS-stimulated HUVEC controls using one-way ANOVA and Bonferroni post-hoc test. Each group was represented by three biological replicates. The results were reported as mean ± SD. Viability of HUVEC was monitored microscopically.

Results and Discussion

LPS induced the expression of E-selectin and VCAM-1 in HUVEC in a time-dependent manner. E-selectin and VCAM-1 were maximally expressed on HUVEC at 4 respectively 6 hours after LPS exposure. E-selectin expression was attenuated throughout the 6 hours' duration of LPS exposure upon post-LPS treatment with RM at 0.5, 1, 1.5, and 2 hours after LPS exposure. VCAM-1 expression was not affected upon post-LPS treatments with RM. These findings suggest that MAPK p38 to be an important signaling cascade mediating LPS-induced E-selectin expression and can be pharmacologically targeted to attenuate E-selectin, but not VCAM-1 expression.

Conclusion

RM can be used to pharmacologically target p38 MAPK signaling pathway to attenuate the expression of E-selectin, but not VCAM-1, in LPS-activated EC. Follow-up *in vivo* study should be done to investigate the effect of RM on the expression of E-selectin and VCAM-1 in animal model of experimental sepsis.

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P-20

AST, ALT, Bilirubin and AST/ALT Ratio Role; COVID-19 Patients

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Impaired liver function upon admission has been linked to the severity of COVID-19 infection, yet the data is debated [1]. Therefore, this retrospective study aimed to evaluate the liver function among COVID-19 patients during hospitalization and its association with the disease severity.

Methodology

The patient aged 18 to 80 with positive COVID-19 at Hospital Raja Perempuan Zainab II (HRPZ II), Kota Bharu, Kelantan, with available AST, ALT, Bilirubin, and AST/ALT ratio data on admission, were retrospectively evaluated from March 2021 to March 2022. Disease severity was categorized based on Annex 2e guidelines by Malaysia's Ministry of Health, which further classified them into mild to moderate disease (Stage 1-3) and severe to critical illness (Stage 4-5). The AST, ALT, Bilirubin, and AST/ALT ratio levels on Day 1 admission were archived from the electronic medical record system and compared between the two groups. The statistical analysis was using SPSS version 27. This study was approved by (JEPeM-USM) protocol code USM/JEPeM/21100691 and Ministry of Health Malaysia NMRR-21-762-58458 (IIR).

Results and Discussion

The study included a total of 168 COVID-19 patients with a mean (SD) age of 46.67(16.10) for mild to moderate and 56.66(12.41) for severe to critical. There is a significant age group for both groups (p-value=0.002). During hospitalization, 16(14.41%) patients progressed to death from severe to critically ill patients. Upon admission, the median (IQR) of AST and ALT were significantly higher in the severe to critical group compared to in the mild to moderate group, [AST; 39.0(49.0) and 24.0(14.0), ALT 38.0(43.0) and 21.0(18.0)], $p<0.05$. However, no significant difference between both groups for bilirubin level and AST/ALT ratio. Non-survivors had a higher AST and ALT level compared to survivors, with a median (IQR) of [AST 98.0(88.0) and 32.0 (26.0), ALT of 67.5(90.0) and 28.0(31.0), ($p<0.05$). Similarly, no significant difference between non-survivors and survivors for bilirubin and AST/ALT ratio. Our study support that, abnormal liver function at admission has been shown to be associated with the disease severity and mortality of COVID-19 infection. However, there is also a need to observe the COVID-19 survivors' hepatobiliary sequelae and dynamic liver function changes following hospital discharge.

Conclusion

Abnormal AST and ALT level at admission has been shown to be associated with the disease severity and mortality of COVID-19 infection. Further study needed to evaluate liver damage in COVID-19 post-discharge.

Acknowledgements

This study is funded by the Ministry of Higher Education Malaysia for the Fundamental Research Grant Scheme with project code: FRGS/1/2021/SKK06/USM/03/6 and External Agencies UTAS MAJU SDN BHD, 304.PPSP.6150238.U167.

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P-21

MALDI-TOF mass spectrometry-based strategy in discovery reproducible *N*-glycans in human fibroblast

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Mass spectrometry (MS) has evolved over the years into a powerful tool which facilitates the identification of glycoproteins and *N*-glycans. *N*-glycans are essential for controlling metabolic pathways and cellular communication between cells, tissues, and organs. Because of this, there is a lot of interest in the study of glycosylation and *N*-glycans as biomarkers for a number of diseases [1]. Thus, the reproducible release and identification of *N*-glycans from tissue are important criteria for biomarker discovery. The aim of this study is to explore a general out-look on *N*-glycans reproducibility in fibroblast using matrix assisted laser desorption ionization time-of-flight (MALDI/TOF) mass spectrometry.

Methodology

Two different batches (batch 1 and 2) of the human fibroblast cell lines were grown to confluence subjected to FANGS protocol [2] and permethylated before mass spectrometry analysis. Sample from these two batches (Batch 1: A1, B1 and C1; Batch 2: A2, B2 and C2) were processed on different days and the released permethylated glycans analysed on a different day using MALDI/TOF. Triplicate spots from each sample were spotted onto a MALDI plate and each spot analysed three times. The resulting [M+Na]$^+$ glycan intensities from the spectra corresponding to the same sample were averaged and used to prepare bar charts.

Results and Discussion

Results showed that there are no significant differences between the different spots of the same sample in all samples (p>0.05). This finding is similar to the recently published paper [3], in which the plasma *N*-glycan analysis of control samples was used to evaluate the reproducibility of sample processing. Reproducibility of sample processing on different days showed a few sialylated glycans missing in A2 and B2 compared to sample A1 and B1. This is possibly due to the low levels of the collected sialylated glycans that can be lost during sample processing or possibly due to biological variation during cell growth. There are differences in the signal intensities of the Hex5HexNAc2 and NeuAcFucHex5HexNAc4 species between the two batches. Most of the signal intensities are similar in the two batches prepared from the C cells.

Conclusion

In conclusion, this technique of *N*-glycan identification from fibroblast using mass spectrometry can be applied in investigations using human fibroblasts. Further features have to be improved such as to reduce the variability of *N*-glycans obtained during sample processing or cell growth.

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P-22

Spray-dried andrographolide nanoparticles for the treatment of Alzheimer's disease: Preparation and cytotoxicity evaluation

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Andrographolide is a major bioactive constituent found in *Andrographis paniculate* (Hempedu bumi in Malay). It has been reported to possess a promising neuroprotective effect that has the potential for the treatment of Alzheimer's disease[1]. However, the penetration of andrographolide into the central nervous system via the blood-brain barrier is one of the main obstacles for the treatment of Alzheimer's disease. Oral administration of *A. paniculata* extract has been reported to limit the reach of andrographolide to the brain owing to high plasma protein binding (55%)[2-3]. Andrographolide also readily degraded in acidic and alkaline environments of the gastrointestinal tract. Hence, it is hypothesised that if andrographolide in the nanoparticle form is delivered to the brain, the poor bioavailability limitations could be overcome. The current study aimed to produce spray-dried andrographolide nanoparticles and investigate their cytotoxicity in vitro using a human neuroblastoma cell line.

Methodology

Andrographolide nanoparticles were produced using a nano-spray dryer (Buchi Nano Spray Dryer B-90 HP). The particle size was measured using laser diffraction technology and electron microscopy was used to observe the morphology of the particles. The cytotoxicity of the nanoparticles was tested on SH-SY5Y neuroblastoma cells. The nanoparticle suspension at various concentrations was added into the cells in two-fold dilutions and incubated for 48 hrs. 0.05% resazurin was then added and further incubated for 4 hrs. Fluorescence readings were taken at a wavelength of 590nm following excitation at 544nm using a microplate reader. The half-maximal inhibitory concentration ($IC_{}$) was determined by comparing the inhibition to the untreated cells. In addition, lactate dehydrogenase (LDH) assay, nitrite oxide (NO) level and reactive oxygen species (ROS) level were determined using the supernatant of the cells following the assay kit guidelines. An andrographolide solution was included as a control. Three independent experiments were performed in triplicate.

Results and Discussion

The spray-dried andrographolide nanoparticles were spherical with a rough surface with a hydrodynamic diameter of 724 ± 39 nm with a polydispersity index of 0.3. The spray-dried nanoparticles showed lower toxicity (IC50 of 15.22 ± 0.61 µg/ml) as compared to the solubilised andrographolide control (IC50 of 10.82 ± 0.61 µg/ml). As anticipated, cells treated with solubilised andrographolide showed a higher amount of LDH release compared to the formulated nanoparticles, indicating that the formulation slightly reduced the toxicity of the andrographolide. The nitrate level in the supernatant did not increase dramatically compared to the untreated control, indicating that the cellular death did not involve the release of NO level in the cells. Similarly, the cells did not show any differences in ROS between the treated and untreated cells, suggesting that cellular death might not be induced through the ROS pathway.

Conclusion

These findings warrant further in vivo studies to establish the efficacy of the spray-dried andrographolide nanoparticles for the treatment of Alzheimer disease.

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P-23

Cross-reactivity between Leptospira Serovars in microscopic agglutination test for Leptospirosis

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Leptospirosis is a zoonotic disease resulting in high morbidity and mortality in Malaysia and other endemic countries. Microscopic agglutination test (MAT) is the gold standard method in diagnostic leptospira armamentarium. MAT is a laborious serological test performed by observing agglutination reaction of patient sera when incubated against a panel of live Leptospira serovars. This study aimed to analyze degree of cross-reactivity of sera for MAT conducted in Institute for Medical Research (IMR).

Methodology

The study included all sera for MAT in 2019. MAT was performed using an extended WHO panel comprising of 20 serovar and 4 strain of *Leptospira species.* A titer of more than or equal to 1:400 is considered as seropositive. Data analysis for serological cross reactivity was performed using Microsoft Excel for descriptive statistic.

Results and Discussion

96 sera (2.4%) out of 3965 sera were tested positive for MAT. Single seropositivity observed in 40.6% of the sera and the remaining demonstrated cross-reactivity ranging from 2 to 9 serovars (average of 3 serovars). High degree of cross reactivity observed may be a unique interplay between acute febrile illness (AFI) sera reacted to an extended MAT panel. Single serovar seropositivity involving serovar Fugis, Whitcombi and Lai Langkawi, *L. meyeri, L. borgpetersenii* serovar Batavae, may reflect a convalescent sera reacting to specific infecting serovar. Our finding is in parallel with other studies that support optimization of extended MAT panel to suit locally dynamic circulating serovar. Limited number paired sera in this study hinder the optimal determination of important infecting serovars.

Conclusion

Extended MAT panel tailored for local endemic serovar display high degree of cross-reactivity in AFI sera that serve as a more sensitive tool for diagnosis of leptospirosis.

