REVIEW ARTICLE

Unlocking Early Detection: The Role of DNA Methylation Biomarkers in Colorectal Cancer Tumorigenesis – A Systematic Review

Nurul Izzati Mohd Shukri[1], Mohd Arifin Kaderi[1], Adel Alhabbal[2], Norafiza Zainuddin[1,*]

[1]Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Bandar Indera Mahkota, Jalan Sultan Haji Ahmad Shah, 25200 Kuantan, Pahang, Malaysia
[2]Department of Microbiology & Biochemistry, Faculty of Pharmacy Arab Private University for Science and Technology, 2Q85+R2Q, Hama, Syria

Keywords:
colorectal cancer; DNA methylation; biomarkers; early detection

ABSTRACT

Background: DNA methylation is an epigenetic mechanism that holds promise for improving disease detection, particularly in the early stages of neoplastic transformation. Although colonoscopy is currently the most effective method for detecting colorectal cancer (CRC) due to its high sensitivity, patient compliance is often hindered by its invasive nature, high cost, and inconvenient preparation process. This systematic review aims to systematically identify DNA methylation-based biomarkers used in early-stage CRC detection and to systematically compile evidence on the roles of DNA methylation-based biomarkers in CRC tumorigenesis. **Methods:** Data were collected via electronic searches for relevant citations from 2018 to 2023 in PubMed, Scopus, and Cochrane Library, using relevant and specific keywords for the search strategy. The selection of relevant articles is associated with the inclusion and exclusion criteria. The quality of the articles was assessed using the Crowe Critical Appraisal Tool (CCAT). **Results:** From an initial pool of 121 articles, 14 articles were selected based on the inclusion criteria and PRISMA guidelines. This systematic review successfully identified relevant DNA methylation-based biomarkers that have potential in early-stage CRC detection which are SDC2, KCNQ5, C9orf50, CLIP4, a combination of SEPT9 and SDC2, and a combination of GALNT9 and UPF3A. These biomarkers have been shown to have high accuracy and can be identified in a non-invasive approach such as stool and blood, demonstrating their potential as an effective tool for early CRC detection. Additionally, DNA methylation biomarkers were shown to be involved in key processes of CRC tumorigenesis, including cell proliferation, migration, transformation, metastasis, and angiogenesis. **Conclusion:** This systematic review highlights the promising role of DNA methylation-based biomarkers in the early detection of CRC, offering a non-invasive approach and highly accurate alternative to traditional methods.

INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers worldwide and the second leading cause of cancer-related deaths, according to the World Health Organization (2023). It ranks third among cancers in men and second in women after breast cancer (WHO, 2023). CRC risk increases with age, but healthier lifestyles and regular screening have contributed to decreasing incidence rates in some countries (Miller et al., 2019). In line with this, The American Cancer Society (2024) stated that the mortality rates of CRC have been declining for some decades among males and females due to the reason for getting a screening. Getting a screening could increase the identification and removal of colorectal polyps before they develop into cancer and facilitate more accessible treatment for CRC.

Colorectal cancer often arises due to a combination of genetic and epigenetic modifications (Ye et al., 2024) . One of the most common epigenetic modifications linked to CRC is DNA methylation. Changes in DNA methylation pattern which leads to aberrant methylation can serve as cancer biomarkers (Yuan, 2024). This aberrant methylation manifests in the initial phase of cancer progression, making them potentially valuable for screening purposes (Locke et al., 2019).

Various CRC screening methods exist, each with strengths and weaknesses. The current gold standard for CRC detection is colonoscopy, which significantly reduces CRC mortality by 67% (Doubeni et al., 2016). Despite its high accuracy, colonoscopy's invasive nature, cost, and preparation process often deter patients from getting screened (Pontone et al., 2022). Non-invasive stool-based tests like the guaiac-based fecal occult blood test (gFOBT) and fecal immunochemical test (FIT) are easier to use but have limited sensitivity, particularly for early-stage CRC (Zhang et al., 2023).

Given these limitations, there is a need for more effective, non-invasive screening methods. DNA methylation-based biomarkers show promise for early CRC detection. Changes in DNA methylation patterns occur early in cancer progression and could serve as reliable biomarkers. These biomarkers could improve detection accuracy and patient

*Corresponding author.
E-mail address: znorafiza@iium.edu.my

compliance, complementing existing CRC screening methods.