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P-24

Susceptibility of human lung-derived cell lines MRC-5 and A549 to Malaysian SARS-CoV-2 isolate

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background
Severe Acute Respiratory Disease 2 (SARS-CoV-2) has been identified as the causal factor to the recent COVID-19 pandemic (MacKenzie and Smith, 2020). One of the capacities required to study this virus is to isolate and propagate them according to the requirement of the studies. To date, majority studies utilized monkey-derived Vero cells to rapidly propagate the virus. However, due to its highly susceptibility to SARS-CoV-2 infection and limited capacity to metabolize drugs, this cell lines are not suitable for pathophysiology and anti-viral research. Vero cells are not preferred for investigation of pathological mechanism of host cell's response to virus infection because they are not derived from human lung tissue. Furthermore, they are difficult to be used in the study to assess cytopathic effects as they do not express type 1 interferon genes (Konishi *et al.*, 2022). In this study, we adapted the SARS-CoV-2 isolates into selected human lung-derived cell lines, i.e., MRC-5 and A549, to investigate the capacity of these cell lines as the platform for the characterization of SARS-CoV-2 isolated in Malaysia. MRC-5 has already been identified to be highly susceptible to the infection of various human coronaviruses, including HCoV-OC43, HCoV-229E and Middle East respiratory syndrome coronavirus (MERS-CoV) (Shirato, Kawase and Matsuyama, 2013). Meanwhile, A549 was selected due to its origin of lung derived.

Methodology: To determine the growth profile of SARS-CoV-2 in human-derived cell lines, a local SARS-CoV-2 clinical isolate obtained from the IMR archive was first adapted into Vero E6, thus resulted in the generation of passage 1 (P1). These P1 isolate was used to study the growth profile of the virus in MRC-5 and A549 cell lines. P1 isolate was subjected to Whole Genome Sequencing (WGS) to identify the genomic sequence of the isolate. Replication kinetics of these isolates in MRC-5 and A459 were evaluated based on the formation of cytopathic effect (CPE), Cq value, plaque forming unit (pfu) as well as observation by electron microscope (EM). Whole Genome Sequencing (WGS) was repeated on the propagated SARS-CoV-2 passages to examine the genomic stability.

Results and Discussion: There is no formation of CPE observed after inoculation of SARS-CoV-2 into MRC-5 and A549 cells after 7 days of culture. In parallel, the qRT-PCR results suggested that the virus did not multiply well in these cells. Plaque assay and EM images supported these findings further. In term of genome stability, WGS data revealed several genetic polymorphisms in the genome of the virus adapted in MRC-5 and A549 cells.

Conclusion: MRC-5 and A549 are not susceptible to SARS-CoV-2 infection.

Keyword: MRC-5, A549, SARS-CoV-2, COVID-19

P-25

Elucidating the microproteome uniquely expressed in cell lines derived from multiple stages of colorectal cancer.

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

As non-canonical proteins, microproteins (such as PIGBOS) are increasingly implicated in various cancer hallmarks, including colorectal cancer (CRC), the area of our study which is ranked 3rd globally in all cancer-related mortality [1].

Microproteins (≤100 amino acids) are encoded by short open reading frames (sORFs, ≤300 bases). Traditionally dismissed by genome annotation, the coding potential of sORFs were supported by bioinformatics prediction and RIBO-Seq. A recent reanalysis of RIBOseq datasets identified 7,264 highly confident sORF sequences [2].

Notwithstanding, existing microprotein databases remain haphazard; hence consolidation is required to improve mass spectrometry-based microprotein identification. Furthermore machine learning-based protein structure prediction such as AlphaFold has gained high confidence, and should therefore be advantageous for less characterized microproteins [3].

We hypothesize that differential expression of sORFs is involved in progression of CRC. Therefore, the main objective of this work to elucidate the microproteome from cell lines derived from multiple stages of CRC.

Methodology

1. Cell culture of cell lines derived from multiple stages of CRC (COLO205, COLO320DM, SW48, SW480, SW1116) and normal control (CCD841CoN).
2. Microprotein extraction and enrichment via acid precipitation and 30kDa, 10kDa MWCO filters.
3. Microprotein expression and phosphorylation profiles characterized through LC-MS/MS.
4. Consolidation of microprotein databases (OpenProt, SmProt, sORFs.org) using MATLAB (R2022a).
5. Structural proteomics: using UniProt-matched Mudge et al (2022) dataset against AlphaFoldDB.

Results and Discussion

Acetic acid concentration was optimized to 0.25% as it reduces sample complexity efficiently. This was visualized on Coomassie-stained SDS-PAGE gel. MWCO filters enrich the microproteins by filtering for microproteins through its respective membrane pores. Physicochemical properties of microproteins show most microproteins from existing databases are 2 to 8 kDa, 10 to 40 amino acids. Isoelectric points peak at pH 7.5 and 8.5, at normal intracellular pH. 108 UniProt-matched sequences in Mudge et al. dataset were searched against AlphaFold DB, and their pLDDT scores recorded for confidence evaluation. Only 5 sequences had pLDDT scores of > 90.

Conclusion

Microproteins represent an exciting landscape in cancer proteomics. We optimized microprotein extraction, computationally estimated the physicochemical distribution of microproteins and used AlphaFold to predict microprotein structure.

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P-26

Autoantibodies profile of Glioblastoma patients in Malaysia: A preliminary study

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background
Glioblastoma (GB) is a very aggressive and highly malignant subtype of glioma. Gliomas constitutes around 2.4% of all cancer deaths despite a 1.6% incidence recorded in Malaysia in 2020. Difficult detection of GB has been a main concern in treating this disease. Additionally, gliomas are considered as immunologically "cold", making it more difficult to target. Among the many ways to detect cancer, liquid biopsies have garnered considerable attention for being minimally invasive. Autoantibodies, which is often found in the blood may serve as a potential serum biomarker, possibly allowing a less invasive, earlier identification of GB. This study aims to perform autoantibody discovery via Sengenics Immunome protein microarray against sera of higher-grade GB patients from Hospital Canselor Tuanku Muhriz, UKM.

Methodology
Ten GB patients' and ten age-matched, sex-matched healthy controls' sera were assayed using the Sengenics i-Ome Protein Array to screen for autoantibodies. Following that, Loess normalization and fold change analysis with cut-off value of 1.5 were conducted to determine for differentially expressed autoantibodies. STRING analysis was also run on differentially expressed autoantibodies to identify co-expression. Receiver operating characteristics (ROC) curve analysis of the selected autoantibodies was also performed to determine the discriminatory potential as biomarkers.

Results and Discussion
23 autoantibodies were found to be differentially expressed in GB patients' sera compared to healthy controls. Upregulated autoantibodies include anti-HSP90AA1, HSPE1 and ALDOA which shows co-expression. Among the selected autoantibodies, ALDOA, KRT19, ACAT2 and HSP90AA1 had among the highest AUC values.

Conclusion
This study shows a potential usage of autoantibodies as serum biomarkers for GB among the Malaysian populace

P-27

Effects of kaempferol on cell morphology, migration and microtubule functions in colorectal cancer cell HT-29

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Kaempferol, a naturally-occurring flavonoid, exhibits similar structure to estrogen and exerts estrogen-like activity. Extensive studies have been done to investigate its anticancer properties against various cancers [1,2]. Here we report effects of kaempferol on cellular activities that are closely regulated by microtubules. We also reveal our finding on expression of main microtubules components following kaempferol treatment in colorectal cancer cell line HT-29.

Methodology

We carried out cell viability assay to examine whether kaempferol inhibits HT-29 cell growth by estimating viable cell numbers. Next, we performed cell spreading and scratch wound assay to investigate the effects of kaempferol on HT-29 cell morphology and migration activity. To obtain a clearer understanding of how kaempferol causes alterations in microtubule network and cell motility, we performed whole cell microtubule analysis by flow cytometry.

Results and Discussion

Kaempferol decreased viable HT-29 cell numbers in a dose-dependent manner with almost 66% decrease in the viable cell number within 24 hours after the addition of 240 µM kaempferol. During cell spreading, HT-29 cells treated with IC_{50} of kaempferol (143 µM) were found to be significantly reduced in cell area and increased circularity. This suggests kaempferol inhibits cell spreading and promotes a more circular-shaped cells. Further investigation on microtubule organization revealed that kaempferol suppressed normal microtubule spreading in HT-29 cells, consistent with reduction in the cell area. Kaempferol-treated cells were having intense anti-tubulin staining around the cell edge, almost identical to the effects seen in taxol-treated HT-29 cells. Taxol treatment resulted in a more intense anti-tubulin staining, in agreement with its well-known role in promoting microtubule stabilization. More detailed microtubule analysis revealed that expression of α-tubulin and β-tubulin in kaempferol-treated cells was significantly lesser than untreated cells. Given the important roles of microtubule cytoskeleton in controlling cellular shape and cell motility, altered cell morphology and inhibition of cell migration found in kaempferol-treated HT-29 cells could be attributed to perturbation in microtubule functions.

Conclusion

Collectively, our findings show that kaempferol leads to alteration in cell spreading and hinders cell migration of cancer cells. These changes could be associated with decrease expression of α-tubulin and β-tubulin. Further work will be required to elucidate underlying mechanisms of kaempferol effects on microtubule dynamics.

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P-28

Genomic Characterization of the First Case of Extensively Drug-Resistant, Travel-Related *Salmonella enterica* Serovar Typhi in Malaysia

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Typhoid fever, caused by *Salmonella enterica* serovar Typhi (*S*. Typhi) remains a serious global health concern. The effectiveness of antimicrobial therapy has been threatened by the emergence of Extensively Drug-resistant (XDR) *S*. Typhi exhibiting resistance to the first line and second-line antibiotic options. The aim of this study was to characterize an XDR *S*. Typhi isolate from a foreign worker with a history of recent travelling to Pakistan.

Methodology

Whole-genome sequencing (WGS) was performed on the isolate with Illumina MiSeq and data was analysed using Resfinder tool to detect the presence of genes associated with antimicrobial resistant profile. Pathogenwatch was used to characterize the genomic clonality in relation to the XDR outbreak of *S*. Typhi.

Results and Discussion

WGS analysis detected 10 antimicrobial resistance genes including aminoglycoside resistant gene (*aph(3'')-Ib, aph(6)-Id*, *aac(6')-Iaa*), trimethoprim resistant gene (*dfrA7*), sulphonamide resistant gene (*sul1, sul2*), beta-lactam resistant gene (*blaCTX-M-15*, *blaTEM-1B*), phenicol resistant gene (*catA1*) and quinolone resistant gene (*qnrS*). Similar gene profiles were seen for the XDR *S*. Typhi from Pakistan. A core genome phylogeny tree constructed using selected genomes from Pathogenwatch collection clustered Malaysia XDR *S*. Typhi isolate with XDR isolates from Pakistan, United Kingdom and Italy.

Conclusion

This first case of typhoid fever due to XDR *S*. Typhi detected in Malaysia highlights the need to be vigilant for future cases. Genomic characterization of *S*. Typhi using WGS as a tool in surveillance program is important to manage outbreak investigation by studying the pathogen population structure, identification and tracking of potential source both local as well as global.

P-29

Suggestive SNPs of Dengue Pathogenesis Towards Severity in Single-Centre Case-Control Study

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Dengue has persistently served as a major public health concern especially in the tropical and sub-tropical areas. In Malaysia itself, dengue is a hyperendemic disease and the incidence rate continued to increase exponentially since the past ten years [1]. Manifestations of the dengue symptoms can range from asymptomatic, mild or severe. Dengue fever that progresses to severe dengue, either dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS), are usually life-threatening. The mechanisms of how this disease progresses to severity is incompletely understood. Early identification of cases that are likely to progress to worse health conditions remains challenging. Multiples studies have highlighted the role of host genetics as a crucial factor towards severity. Thus, this study aimed to identify single nucleotide polymorphism(s) that predisposes dengue patients to severe disease outcome among the Malaysian dengue cohort.

Methodology

This is a case-control study that includes a total of 188 dengue patients; 86 with dengue fever and 102 with severe dengue. Patients were recruited from Hospital Kuala Lumpur from year 2018 till 2020. Classification into dengue fever (DF) or severe dengue (SD) were made based on the WHO 2009 classification. DNA extracted from whole blood samples, were genotyped using Infinium™ Asian Screening Array. Single variant association analysis was performed on 617,245 single nucleotide polymorphisms (SNPs). Both sample- and SNP-levels data were subjected to stringent quality control checking for high quality genotype data.

Results and Discussion

We found genetic variations that are suggestively associated with dengue severity, namely rs9872672 and rs148681490. These SNPs reached the p-values of ~106, with their odds ratio of 4.574 (rs9872672) and 0.092 (rs148681490) were mapped to chromosome 3p14.2 and 11p11.2, respectively. Our findings at position 3p14.2 indicate a change of allele from C to T that occurred at a frequency of 0.143 globally [2]. Suggestive allele at this position identified an intronic region of the synaptoporin (SYNPR) gene that is predicted to be an integral component of synaptic vesicle membrane, whose probable function is linked with vesicular channel protein. Likewise, suggestive SNP found at chromosome 11 showed a change from A to G. The alleles at 11p11.2 encode for tetratricopeptide repeat domain 17 (TTC17) protein that are involved in the actin filament polymerization and cilium organization.

Conclusion

In conclusion, the findings from this study support the notion that host genetic factor contributes to the disease pathogenicity of dengue severity. Although the potential roles of the suggestive SNPs are still uncertain, further validation is highly warranted.

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P-30

Probiotics Alter Biofilm Formation of Periodontal Pathogen, *Porphyromonas gingivalis* virulence-associated genes

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Probiotics, a group of beneficial live microorganisms are suggested as an adjunct therapy to control the colonization of periodontal pathogens. Periodontal pathogens, especially *Porphyromonas gingivalis*, can form biofilm with other pathogens in the oral cavity and exhibit virulent factors that are detrimental to human gum health [1]. This study was carried out to assess the anti-biofilm activity of probiotics isolated from local fermented food and to investigate the underlying molecular activity behind the anti-biofilm activity.

Methodology

Cell-free supernatant (CFS) was prepared by centrifugation and filter sterilisation of the overnight culture of *Lactobacillus plantarum* FT 5. The probiotics culture was isolated from local fermented food and is kept as a private collection [2]. Anti-biofilm assay was performed against *P. gingivalis* where the optical density of *P. gingivalis* biofilm stained with crystal violet solution in the treated and untreated groups was measured. Sterile MRS and 0.2% chlorhexidine acted as negative and positive control respectively. Then, RT-qPCR procedure involving *fimA, mfa1, kgp,* and *rgp* genes was carried out on the treated and untreated *P. gingivalis*. Additionally, the 16S rRNA gene was employed as the normalization gene.

Results and Discussion

The anti-biofilm assay showed a significant reduction of *P. gingivalis* biofilm with a 90.5% (p-value < 0.05) reduction at the highest concentration of *L. plantarum* FT 5 CFS. The RT-qPCR revealed that biofilm reduction might be related to the downregulation of two biofilm-related genes, *fimA* and *mfa1*. Interestingly, *P. gingivalis* treated with *L. plantarum* FT 5 CFS showed upregulation of *kgp* and *rgp* genes. The results implicate that the inhibition of *P. gingivalis* biofilm formation by *L. plantarum* FT 5 CFS are due to the downregulation of fimbriae-related genes, *fimA* and *mfa1* genes. Aside from being vital virulent genes, *kgp* and *rgp* also contribute to the maturation of fimbrial protein [3]. Thus, the upregulation of *kgp* and *rgp* in *P. gingivalis* is probably necessary to overcome the downregulation of fimbriae-related genes.

Conclusion

In conclusion, *L. plantarum* FT 5 isolated from fermented foods can reduce the biofilm formation by *P. gingivalis* and the underlying mechanism might be related to biofilm and virulent-related genes regulation.

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P-31

Survival analysis of TNBC patients: A retrospective study from Hospital Universiti Sains Malaysia (HUSM)

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Triple-negative breast cancer (TNBC), characterised by the absence of ER/PR expression with lack of HER2 overexpression, has drawn more attention both clinically and experimentally due to its aggressive biological characteristics and lack of effective treatment methods. There are very limited population-based breast cancer survival and prognostic factors studies for Malaysian population, particularly that focus on TNBC patients. Hence, the purpose of this study was to determine the survival of TNBC patients at HUSM and to identify potential prognostic factors for their overall survival (OS).

Methodology

This retrospective study was conducted using primary data of 50 women diagnosed with malignant breast cancer at HUSM between January 2017 and December 2019 (USM/JEPeM/18120775). The patients were divided into two groups: TNBC and non-TNBC. Overall survival curves were estimated using the Kaplan-Meier method and a log-rank test was used to compare the survival functions among the two groups. Simple Cox's proportional hazard regression model was employed to identify the important prognostic factors of death.

Results and Discussion

The OS for TNBC was significantly lower than that for non- TNBC, at 45.0% vs 83.3% (p = 0.003). The mean survival time for TNBC was 38.7 months compared to 73.4 months for non- TNBC. Molecular subtype was a significant prognostic factor for malignant breast cancer patients whereby diagnosis with non- TNBC reduces the death hazard by a factor of 0.23 or 77.0% (95% CI 0.079–0.664; p = 0.007). Only staging status was statistically significant for OS of TNBC patients. The OS for early-stage TNBC was significantly higher than that for advanced-stage TNBC, at 66.7% vs 27.3% (p = 0.021). The median survival time was 57.0 months (95% CI 50.5–63.5 months) for early-stage TNBC vs 24.0 months (95% CI 19.7–28.3 months) for advanced stage TNBC patients. Advanced stage TNBC patients had 5.3 times increased hazard to death compared to early-stage TNBC patients (95% CI 1.096–25.647; p = 0.038). Since no other variables showed statistical significance on OS of TNBC or non-TNBC patients, multivariate analysis was not conducted. The OS and important prognostic factors reported in this study are in accordance with other studies [1,2] that have recorded worse OS for TNBC patients diagnosed at advanced stage. Nonetheless, other factors including patient's age, surgical treatment methods and family history of malignancy have been shown to be important prognostic factors for breast cancer [1,2], which was not the case for this study.

Conclusion

At HUSM, TNBC patients, particularly those diagnosed at advanced stage, have worse prognosis compared to non-TNBC patients. Future studies with larger sample size and comparative studies with other hospitals in Malaysia are necessary to enable the identification of more potential prognostic factors for TNBC and non-TNBC patients, with the aim to improve breast cancer management in this region.

Acknowledgment

This work was funded by Agilent Technologies (304/CIPPM/6150161/A146).

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P-32

The shark VNAR single-domain antibody as potential binder for SARS-CoV-2

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as an emergent zoonotic virus since December 2019, has caused coronavirus disease 2019 (COVID-19) as the first coronavirus pandemic in history [1]. Neutralizing antibodies against SARS-CoV-2 is useful, however with the large size (~150 kilodaltons (kDa)), complex hetero-tetrameric structure and susceptibility to extreme ambient temperature for the conventional antibody may limit their performance in therapeutic and diagnostic applications [2]. Antibody surface display technology allows the exploration of antibody fragments from other organisms, including VNAR (variable domain of immunoglobulin new antigen receptor (IgNAR)) from shark with better thermostability [3]. The advantages of shark VNAR single-domain antibody are due to possessing smaller size (~12 kDa) and simpler homodimer structure, with its antigen-binding capability comparable to conventional antibody [2, 3]. The aim of this study is to evaluate the potential of shark VNAR as binders towards SARS-CoV-2 as target antigen.

Methodology

A proprietary phage-displayed semi-synthetic shark VNAR library was subjected to 3 rounds of biopanning. The commercial wild-type SARS-CoV-2 receptor binding domain (RBD) diluted in various concentration was used as target antigen in this study. The biopanning steps per round involved immobilizing antigen on immunotube, blocking immunotube with skimmed milk, incubating phages from antibody library in immunotube, washing off unbound phages while eluting bound phages, followed by the re-propagation of specific phage clones in *Escherichia coli* TG1. The phage particles harvested from culture supernatant were applied in the next round of biopanning. Polyclonal phage pool and monoclonal phage clones from each biopanning round were analysed by agarose gel electrophoresis, to detect the presence of VNAR insert.

Results and Discussion

Throughout rounds of biopanning, the amplification of phage clones was observed in Round 3, with 11-fold enrichment as compared to Round 1. Based on agarose gel electrophoresis result, the presence of VNAR insert increased from 60% (Round 1) to 100% (Round 3), indicated that SARS-CoV-2 RBD-targeted phage clones have been enriched throughout subsequent rounds of biopanning. The identification and functional characterization of potential clones are in process.

Conclusion

Shark VNAR has been proven as potential binders targeting towards SARS-CoV-2. Thus, further biological characterization is required to determine the specificity and sensitivity of the potential binders.

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P-33

Selecting antibacterial aptamers against an essential outer membrane protein in *Pseudomonas aeruginosa* via aptamer repurposing approach

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Antimicrobial resistance is one of the major threats to global health, resulting in an increasing number of people suffering from severe illness or dying due to infections that were once easily curable with antibiotics. *Pseudomonas aeruginosa* represents one of the most concerning pathogens involved in antibiotic resistance where the World Health Organisation has classified this gram-negative bacterium as an ESKAPE organism, and it is also listed in the Priority 1: Critical list. Hence, in this project, we opted for a novel intervention by using aptamers to inhibit the growth of *Pseudomonas aeruginosa,* which can be cost-effective and less laborious. Besides, antimicrobial agents targeting the outer membrane protein (OMP) in Gram-negative bacteria can also be effective at killing or inhibiting bacterial growth as these proteins play an important role in bacteria survival as well as being exposed to the surface, which facilitates direct binding. Aptamer repurposing also reduces the cost and length of new drug development.