MATERIALS AND METHODS

Materials and methods

Protocol and registration

This systematic review was conducted according to a protocol registered in the International Prospective Register of Systematic Reviews (PROSPERO—https://www.crd.york.ac.uk/PROSPERO/) under registration number CRD42024487883. This study closely adhered to the guidelines provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-P) 2020.

Selection Procedure

The articles have been reviewed by evaluating their titles, objectives, abstracts, discussions, and research designs to assess their relevance to the research subject. Furthermore, any duplicates present in the list of relevant articles were identified and removed. Additionally, each article was evaluated based on the inclusion and exclusion criteria. The authenticity of articles was then ultimately verified by quality evaluation.

Systematic Review Process

Identification

Articles were retrieved from the chosen databases which are PubMed, Scopus, and Cochrane Library using specified keywords, including colorectal cancer, DNA methylation, biomarker, and early screening. The search strategy design involved integrating text words (keywords) and MeSH terms. All possible variations of the terms were considered and combined with Boolean operators (AND, OR) and truncated search terms according to the PubMed User Guide. In PubMed, the truncation symbol is represented by an asterisk (*) where this truncation retrieves all terms that contain the root which is the base part of the word.

Screening

The articles retrieved from the databases were further screened for any presence of duplications, and those that were identified were excluded from inclusion. Subsequently, the titles and abstracts of the remaining articles were thoroughly assessed, and any articles that were found unrelated to the research objectives were excluded.

Eligibility

After the initial screening of articles, the inclusion and exclusion criteria were applied to determine the eligibility of the remaining full-text articles (Table 1). Only articles that satisfied all the criteria were included in this study.

Inclusion

Data analysis was conducted on the remaining selected articles that met all the criteria and previous assessments.

Table 1: Inclusion and exclusion criteria for systematic review

Inclusion criteria	Exclusion criteria
• Studies published in English.	• Studies published in other languages.
• Studies published in 2018-2023.	• Studies published before 2018 and after 2024.
• Randomized controlled trials, clinical trials, validation studies, observational studies (cohort, cross-sectional and case-control studies), prospective studies, prospective-retrospective studies, and multicentred studies.	• Unpublished studies, hand-searched articles or grey literature, technical reports, web-based guidelines, letters, editorials, reviews (systematic, scoping, narrative reviews), and meta-analysis.
• Must contain samples from CRC patients.	• Studies on patients with adenoma, precancerous polyps, or other types of cancer.
• Studies conducted on humans.	• Studies conducted on animals.

Data Extraction

Data from the final full-text publications were assessed, summarized, and presented in the form of tables to enhance readability. Besides, the main findings were retrieved from the articles that discuss on DNA methylation-based biomarkers used in CRC detection and their roles in CRC tumorigenesis. Data was also retrieved in a pre-defined form including the specimen type, sensitivity, specificity, and analysis method. The extracted effect measures are restricted to the area under the receiver operating curve (AUC), sensitivity and specificity.

Quality Assessment

For quality assessment, the Crowe Critical Appraisal Tool (CCAT) (Crowe, 2015) version 1.4 has been used to systematically assess research papers' reliability, validity, and overall quality. The CCAT comprises a form and a user guide that must be used together to ensure the scores obtained are valid and reliable. To ensure that the systematic review includes only high-quality publications, a quality score of 75% or higher is only included in this systematic review. Any discrepancies during the assessment of the risk of bias process were resolved by discussion and consensus among all reviewers.

RESULTS

Literature Search

121 articles in total were identified through database searching on PubMed, Scopus, and Cochrane Library. Six duplicate articles were identified and removed, leaving 115 articles for further assessment. Subsequently, these articles underwent a screening process based on the titles, which led to the removal of 22 articles. The remaining 93 articles underwent abstract screening, resulting in 51 of the articles being excluded. 42 articles from the abstract screening underwent an eligibility process which involved predefined inclusion and exclusion criteria. Eight articles did not meet the criteria and have been removed, resulting in 34 articles. These articles were then assessed for their quality by using CCAT tools. Of 34 articles, 14 of the articles were qualified and included in this systematic review. Figure 1 shows the comprehensive view of the selection procedure in a PRISMA flow diagram.