Methodology

Unmodified functional DNA aptamers from the Aptagen database were docked with the essential OMP using various docking tools such as HADDOCK 2.4 and HDOCK web server to determine the binding site and binding score of the various aptamers. Aptamers that bind to the active site with a good binding score were synthesised and folded into their 3D structures. *P. aeruginosa* cells were incubated with the aptamers overnight and the inhibitory effects on the bacterial growth curve were investigated.

Results and Discussion

Apt31 had the best HADDOCK score, and was found to bind near the active site of the OMP. Interestingly, this aptamer also exhibited significant antibacterial activity from the early stationary growth phase onwards. The addition of the aptamer after the late log phase also exhibited a similar effect. Besides, the antibacterial activity of apt31 was also dose-dependent. Hence, we can deduce that apt31 binds to the active site region and blocks the OMP activity. The major role of this OMP is to fold and insert β-barrel proteins into the outer membrane layer. As apt31 hinders the OMP activity, we postulate that the bacteria cells eventually die due to a lack of OMPs, which are essential for survival and virulence.

Conclusion

We show that the apt31, an aptamer that binds to an antitumor, is capable of binding to OMP in *P.aeruginosa.* The aptamer-OMP complex exhibits a significant antibacterial effect in a dose-dependent manner. Future experiments will include the expression of the recombinant OMP and determination of the dissociation constant (K) of the aptamer-OMP complex.

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Philadelphia Chromosome and FISH- negative, cryptic BCR/ABL1 positive Acute Myeloid Leukaemia: A Diagnostic Dilemma

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Acute myeloid leukaemia (AML) with BCR/ABL1 is a provisional entity classified in the most recent WHO classification of AML with recurrent genetic abnormalities. This entity can pose diagnostic dilemmas as it can be confused with blastic phase of chronic myeloid leukamia (CML) or mixed phenotype acute leukaemia (MPAL) with BCR-ABL1. We present here a patient with Philadelphia chromosome negative AML and BCR/ABL1 fusion detected by PCR.

Case Report

A 65-year-old man presented with constitutional symptoms for three weeks. Physical examination revealed a thin man with hepatomegaly and no appreciable splenomegaly. The full blood picture showed bicytopenia with leucoytosis and 58% blasts cells. The bone marrow aspirate was hypercellular, with 48% MPO-positive blasts cells and heterogenous background of granulocytic cells and abundant eosinophils. Cytogenetic analysis showed 46 XY, del (7q) (q22q23) and no BCR/ABL1 was detected using FISH probe BCR/ABL1. However, using multiplex ARMS PCR, both b3a2 and b2a2 fusion genes were detected. This patient is currently on treatment with standard chemotherapy regime and Nilotinib.

Discussion

AML with BCR/ABL1 usually have equal distribution of p190 and p210 transcripts and clinically, is associated with no splenomegaly or basophilia and lower bone marrow cellularity. AML with BCR/ABL1 show aberrations that are normally seen in lymphoid pathologies such as deletions of IKZF1 and/or CDKN2A/B genes. IGH and TCR can also show cryptic deletions. These events along with the findings of different rearrangement of BCR/ABL1 reflects the genetic heterogeneity of this subtype. In this patient, the presence of two PCR product both representing major transcript (p210), raises suspicion that there are two different breakpoints. The translocation of BCR exon 13 with ABL1 exon2 (leading to b2a2 transcript) and BCR exon 14 with ABL1 exon2 (leading to b3a2 transcript) would normally be seen on FISH.[2] Factors that could contribute to non detection on FISH include these novel breakpoints may not be covered by the standard BCR/ABL1 FISH probes and cryptic rearrangement involving multiple chromosomes. Masked Ph positivity in this patient may be due to insertion of ABL1 into BCR region or the translocation of 9;22 is followed by another translocation of both products leading to fairly normal chromosome morphology.[3]

Conclusion

Philadelphia chromosome negative BCR/ABL1 positive AML is extremely rare. Moving forward, the utilisation of FISH mapping using Bacterial Artificial Chromosomes (BAC) probes, which can cover both minor and major breakpoint, Whole Chromosomes Painting and direct sequencing can offer new insights into the formation of masked Ph chromosomes.

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Production of recombinant shark V_{NAR} single domain antibody specific against DENV Type 2 NS1

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Dengue virus (DENV)) infection is one of the main global health issues leading to high morbidity and death rate. The accuracy of early diagnosis is the key for effective treatment. However, early diagnosis for DENV infection is a great challenge due to the similar onset symptoms of Dengue with other flaviviruses, concurrent infection and cross-reactivity among dengue serotypes and other flaviviruses [1]. Besides, the limitations of conventional monoclonal antibodies (mAbs) also affect the efficiency of diagnostic kits [2]. Alternatively, the variable new antigen receptor (V_{NAR}) found in shark, has recently been identified as a promising diagnostic tool due to its excellent thermostability and capability to target on cryptic epitopes [3]. In this study, a potential clone that recognizing DENV Type 2 NS1 has been isolated and expressed. This recombinant protein was determined to be soluble and potentially developed as a novel reagent for DENV immunodiagnostic platform.

Methodology

A semi-synthetic library of shark V_{NAR} was used for antibody selection. Total of 3 rounds of biopanning was undertaken in respect to isolate potential binders which can specifically recognize DENV Type 2 NS1. The eluted phage from each round of biopanning were subject to polyclonal and monoclonal phage ELISA. After verified through DNA sequencing, the selected clone was expressed using bacteria expression system, followed by immobilized metal affinity chromatography (IMAC) purification process. The biological functions of recombinant V_{NAR} will then be characterized, including thermostability, specificity and sensitivity towards DENV Type 2 NS1.

Results and Discussion

For monoclonal phage ELISA, G3, Z3 and Z8 clones were identified to possess good affinity towards NS1 and lower cross reaction towards BSA. Thus, Z8 clone was selected to express as a 12 kDa protein with some optimization during protein expression. To verify target protein, the recombinant antibody reacted with anti-His6 antibody was performed using Western Blot. The functional assays of recombinant protein Z8 are currently underway.

Conclusion

Z8 clone isolated from biopanning has been identified as a potent binder. The recombinant anti-NS1 V_{NAR} Z8 antibody was successfully produced in a bacteria expression system. This new binder can be developed as new reagent for DENV immunodiagnostic platform.

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P-36

Vasoprotective Effects of Red Yeast Rice Supplementation in Spontaneously Hypertensive Rats

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background
Hypertension is associated with endothelial dysfunction, a condition best described as an impaired endothelium-dependent relaxation due to reduced nitric oxide (NO)-cyclic GMP signaling. Red yeast rice (RYR) is a traditional folk medicine and supplement produced from the fermentation of rice with *Monascus purpereus* mould. RYR is reported to have anti-hypertensive, anti-hypercholesterolemia and anti-atherosclerotic properties in both human and animal models. However, its vasoprotective effects have yet to be fully elucidated. This study aims to examine the vasoprotective effects of RYR supplementation on the vascular function in Spontaneously Hypertensive Rats (SHR) through oral supplementation.

Methodology
Male SHR of age 10-12 weeks old were grouped randomly and were administered with three different dosages (100, 300, 1000 mg/kg/day) of RYR respectively for 12 weeks via oral gavage. Blood pressure readings were obtained weekly from pre-warmed, restrained animals using the tail-cuff method. Wistar-Kyoto Rats (WKY) were used as strain control. At the end of the treatment period, the vascular reactivity of sectioned aortic rings was studied by organ bath. Both endothelium-dependent and independent relaxations were evaluated. The vascular cGMP levels were determined using aortic segments collected.

Results and Discussion
SHR treated with 100 mg/kg/day of RYR showed a significant decrease in systolic blood pressure (SBP). However, no significant changes were observed in the SBP of SHR treated with 300 and 1000 mg/kg/day of RYR. Conforming to the blood pressure observation, vascular reactivity studies also presented significant improvement in endothelium-dependent relaxations in SHR treated with 100 mg/kg/day of RYR, while SHR supplemented with 300 and 1000 mg/kg/day did not have significant improvements in endothelium-dependent relaxations. Together with improvement in vascular function, treatment with 100 mg/kg/day of RYR also had increased the cGMP levels of hypertensive animals. Indeed, the blood pressure lowering effects seen in SHR supplemented with 100 mg/kg/day can be partially contributed by its improvement in endothelial function.

Conclusion
In the current study, it is evident that 100 mg/kg/day of RYR attenuated the blood pressure of SHR. The decrease in SBP is associated with an improvement in endothelial relaxation of the treated animals

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A fatal case of cerebral malaria complicated with *Gemella bergeri* bacteremia: Role of *Plasmodium* mitochondrial *cox3* gene and MALDI-TOF Mass Spectrometry for species identification

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background
Here, we described a fatal case of a 37-year-old returning traveller from Burkina Faso, West Africa, who presented with an acute fitting episode later diagnosed as cerebral malaria induced by *Plasmodium falciparum* based on microscopic examination and *Plasmodium* mitochondrial cytochrome c oxidase III (*cox3*) gene PCR target. Unfortunately, the patient passed away due to severe malaria with multiorgan failure complicated with *Gemella bergeri* bacteremia. *G. bergeri* was identified using MALDI-TOF mass spectrometry.

Methodology
Preliminary evaluation of blood film malaria parasite showed coinfection of *P. falciparum* and *Plasmodium malariae*. Further PCR-based detection analysis using the *Plasmodium* mitochondrial *cox3* gene was used to identify the malaria species. The patient's blood culture revealed gram-positive cocci in clusters, and further identification using MALDI-TOF Mass Spectrometry was done.

Results and Discussion
The patient blood sample was positive for *P. falciparum* based on analysis using a PCR assay targeting the *cox3* gene. Further, analysis done at Makmal Kesihatan Awam Kebangsaan confirmed *P. falciparum* infection. His blood culture revealed gram-positive cocci in clusters, which was later identified as *G. bergeri* using MALDI-TOF mass spectrometry. Despite the initiation of intravenous artesunate and subsequent parasite count responding and showing parasite clearance, the complication is detrimental. As shown in our case, cerebral malaria induced by *P. falciparum* causes coma, long-term neurological consequences and death. The patient also had concomitant fatal *G. bergeri* bacteremia. Initial examination revealed that the patient had poor oral hygiene and a missing tooth which could be the source of *Gemella* infection, which is common in the oral cavity and a causative organism of septic shock.

Conclusion
This report is the first fatal case of cerebral malaria caused by *P. falciparum* coinfection with *G. bergeri* bacteremia. Therefore, it is critical to determine the exact aetiology for appropriate medical management. *Plasmodium* mitochondrial *cox3* gene and MALDI-TOF Mass Spectrometry are useful for species identification, as illustrated in this case.

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Association of *MTHFR* polymorphism in stroke risk and rehabilitation outcomes

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

There are 12.2 million new stroke cases annually and about 101 million people worldwide are living with stroke aftermath [1]. In Malaysia, stroke is the third leading cause of death. The rs1801133 single nucleotide polymorphism (SNP) in the methylenetetrahydrofolate reductase (*MTHFR*) gene has been linked to stroke pathogenesis in a recent meta-analysis [2]. To date, there is a lack of study addressing this SNP towards stroke risk and rehabilitation outcomes in the Malaysian population. Hence, this study aims to investigate the association of *MTHFR* rs1801133 SNP with the risk and rehabilitation outcomes in Malaysian stroke patients.

Methodology

The peripheral blood sample was collected from 251 age-matched individuals (113 stroke patients and 138 healthy controls without a stroke history) with written consent. The genomic DNA was extracted from these blood samples and the rs1801133 SNP was genotyped using a TaqMan® assay approach. The odds ratio and 95% confidence interval were calculated for risk association analysis. The stroke patients were subjected to a special-designed rehabilitation exercise and their pre- and post-exercise assessments including the Barthel index, Fugl-Meyer assessment-upper extremity, and Fugl-Meyer assessment-lower extremity were compared based on their genotypes.

Results and Discussion

This study showed that the presence of a T allele of the rs1801133 SNP had at least a 0.4-fold reduced risk of stroke. This suggests that the T allele of the rs1801133 SNP is protective against a stroke in the Malaysian population. Surprisingly, the finding of this study is in contrast with those reported in the meta-analyses [2,3]. Out of the 31 stroke patients who had completed their special-designed rehabilitation exercises, patients with a homozygous (C/C) genotype showed significant improvement in the post-rehabilitation assessments including the Barthel index, Fugl-Meyer assessment-upper extremity, and Fugl-Meyer assessment-lower extremity.

Conclusion

In conclusion, this study suggests that the *MTHFR* rs1801133 SNP is a potential biomarker for stroke risk and rehabilitation outcome predictions in Malaysian stroke patients. Data of this study could be useful for stroke management in the country.

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P-39

RNU6B and miR-16 as Stable Normalisation Control for Relative RT-PCR of Urinary microRNA in Patients with Colorectal Polyps

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Relative real-time polymerase chain reaction (RT-PCR) is an experimental technique widely used in quantifying the expression of a particular gene of interest. However, the reliability of gene expression data obtained through this technique is highly dependent on the stability of the housekeeping genes being used. Housekeeping genes act as a normaliser or endogenous reference in the relative quantification of a target gene and its expression may vary between different types and sources of samples. Studies on urinary microRNA as a potential disease biomarker are expanding, and the suitable housekeeping genes are yet to be confirmed. In this study, we assessed the stability of three commonly used references for microRNA i.e., hsa-miR-26b, hsa-miR-16, and RNU6B.

Methodology

This study was registered (NMRR-19-1188-47227), approved by the Medical Review and Ethics Committee (MREC) of the Ministry of Health Malaysia, and the Human Research Ethics Committee of Universiti Sains Malaysia (JEPeM, USM), and conducted following the Good Clinical Practice Guideline. The urine samples were collected from 24 patients (12 with colonic polyps and 12 without colonic polyps) scheduled for colonoscopy at Hospital Sultanah Bahiyah, Kedah, Malaysia. Colonic polyps' diagnosis was confirmed by colonoscopy and histopathological report. MicroRNA was extracted from the urine supernatant based on the acid guanidinium thiocyanate-phenol-chloroform-potassium acetate-lithium chloride method described by Zununi Vahed et al. (2016) with a slight modification. Reverse transcription and primer design for RT-PCR were performed following protocol by Balcells et al. (2011) and Busk (2014), respectively. We performed RT-PCR with cycling conditions of initial denaturation (95°C, 5 min) followed by 45 cycles of denaturation (95°C, 15 sec) and annealing (60°C, 30 sec). The analysis was extended with melting curve analysis (60°C to 95°C) to assess RT-PCR efficiency. The stability of Ct values was evaluated using three algorithms i.e., BestKeeper, DeltaCq, and NormFinder. T-test was used to analyse whether a significant difference in mean Ct values of hsa-miR-26b, hsa-miR-16, and RNU6B existed between those two groups of patients.

Results and Discussion

There is no significant difference in mean Ct values of RNU6B, hsa-miR-26b, and hsa-miR-16 between the group of patients with colonic polyps and without colonic polyps (P-value = 0.204, 95% CI, -2.56, 0.58). This suggests that these three microRNAs are suitable candidates for normalisation control for relative RT-PCR. Analysis with all three algorithms showed that hsa-miR-16 alone had the best stability among the three microRNAs tested, while the stability improved when miR-16 and RNU6B were combined.

Conclusion

The findings in this study could be used as a guide to other research applying the relative RT-PCR technique in measuring gene expression of microRNA in human urine samples.

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P-40

Vernonia amygdalina leaf extract and luteolin inhibits nasopharynx cancer cells and display synergism with cisplatin

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Nasopharyngeal cancer (NPC) is a cancer that develops from the epithelium of the nasopharynx. NPC is the fifth most common cancer among Malaysians. NPC is treated with radiotherapy and cytotoxic chemotherapeutic drugs, such as cisplatin and 5-fluorouracil. However, treatment-resistance, treatment of recurrent NPC and distant metastasis remain challenging; whilst NPC patients have poor survival. There is growing interest in natural products with therapeutic potential to circumvent toxicity associated with synthetic anti-cancer drugs and radiation. Epidemiological and dietary intervention studies showed that diet-derived phenolics, especially the flavonoids, can inhibit or reverse tumorigenesis. Luteolin (3',4',5,7-tetrahydroxyflavone) is a biological compound that belongs to the flavone group of flavonoids. Luteolin is found in celery, broccoli, carrots, apple skins, parsley and green peppers. *Vernonia amygdalina* belongs to the family Asteraceae; and is found primarily in Africa. Phytochemical constituents of *V. amygdalina* include saponins, triterpenoids, flavonoids, sesquiterpene lactones, terpenes, coumarins, phenolic acids, lignans, xanthones, and anthraquinones. The leaf contains abundance of flavonoids, including luteolin, the major bioactive component. This study evaluated the activities of standardized methanol extract of *V. amygdalina* and luteolin, against NPC cell lines used as models for NPC.

Methodology

Leaf powder of *V. amygdalina* was extracted with methanol, then the content of luteolin was determined using HPLC. Methanol extract of *V. amygdalina* and luteolin were screened for free radical-scavenging activity using DPPH assay. Methanol extract of *V. amygdalina* and luteolin were evaluated for inhibition of NPC cells in a concentration- and time-dependent manner using MTS assay. Cell growth kinetics was monitored dynamically using the xCELLigence real-time, impedance-based cell analyser. Morphological changes of cells were observed microscopically. Apoptosis was evaluated by flow cytometry and high content analysis. Effects of combining luteolin with cisplatin on NPC cells were studied.

Results and Discussion

Using HPLC analysis, the content of luteolin in methanol extract of *V. amygdalina* leaves was standardized to 12.10 µg/ml. DPPH free radical-scavenging activity of luteolin was approximately 95%. *V. amygdalina* standardized methanol extract and luteolin inhibited NPC cells in a concentration- and time-dependent manner. Morphological observation and cell growth kinetics were consistent with the MTS assay. NPC cells were clearly inhibited within 24 hours of treatment. Apoptosis was not observed in luteolin-treated NPC cells. The Combination Index of luteolin and cisplatin was < 1, indicating that simultaneous treatment of NPC cells with luteolin and cisplatin resulted in synergistic effects.

Conclusion

Combinations of luteolin and cisplatin produced synergistic effects. This synergism suggests the possibility of reducing the dosage of cisplatin required to treat NPC, with the addition of luteolin. In turn, this dosage reduction could lessen the risk of cisplatin-associated toxicity.

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A novel mutation of the *NOTCH3* Gene in Malaysian patient with Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL)

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Mutations in the *NOTCH3* gene are responsible for cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), an adult-onset hereditary angiopathy leading to ischemic episodes, vascular dementia and other neurologic deficits. The majority of *NOTCH3* mutations that have been reported so far are rigidly stereotyped, involving the gain or loss of a cysteine residue in a specific *NOTCH3* epidermal growth factor (EGF)-like repeats on chromosome 19.p13.12 [1,3].

Methodology

More than 100 blood samples of unrelated Malaysian patients with clinical/neuroimaging picture suggestive of CADASIL referred to our laboratory for molecular investigation. Genomic DNA was extracted from whole blood using Prepito DNA Blood250 Kit (PerkinElmer). Genetic diagnosis of CADASIL was performed by targeted PCR amplification of exons 3, 4, 5, 6, 11, 16 and 19 including exon-intron boundaries of *NOTCH3* gene followed by Sanger sequencing to detect causative mutations. Sequence analysis was performed using 3500 Genetic Analyzer (Applied Biosystems). To confirm the pathogenicity of new nucleotide variants, the identified sequence variants were assessed against several public databases and *in silico* prediction tools such as VarSome in accordance with ACMG guidelines.

Results and Discussion

Out of the 108 samples tested, 23 samples were found to harbour variants suggestive of CADASIL. Using PCR-directed sequence analysis, a total of seven mutations were identified. One of which was a novel missense mutation, C260S and six previously reported mutations, R110C, R141C, R182C, C194S, R544C and R1006C were listed in the Human Genome Mutation Database (HGMD). In this study, a 51-year-old female patient with novel missense mutation C260S is presented with sudden onset of left sided weakness and MRI brain scan findings suggestive of CADASIL. VarSome predicted C260S as pathogenic, located in a mutational hotspot and two different missense change determined to be pathogenic have been seen before. The nucleotide position was strongly conserved and the variant was not found in gnomAD, a population database for normal individuals. Furthermore, the pathogenicity score was 95% from multiple *in silico* predictors. This result is consistent with CADASIL diagnosis for this patient.

Conclusion

Careful assessments of genealogical, clinical, and neuroimaging data in patients with lacunar stroke can aid in the selection of patients with a high probability of finding mutations by genetic screening [2]. In our samples, 21% of Malaysian patients with 'clinically suspected' CADASIL received the definitive molecularly proven diagnosis while the remaining undiagnosed patients should be recommended for further testing of *NOTCH3* exons which were not included in this study, if clinical suspicion is high. In addition, the discovery of new mutation expands the genetic spectrum of *NOTCH3*-related diseases, which will contribute to further study of this disease in the future. Correct diagnosis is not only important for the management of the patient, but also crucial for genetic counseling and early diagnosis of at-risk family members.