Data Selection and Study Characteristics

The primary author, publication year, journal, study title, study design, study population, and country were extracted, summarized, and tabulated based on the 14 eligible full-text articles (Supplementary Data). Furthermore, the DNA methylation-based biomarkers used in early-stage CRC detection, the biomarkers' performance, and the roles of biomarkers were analyzed and extracted. This study exclusively focused on articles written in English and published in 5 years from 2018 to 2023.

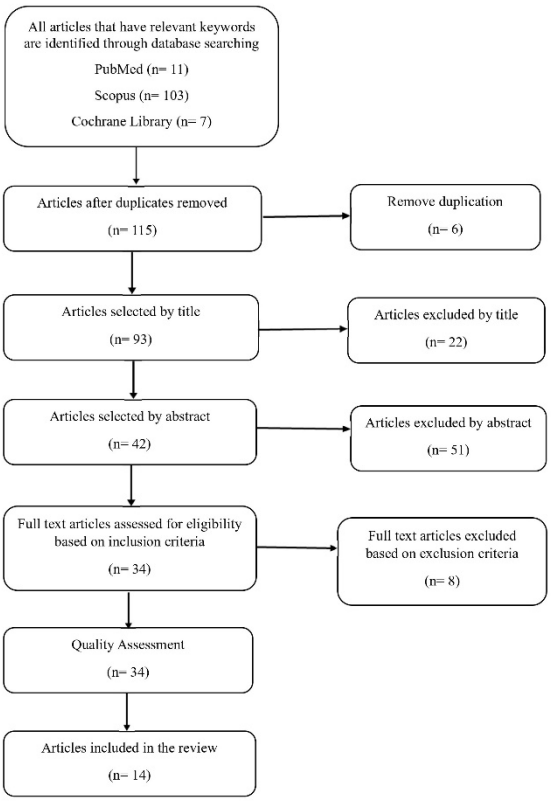

Figure 1: Flow diagram based on PRISMA 2020

Main Findings

DNA methylation-based biomarkers in early-stage CRC detection

DNA methylation which serves as a biomarker for CRC detection was identified from the included studies, comprising of single gene and panel gene biomarkers. The sample used, detection method, and early-stage detection status of DNA methylation biomarkers were extracted and tabulated (Table 2), and the performance of biomarkers in terms of sensitivity, specificity, and AUC value, were extracted (Table 3). Among the included studies, several potential biomarkers for early-stage CRC detection were identified such as *SDC2*, *KCNQ5*, *C9orf50*, combination of *SEPT9* and *SDC2*, *CLIP4*, and combination of *GALNT9* and *UPF3A* (from Study no. 2, 3, 7, 8, 9, and 12).

Role of DNA methylation biomarkers

DNA methylation-based biomarkers play important roles in the progression of CRC. Table 4 demonstrates the findings on the role of DNA methylation biomarkers which influence various processes in CRC tumorigenesis such as proliferation, migration, cell transformation, metastasis, and angiogenesis (from Studies no. 1, 5, 6, 11 and 14).

Table 2: DNA methylation-based biomarkers used in early-stage CRC

Study no.	Gene (s)	Sample/ Material	Method to detect methylation	Early-stage sensitivity for CRC detection
1.	*SM22α*	Tissue	Methylation-specific Polymerase chain reaction	-
2.	*SDC2*	Stool	Linear target enrichment-quantitative methylation-specific real-time PCR using me*SDC2* LTE-qMSP	- In stage 0-II: 89.1%
3.	*SDC2*	Stool	Real-time quantitative methylation specific PCR using sDNA test	- In stage 0-II: 87.0% sensitivity
4.	*SMAD3*	Tissue Plasma	Quantitative methylation-specific polymerase chain reaction	-
5.	*TMEM240*	Tissue Plasma	Quantitative methylation-specific polymerase chain reaction	-
6.	*WIF1* *NPY*	Tissue Plasma	Crystal Digital PCR™	-
7.	*KCNQ5* *C9orf50*	Stool	Methylation-specific quantitative PCR	- For *KCNQ5,* in stage 0:84.4%, stage I: 82.8%, stage II: 69.5% - For C9orf50, in stage 0: 90.6%, stage I: 87.9%, stage II: 84.7%
8.	*SEPT 9/SDC2*	Plasma	Quantitative real-time PCR using ColoDefense test	- In stage 0-II: 81.8% using ColoDefense test
9.	*CLIP4*	Stool	Quantitative real-time PCR using m*CLIP4* test	- In stage I: 96.2% and stage II: 83.1%
10.	*SDC2*	Whole blood	Methylation quantification endonuclease-resistant DNA	-
11.	*LINC00473*	Plasma	Quantitative methylation-specific PCR and droplet digital PCR	-
12.	*GALNT9* *UPF3A* *WARS* *LBD2*	Serum	Bisulfite pyrosequencing	-In stage I: 54.2% and stage II: 75.0% detection If *GALNT9*/*UPF3A*: 87.5% stage I detection and 100% stage II detection
13.	*SEPT9* *BMP3*	Plasma	Droplet digital PCR	-
14.	*FOXF1*	Plasma	MethyLight PCR	-