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P-42

Screening of *Lactobacillus* species in stingless bee honey

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Stingless bee honey is a natural substance produced by stingless bee of genera *Meliponini*. Comparing to honeybee honey, stingless bee honey has significantly higher moisture content, water activity, ash content, while the pH and total soluble solid content are slightly lower. Other components such as proteins, amino acids, enzymes, organic acids, mineral elements, and vitamins are also found to be present in small amounts. Being rich in nutrients, the honey provides a very suitable environment for microflora growth. Microflora in honey includes yeasts, moulds, and bacteria with bacteria being the major microbes associated with stingless bee honey. Many studies had proven the presence of probiotic amongst the microflora, which includes *Lactobacillus, Lactococcus*, and *Bifidobacterium*. This founding had provided additional commercial value to stingless bee honey. Hence, the aim of this study is to screen the presence of *Lactobacillus spp.*; one of the most accepted probiotic, in stingless bee honey of *Heterotrigona itama* collected from a plantation in Mantin, Negeri Sembilan.

Methodology

Samples were obtained from July to November 2018 randomly from the same plantation area. The samples were cultivated in lactobacilli de Man, Rogosa, Sharpe (MRS) broth before subjected to isolation on MRS agar. This step was carried out 3 times to further isolate the potentially different species by differentiating them according to their colonies morphology. 5 colonies isolated from each month were subjected to DNA extraction using QiaAmp DNA kits. Identification of the colonies was done via polymerase chain reaction (PCR) using a set of primer pairs targeting the region of 16S rRNA; LF 5'-AGCAGTAGGGAATCTTCCA-3' and LR 5'-ATTACACCGCTACACATG-3', targeting amplicon sizing 430bp. PCR products were sent to 1st Base Laboratories for sequencing which was then followed by sequence alignment using BLAST on NCBI website.

Results and Discussion

Growth on MRS agar showed growth of a few species that could be differentiate by their colony morphologies. Colonies colour ranging from whitish to brownish yellow, while other characteristics observed were sliminess, size, and shape; where there was round and slightly oval shape. Most samples subjected to PCR had gave out clear single band sizing around 400bp during gel electrophoresis, while some other gave two to three, clear but faint bands which were also ranging around the same size; 400 - 500bp. This could be explained by mix species that co-inhibit the same honey pot. Sequencing and alignment of PCR products in the database showed that there were several *Lactobacillus* species repeatedly emerged on the list. This suggested that these species are dominant in the stingless bee colony of that plantation. Said species were *Lactobacillus kefiri* strain JK5, *Lactobacillus timberlakei* strain HV, *Lactobacillus malefermentans* JCM 12497, *Lactobacillus kefiranofaciens* strain IMAU98303 and *Lactobacillus salivarius* strain JCM1046.

Conclusion

From this finding, it is confirmed that stingless bee honey provides a good variety of beneficial bacteria especially from *Lactobacillus* strains

P-43

Interaction of UV-VIS optical properties towards enterovirus 71 RNA for detection of hand footmouth disease using optical spectroscopy

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

1. Background

Hand foot mouth disease (HFMD) is a common infectious disease that occurs in children age below 5 years old. The transmission of the disease is through nasal fluids, the saliva as well as the contact on surfaces contaminated with the HFMD patients. HFMD is caused by viruses known as Enterovirus A71 (EV71). Generally, all viruses are biological things that contain specific genes of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). In this project, RNA extraction is used instead of DNA. RNA extraction is safer, stays inactivate at room temperature, and has resistance towards the ultraviolet sensitivity compared to the DNA. There are various types of light with different wavelengths that are represented as the electromagnetic spectrum and this research will be using Ultraviolet (UV) until Visible (VIS) spectra. This project aims to study the optical properties of EV71 RNA via UV-VIS spectroscopy for HFMD detection and to differentiate the UV-VIS light response between various sampling of EV71 RNA through spectra analysis. The UV-VIS spectroscopy requires specialized methodologies to be performed. This project was implemented using absorption, transmission, and reflectance methodologies.

2. Methodology

In this study, EV71 RNA with different concentration were used. Optical spectroscopy in the ultraviolet-visible (UV-VIS) range was used to observe the optical properties of all of the RNAs, including absorbance, transmission and reflectance. The Ocean View software was used to capture the spectra of all honey. To improve the accuracy of the results, the spectra was collected more than 10 times for each concentration. The average of the results was plotted, and analysis was performed for all spectra results.

1. Results and Discussion

As a result of this project, the value of absorbance and reflectance is directly proportional to the concentration whereas the value of transmittance is inversely proportional to the concentration. The results of this experiment were supported by theoretical and previous studies. The data of this research need continue to be discussed and improved from time in developing a diagnostic instrument that is responsive, mobile, lowcost, and accurate. A rapid, adaptive test, such as a "test kit" capable of identifying the EV71, will significantly improve the detection of HFMD. Thus, the annual number of HFMD cases can be controlled since the appropriate treatment can be administered due to the early detection of HFMD in the early stages.

2. Conclusion

Three optical properties, which are absorbance, transmission, and reflectance were studied for all of the EV71 RNA using optical spectroscopy in the UV-VIS ranges. An analysis was performed on the spectra results to compare the mean value of the peak spectra. Absorption and reflection have demonstrated a similar relationship between the sample concentration used and the absorbance or reflectance values. This means, when the concentration of the sample was high, the absorbance or reflectance value will be high as well. Contrarily, the association between sample concentration and transmittance were different. This signifies that the transmission value was low when the sample concentration used was high.

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P-44

Analysis of microsatellite instability in colorectal cancer patients from Hospital USM

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Microsatellites are short, repeating DNA segments that are vulnerable to mismatches during replication. Mismatch repair (MMR) protein complexes often correct DNA mismatch errors. DNA errors build up as a result of the MMR system's loss of function, causing microsatellite instability (MSI), a genetic hypermutability disorder. MSI is a molecular phenotype due to a defective DNA MMR system. Recent years have seen a rise in interest in DNA MMR deficiency as a key biomarker to predict the therapeutic efficacy of immune checkpoint inhibitors for various malignant neoplasms. The aim of our study was to investigate the profile of MSI status in colorectal cancer (CRC) patients from Hospital USM.

Methodology

The formalin-fixed, paraffin-embedded tissue blocks from archival samples of colon cancer and adjacent normal tissue (n = 43) were randomly selected between 2018 and 2020. Genomic DNA extracted from tumors and corresponding normal tissues were used for MSI analysis using the Promega panel (5 mononucleotide markers: BAT-25, BAT-26, NR-21, NR-24, and MONO-27; and 2 pentanucleotide markers: Penta C and Penta D).

Results and Discussion

In the 43 tissue samples that were studied, 21 samples came from men and 22 samples came from women. The median age of patients in the study group was 62 years. Four tumors (9.3%) demonstrated high MSI (MSI-H), one demonstrated low MSI (2.3%), and the remaining 38 (88.4%) tumors were microsatellite stable (MSS). Our finding was comparable with other studies which is show that the incidence of MSI in tumors in CRC varies from 8.8% to 20.3%. Interestingly, patients in the MSI-H group were younger with a median age of 39 years compared with the study population with a median age of 62 years, whereas median age 63 years was observed in MSS group. According to a study, patients with CRC who had MSI-H were diagnosed earlier than those with MSS (1). Majority of selected tissue samples arise from distal colon where smaller percentage were from proximal colon (16%). Notably, the incidence of proximal lesions in the MSI-H group (75%, 3/4) was higher than in the MSS group (9%, 4/39). The findings of authors from several countries have demonstrated that MSI is more frequent in patients with tumors localization in the proximal of the colon (2), which is also in agreement with our findings. It has been reported that MSI-H tumors respond well to immunotherapy, PD-L1 (programed cell death ligand 1) with FDA approved to treat MSI-H/MMR patients.

Conclusion

This is an exploratory study employing a Promega panel to look at the MSI profile in our CRC patients. We discovered that the MSI rate was 9.3%. It is necessary to do further research with a larger sample size to associate clinical characteristics with MSI status and it would be beneficial to screen the patients with MSI-H status that has a potential role in treatment management.

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P-45

Integration of Nanotechnology and Biomedical Sciences – A Perspective Review

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

The emergence of nanotechnology and its integration in biomedical sciences have elevated expectations as it can solve vital issues of biological systems at the nanoscale. Nanotechnology has several applications and is gaining importance in biomedical sciences due to its small size and targeted effects. Researchers have identified and confirmed that nanotechnology products have become increasingly valuable for biomedicine and have led to the advent of a hybrid science. Studies have also confirmed that nanotechnology and biomedicine have a significant role in diagnostics, monitoring, treatment, and progression management. The primary justification is that particles of the size of a few nanometers, like iron oxide nanocrystals. This exhibit optical, magnetic, or structural features that are not present in molecules or bulk materials. These nanoparticles can target diseased cells and organs (such as various cancers) with high affinity and specificity when combined with targeting ligands like monoclonal antibodies.

Methodology

The research relies on studies made public by health organisations that validated the important part nanotechnologies play in managing the progression of diseases as well as their surveillance and treatment. To better understand how nanotechnology is used in biomedicine, excerpts have been chosen.

Results and Discussion

Medical and biological nanotechnologies have made significant advances in the following areas: Creating novel biomedical sensors based on nanostructured materials may serve as diagnostic medical equipment components. Use of nanotechnology-integrated biomedical devices, such as nanoporous silicon membranes, for biofiltration. Polymer nanoparticles are utilised to transport therapeutic drugs to specific body locations. Quantum dots, a type of semiconductor nanocrystal, are proving to be a very effective novel reagent for biomedical imaging. Combination therapy with nanotechnology support is quickly becoming one of the most excellent alternatives to traditional methods. Nanobiomechanics is proving to be a potent tool to characterise the mechanical properties of biological materials and structures and track pathological and physiological effects.

Conclusion

Biomedical nanotechnology is needed for a better future. The foundation of biomedical nanotechnology is a nano-scaled platform which is extremely useful for early-stage diagnostics and therapy efficacy evaluation and is dedicated to exploring nanoscience and nanotechnology for health wellness, with the goal of personalised health management. The Revolution of nanotechnology-assisted approaches makes diagnostics and treatment of a targeted disease affordable, sensitive, and accessible.

P-46

Antimicrobial activity of astaxanthin extracts of wild type and mutant *Xanthophyllomyces dendrorhous*

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

There is worldwide concern about the increase in antimicrobial resistance which affects both developed and developing countries. It is a public health issue with immense societal and economic implications (Dhingra *et al*., 2020). Antibiotic resistant pathogens pose an enormous threat to the treatment of a wide range of serious infections. To prevent this exponential emergence, a periodic replacement of the existing antibiotic is necessary. *X. dendrorhous* extracts were signified on the benefits of astaxanthin in antioxidant activity, but less emphasized for its antimicrobial activity. This study aims to assess the antimicrobial ability of astaxanthin extract of wild type and mutant *X. dendrorhous* obtained in our research.

Methodology

Antimicrobial activity of astaxanthin extracts was evaluated through zone of inhibition using paper disc assay against seven microorganisms of industrial importance namely Gram negative bacteria: *Shigella boydii* and *Escherichia coli*, Gram positive bacteria: *Bascillus subtilis* and *Staphylococcus aureus*, yeasts: *Saccharomyces cerevisiae* and *Candida albinans*, and fungus: *Asperigillus niger*. Both the wild type and mutant carotenoid extracts were prepared at the concentration of 30ug/mL and dotted on autoclaved paper discs. The experiment also included a positive control of 30ug/mL Chloramphenicol for antibacterial drugs and Miconizole as standard antifungal drug. All the plates containing bacteria were incubated at 37°C for 24 h and that of yeast and fungi at 28°C for 48 h.

Results and Discussion

The standard antibiotic chloramphenicol produced inhibition zones of 12, 14, 9 and 8mm against *B. subtilis*, *S. aureus*, *S. boydii* and *E. coli* respectively. Meanwhile positive control Miconizole produced inhibition zones of 28, 25 and 23 mm against *S. cerevisiae*, *C. albicans* and *A. niger* respectively. The antimicrobial activity of both wild type and mutant extracts showed significant growth inhibition against pathogenic microbial strains. The mutant carotenoid extract showed slightly better inhibition effect on all the pathogens compared to wild type extract but the difference was not significant. The extracts were found to exhibit excellent inhibitory effect against Gram positive *B. subtilis* and *S. aureus*, nearly as good as positive control. They exerted a milder antimicrobial capability against Gram negative *S. boydii* and *E. coli*. For yeast and fungus, the extracts showed moderate inhibition in *S. cerevisiae*, *C. albicans* and *A. niger* compared to positive control. Limited antimicrobial reports on astaxanthin are mostly extract from sources like microalga *H. pluvialis* and crustaceans so far. Some contradictions can be found in the literature as a result of the different strains used in the determination of antimicrobial activity, extraction methods, extraction solvents and extract concentration used in the assays (Irna *et al*., 2017).

Conclusion

Based on controls, both the wild type and mutant carotenoid extracts showed impressive inhibition on Gram positive and Gram negative bacteria compared to yeast and fungus. The twofold use of axtaxanthin extracts as antioxidants and antimicrobials has good prospects in response to the consumer demand for more sustainable and natural ingredients and products.

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P-47

The potential of *Xanthophyllomyces dendrorhous* wild-type and mutant strains in producing UV absorbing compounds

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Solar ultraviolet radiation is the culprit for molecular and genetic changes that occur to the skin. It generates reactive oxygen species and free radicals that cause skin oxidative damage leading to skin cancers, photosensitivity, and photo-ageing problems. These contribute to synthetic sunscreens formulated with UVR filters that mostly show side effects on human health and ecology. Hence, research investigations were initiated upon natural resources that can produce UV-absorbing compounds known as mycosporine-like amino acids and mycosporine- glutaminol-glucoside. These molecules are commonly used by organisms growing at high altitudes to protect themselves from extreme UV exposure [1]. In this study, a basidiomycetous yeast, *Xanthophllomyces dendrorhous* wild-type and its mutant strains were analyzed for their ability to produce MAA/MGGs.

Methodology

X. dendrorhous wild type and mutant strains were revived and maintained on yeast malt agar at 4°C. Synthesis of mycosporines was induced by transferring young cultures (24hr) to YM agar plates and incubated for 4 days at 22°C in an incubator (LabCompanion SIF-6000R) with a 12:12 light: dark photoperiod. For light conditions, culture plates were left exposed to PAR+UVR while for dark conditions, plates were covered with aluminium foil. After exposure, the colonies were harvested and transferred to distilled water, centrifuged, and conserved at -20° until mycosporines extraction. Samples were extracted with 10ml of DMSO solution (2%) for 24hrs at 4°C. After 24hrs, glass beads were added to samples and vigorously agitated in a vortex mixer. Later, they were incubated for 24hrs at 45°C in a water bath and centrifuged using a microcentrifuge. The resulting supernatant was immediately measured spectrophotometrically (UV-visible) at 325nm for mycosporines quantification. Experiments were performed in triplicate.

Results and Discussion

With the aid of a UV spectrum, the mycosporine production rate was found to be the highest at 325nm for all four strains leading to a fixed wavelength for further measurements. Yeast colonies that were exposed to PAR+ UVR showed higher absorbance rates concluding more mycosporines production compared to the colonies grown in a dark environment. It showed that strains that are frequently exposed to high UV radiation have an increased tendency of mycosporine production than those with lower intensity of UV exposure. By comparison between the wild type and 3 mutant strains, the results indicated that the red, white and yellow mutant colonies showed almost equal mycosporines production rate as the wild strain initially. The production rate will be further improved by the introduction of different inducers, growth media and culture conditions.

Conclusion

All the four strains tested showed the detection of MAA/MGGs in the DMSO extracts. It is concluded that after photostimulation, both *X. dendrorhous* wild-type and mutant strains have the ability to synthesize UV-absorbing compounds and further investigation should be done to discover its potential as a natural molecular sunscreen.

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P-48

COVID-19 Pandemic and Biomedical Sciences – Current and Future Application

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

The coronavirus infectious disease (COVID-19) pandemic was in 2019 and caused Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), resulting in unprecedented health and economic crisis worldwide. Biomedical sciences have been a promising area for combating the coronavirus due to the unique challenges raised by the pandemic. There was a great challenge to the biomedical scientist for a practical prevention approach leading to rapid and reliable diagnostics, monitoring and effective & safe therapy. Excerpts have been selected to explain biomedical science's current and future applications for pandemics.

Methodology

The applications of biomedical sciences include several factors such as:

1. Dissemination and use of research, development and access to patents.
2. Contemporary inventions in biomedical devices tackle the current as well as future pandemics.
3. Priority medical devices for prevention and treatment of pandemics.
4. Prevention of pandemic infection using Biomedical devices prevents nosocomial Infections in healthcare facilities.
5. Biomedical devices with coatings based on nanotechnology to prevent surface contamination by viruses and pathogens.
6. Diagnostics and Biosensors for the COVID-19 pandemic involving nanobiotechnology and artificial intelligence.

Research have also shown that reliable and cost-effective biomedical devices are also expected to provide affordable, practical, and easy-to-use products accessible to all countries worldwide, with a particular emphasis on low-income countries. Finally, apps, software, the internet, and intelligent technologies are outstanding tools to monitor, control, and predict the evolution of COVID-19 pandemics.

Results and Discussion

Research in the field of biomedical sciences to tackle the COVID-19 pandemic and any other future pandemics, not just from an experimental point-of-view but also from approaches relying on computational simulations, artificial intelligence, and smart devices. Nanotechnology is one of the prominent field that can significantly impact the development of protecting, diagnosis, and therapeutic solutions. Future perspectives in biomedical science and nanomedicine applied to viral diseases are expected to focus on mainly (i) early, highly sensitive, portable and affordable diagnosis kits, avoiding complex infrastructures, sophisticated devices, and skilled professionals, and (ii) development of nanomedicinal tools, including the development of biodegradable vectors suitable to current patients' treatment.

Conclusion

The multidisciplinary field of biomedical sciences and the research for discovering new molecules and manipulating those available naturally could be dazzling in their potential to improve health care. The pandemic has witnessed increasing interest and tremendous developments in biomedical science through adaptative, collaborative, multidisciplinary, and innovative research efforts made by biomedical scientists, which have significantly benefited human health and society.

P-49

Wheat germ agglutinin binding protein (WBP) from mycobacteria modulates macrophage immune functions and gives antibody response in TB patients

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background
Tuberculosis is still a major health problem, and is the leading cause of death from a curable infectious disease in the world. The problem is exacerbated with the emergence of multi drug resistance. To overcome this situation new vaccines and drugs and more specific rapid diagnostic tests must be developed in conjunction with basic research on biology and pathogenesis of *M. tuberculosis*. Several studies have shown that sugars and lipids are mainly responsible for *M.tb* pathogenesis and resistance to numerous drugs. Hence we aimed to identify and characterize wheat germ agglutinin binding glycoprotein along with its role in immunomodulation in macrophages and B cell response in TB patients.

Methodology
Lysates of various strains of *M.tb* including *Mycobacterium bovis* BCG, *M. smegmetis* and CDC 1551 were subjected to SDS-PAGE followed by western blotting using HRP conjugated wheat germ agglutinin. Subsequently, Periodic Acid-Shiff (PAS) reagent was employed for the staining. Further, the WGA bound protein from *M.bovis* BCG was checked for immune response elicitation in macrophages through cytokine measurement using ELISA and nitric oxide production using Griess' reagent. Furthermore, the WGA bound protein of *M.tb* CDC 1551 lysate was tested for its antibody response in various categories of TB patients using human Igg-HRP in ELISA.

Results and Discussion
Wheat germ agglutinin binding protein (WBP) of about 60-65 KDa size was identified through western blotting. Further, PAS staining validated the protein to be glycoprotein, which was found to be competitively inhibited by N-acetyl glucosamine (NAGA). The WGA bound protein from *M.bovis* BCG was found to elevate pro-inflamatory (TNF-α) and anti-inflamatory (IL-10) cytokines along with heightened nitric oxide levels. Interestingly, the WGA bound antigen is found to be sensitive in discriminating active tuberculosis patients from the BCG-vaccinated controls and could be explored as serodiagnostic antigen for active TB. Simultaneously, the antigen showed a good antibody response in other TB categories including smear positive, smear negative and extra pulmonary TB. Additionally, the Latent TB cases also exhibited a strong antibody response.

Conclusion
Altogether these results suggest the WBP to be involved in immune response and antibody response during bacilli infection. Further studies pertaining to biochemical characterization and B- cell activation could provide insights of the role of WBP during TB pathogenesis

P-50

Comparative Evaluation of Three Methods for Protein Extraction Efficiency from Formalin-Fixed Paraffin-Embedded Tissues for Proteomic Analysis

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Formalin fixation and paraffin embedding of tissues (FFPE) have become the most available biopsies in the hospital setting due to their ability to be stored for long periods and may be kept at room temperature without concern of deterioration or decay. However, this fixation process causes a protein alteration known as "cross-links." Consequently, it made it difficult to extract proteins and characterize individual proteins. The study of proteomics is growing quickly because it has the potential to enhance disease diagnosis, risk assessment, prognosis, and therapy targeting. Consequently, as a researcher, it is important to investigate strong and efficient protein identification approaches and improve all aspects of protein analysis, such as the protein extraction method from FFPE tissue and protein digestion for mass spectrometry.

Methodology

For this research, FFPE human tonsillitis blocks were chosen for this research to compare the efficiency of three different protein extraction methods, including sonication, RapiGest, and Sodium Deoxycholate, from Formalin Fixed Paraffin Embedded (FFPE) tissue for proteomic analysis. These sections were cut into 4 µm thin slices of tissue to facilitate the deparaffinization process. Three sets of unstained FFPE tonsil tissue sections were excised into 1.0 cm2 for the triplicate results. For the quantification analysis, Nanodrop 2000 was used to measure the protein concentration from the extracted protein. While the determination of the protein quality, SDS-PAGE analysis was done with three different protein extraction methods. The proteins of interest are digested with an enzyme, trypsin, and the resultant peptides are examined using mass spectrometry. Protein identification will be accomplished using LC-MS/MS analysis of protein extracted from three sets of each extraction technique obtained from FFPE tonsillitis samples.

Results and Discussion

The protein concentration of the samples was determined using the nanodrop 2000c. From the sonication method, the protein concentration was extremely low and inconsistent. The sodium deoxycholate method produced a greater protein concentration than RapiGest. Next, the protein quality was evaluated using SDS-PAGE following the application of the three protein extraction methods for sample fragmentation. The intensity of the band for RapiGest and Sodium Deoxycholate can be observed at 1 cm. However, a very faint band was observed for the sonication method. This shows that the highest abundance of protein loaded into the gel is from the RapiGest and Sodium Deoxycholate, while the lowest is from the sonication method. All the proteins were then analysed via LC/MS and identified using Peak 7.5 software. A total of 668 proteins were successfully identified using the sodium deoxycholate method. In addition, a total of 387 protein samples were obtained using the sonication method. However, for the RapiGest method, the number of proteins acquired from the samples was only 89, which was the lowest. Based on the results obtained from the software, 14 proteins were found to overlap in all three protein extraction methods.

Conclusion

In conclusion, the most efficient protein extraction method is sodium deoxycholate because the protein concentration is the highest. Adding to that, the number of proteins identified using this method is also the highest.

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Optimization of expression and purification of soluble *E. bieneusi* B7XHF2 recombinant protein in *E.coli*

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Worldwide reports of human infections with *Microsporidia* mainly include immunocompromised patients [1]. One of the most prevalent microsporidian species to infect HIV/AIDS patients is *Enterocytozoon bieneusi* [2]. Although stool testing is the most reliable method to diagnose microsporidiosis, the infection is underdiagnosed in patients with disseminated microsporidiosis due to the absence of microsporidia spores in their stool samples. Our previous study has identified two circulating antigens of diagnostic value from the serum of immunocompromised patients. In this study, we aimed to express and purify *E.bieneusi* recombinant protein B7XHF2 in *Escherichia coli* as a potential diagnostic biomarker for detection of disseminated microsporidiosis.

Methodology

The B7XHF2 gene was cloned in the pET-32a(+) vector and the recombinant plasmid was transformed into C41(DE3) *E.coli* host cell. Small scale expression in 200ml Terrific broth (TB) was performed to optimize the parameters of protein expression namely IPTG concentration and post-induction incubation period. Subsequently, protein expression was carried out in 2L TB medium, at a temperature of 37°C, 200 rpm before the temperature was lowered down to 28°C for 4 hours post IPTG indction. The harvested cells were pelleted and lysed by sonication in lysis buffer containing protease inhibitors and lysozyme. The filtered protein lysate was then subjected to affinity purification using Ni-NTA resin at a ratio of 1:10 (resin:lysate). Contaminating proteins were removed by gradient washing using washing buffer containing 20mM, 30mM and 40mM imidazole concentration, and finally the targeted protein was eluted by adding elution buffer with 250mM imidazole. Eluted fractions were pooled and concentrated to 300ul and the protein concentration was measured by RCDC method (Biorad-). Anti-histidine western blot was carried out to verify the presence of the targeted protein.

Results and Discussion

In this study, the rB7XHF2 protein was highly soluble when induced with 0.5mM IPTG compared to 1.0mM IPTG. There was no significant difference in terms of protein expression when incubation was performed at 2,3,4 and 16 hours post-induction, therefore 4 hours was chosen as the best incubation period. The appearance of a 41-kDa protein on anti-histidine western blot confirmed the expression of rB7XHF2 protein, which was close to the theoretical size of 41.7 -kDa.

Conclusion

This preliminary study has successfully expressed the B7XHF2 recombinant protein in *E.coli* C41(DE3) host cell. Further work is needed to improve the yield as well as purity of the target protein for diagnostic applications.

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Production of *Enterocytozoon bieneusi* recombinant protein and reactivity of its polyclonal antibodies toward *Encephalitozoon cuniculi*

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Entercytozoon bieneusi is the most common microsporidia species and has been reported to have the propensity to disseminate in humans. B7XJ00 is a protein of *Enterocytozoon bieneusi* found circulating in the blood of disseminated microsporidiosis patients in our previous study (unpublished data). However, *E. bieneusi* could not be cultivated in culture cells and little is currently known about the function of B7XJ00. Meanwhile, *Encephalitozoon cuniculi* have a high degree of gene homolog with *E. bieneusi,* thus making it a suitable 'surrogate' species in the study of *E. bieneusi.* Polyclonal antibody is a useful tool for assay-specific target discovery and detection. The production of polyclonal antibodies against recombinant B7XJ00 (rB7XJ00) protein could be used to study the protein of *E. cuniculi* that homolog toward rB7XJ00 protein. This study aims to determine the native *E. cuniculi* proteins that are recognized by antibodies raised against the recombinant proteins.

Methodology

B7XJ00 gene was cloned into the expression vector pET32a (+) and transformed into *Escherichia coli* strain BL21(DE3) using the $CaCl_2$ method. rB7XJ00 protein was expressed, purified by Ni-NTA affinity chromatography, and evaluated by Western blot. The protein identity was confirmed by analysis using LCMS/MS. New Zealand white rabbit was immunized with these purified proteins to produce polyclonal antibodies production against the rB7XJ00 protein and the sera were collected after the fourth immunization. The titer of the serum polyclonal antibody was determined by ELISA followed by Western blot to determine *E. cuniculi* lysate that is reactive toward rabbit polyclonal antibodies. The reactive *E. cuniculi* proteins will be characterized by mass spectrometry to elucidate the function of the recombinant B7XJ00.

Results and Discussion

rB7XJ00 protein was successfully transformed in *E. coli* BL21(DE3) and expressed as soluble protein after 5 hours of induction with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 25°C. Purification using nickel-nitrilotriacetic acid (Ni-NTA) resin yielded 0.33mg of rB7XJ00 from 1 L of culture. Western blot with anti-His-HRP shows 3 protein bands with the estimated molecular mass of 10, 25, and 75 kDa on SDS-PAGE. These 3 proteins were identified by LCMS/MS analysis as *E. bieneusi* proteins. The polyclonal antibody at a titer of 1: 2 048 000 was obtained by immunizing a New Zealand white rabbit with purified rB7XJ00 protein. Western blotting showed that the polyclonal antibody could specifically recognize native *E. cuniculi* protein.

Conclusion

B7XJ00 recombinant protein was expressed and the high titer of rabbit polyclonal antibody against B7XJ00 recombinant protein was successfully produced. The polyclonal antibodies of rB7XJ00 were reactive toward *E. cuniculi* lysate.

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Differential expression of E-cadherin and ICAM-1 in triplebreast cancer cell lines

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022) Penang, Malaysia. 11 – 13 October 2022

Background

Triple-negative breast cancers (TNBCs) have a high death rate due to aggressive proliferation, metastasis and ineffective treatment options. Molecular heterogeneity in TNBC cases is the greatest obstacle in treating patients. On the other hand, dysregulation of cell-cell adhesion molecules has been linked to different features of cancer specially, epithelial-to-mesenchymal transition (1). Since ICAM-1 and E-cadherin expression has been proposed as potential molecular targets for treating TNBC cases (2,3), this study aimed to examine the protein expression of E-cadherin and ICAM-1 in two TNBC cell lines.

Methodology

BT-20 and MDA-MB-468 were purchased from ATCC. A total of 1 x 10^{5} cells were seeded into six-well plates. The plates were incubated overnight for adherence. Then, cells were incubated overnight at 4 °C with primary antibodies (Santa Cruz Biotechnology ICAM-1 (G-5) mouse anti-human & E-cadherin (G-10) mouse anti-human), both with a final concentration of 1 µg/ml in 1% BSA buffer. After three washes with PBS, it was followed by incubating with fluorescent secondary antibodies (Santa Cruz Biotechnology Secondary anti-mouse antibody conjugated with CruzFluor 488) with a final concentration of 1 µg/ml in 1% BSA buffer for 1 hour at room temperature. The microscope slides were analyzed to observe DAPI-FITC-stained cells using a Zeiss Axio Vert A1 fluorescence microscope. The fluorescence images were then analyzed with ImageJ software (Version 1.52a) by selecting one cell at a time in a snap and measuring the area, mean gray value and integrated density. For each cell line, 100 of cells was captured for analysis.

Results and Discussion

In this study, results showed that ICAM-1 surface protein in BT- 20 was slightly higher than MDA-MB-468 (1.424 ± 0.09 vs 1.117 ± 0.05) whilst E-cadherin surface protein in MDA-MB- 468 was significantly higher than BT-20 (9.946 ± 0.42 vs 1.719

Conclusion

Differential expression of E-cadherin in two TNBC cell lines might explain some of the heterogenicity in treatment outcomes, progression and survival of TNBC cases.

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P-54

In silico selection of aptamers to cobra venom cytotoxin

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From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background

Snakebite envenomation has been listed as one of the neglected tropical diseases (NTDs) by the World Health Organization (WHO) since 2017 [1]. Cytotoxin (CTX), one of the most abundant venom components, was deemed responsible for the envenomation's local dermonecrosis. Several studies have indicated that the CTX induces cell death through different pathways, yet the molecular mechanism is inconclusive [2]. There is also limited treatment against dermonecrosis. Therefore, there is a need to search for biotherapeutics against CTX-induced dermonecrosis. In this study, we exploit the potential of tRNA-based aptamers as alternate toxin-neutralizing molecules using in silico approach.

Methodology

GtRNAdb database was used to identify the CTX-binding tRNA-based aptamers for the possible interacting regions with CTX [3]. The sequences of the aptamers were subjected to phylogenetic tree analysis to categorize the tRNAs into different clusters using the Clustal Omega. The tertiary structures of the consensus aptamer sequences were modeled using the RNA Composer. Later, the molecular docking of these aptamers and CTX was performed using HADDOCK web server. In addition, RING analysis was performed on each aptamer-CTX complex to determine the interacting amino acids present on the CTX and the type of interactions.

Results and Discussion

Altogether, our results suggested six clusters of consensus aptamer sequences from the phylogenetic tree analysis. Among the six aptamer models generated from their respective consensus sequences, three aptamers demonstrated significant binding to CTX. Additionally, the RING analysis revealed that there were four common interacting regions between the six CTX-aptamer complexes. These four interacting regions were consistent with the CTX's epitopes in our unpublished work. Moreover, the high binding affinity between the aptamer-CTX complexes also
suggested that they were potential aptamers to antagonize CTX's action.

Conclusion

The current work provides insights into *in silico* approach to design aptamers that antagonize CTX-induced dermonecrosis. Three tRNA-based aptamers have been identified as potential aptamers, which showed strong interaction with CTX's epitopes.

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