Table 3: Screening accuracy and AUC value of the biomarkers used in CRC

Study no.	Gene (s)	Sensitivity	Specificity	AUC value
2.	*SDC2*	90.2% (stage 0-IV) 89.1% (stage 0-II)	90.2%	0.90
3.	*SDC2*	83.8% (stage 0-IV) 87.0% (stage 0-II)	98.0%	0.95
4.	*SMAD3*	78.5%	-	-
6.	*WIF1/NPY*	95.5%	100%	0.94 0.98
7.	*KCNQ5* *C9orf50* *KCNQ5/C9orf50*	77.3% 85.9% 88.4%	91.5% 95.0% 89.4%	0.85 0.90 0.89
8.	*SEPT9* *SDC2* *SEPT9/SDC2*	75.8% 60.4% 85.7%	94.7% 86.8% 86.8%	0.86 0.80 0.97
9.	*CLIP4*	90.3%	88.4%	0.96
10.	*SDC2*	81.5%	69.2%	0.85
11.	*LINC00473*	81.0% 90.0%	100% 63.0%	0.88 0.83
12.	*GALNT9/UPF3A/WARS/LBD2* *GALNT9/UPF3A*	62.1% 78.8%	97.4% 100%	0.86 0.90
13.	*SEPT9* *BMP3* *SEPT9/BMP3*	50.0% 40.0% 65.0%	90.0% 90.0% 86.0%	0.68 0.58 0.77
14.	*FOXF1*	78.0%	89.5%	-

Table 4: Roles of the identified DNA methylation-based biomarkers

Study no.	Gene	Original function	Role in tumorigenesis	References
1.	*SM22α*	• Act as a tumor suppressor. • May decrease proliferation and invasion and increase apoptosis in colorectal carcinoma cells. • May prevent the metastasis of CRC	-	Liu Y. et al. (2018)
5.	*TMEM240*	• May repress cell growth, migration, and induce cell cycle arrest in colon cancer cells.	-	Chang S. et al. (2020)
6.	*WIF1*	• A tumor suppressor gene.	• Repression of *WIF1* leads to an overexpression of the Wnt signaling pathway thus promoting cell transformation.	Overs A. et al. (2021)
11.	*LINC00473*	• Able to sponge endogenous miR574-5p or miR15b-5p, inhibit cell proliferation and colony formation capacity, and induce cell apoptosis by activating the APAF1 CASP9-CASP3 pathway.	• Downregulation of pro-apoptotic tumor suppressor properties in CRC.	Ruiz-Bañobre, J. et al. (2022)
14.	*FOXF1*	-	• Associated with angiogenesis in CRC	Dastafkan, Z. et al. (2023)

DISCUSSION

This systematic review focuses on a comprehensive review of DNA methylation biomarkers that hold the potential for early-stage detection of colorectal cancer (CRC). This study includes fourteen articles pertinent to the study objectives, which are to systematically identify DNA methylation-based biomarkers used in early-stage CRC detection and to compile evidence on the roles of DNA methylation-based biomarkers. Every single study that was considered for inclusion had an article quality score of 75% or higher. The countries where the studies were conducted include China, South Korea, France, Iran, Spain, and Brazil. Notably, half of the included studies were accounted for in China. Han et al. (2024) reported a rising incidence of CRC in China, which ranks among the top five causes of cancer mortality in the country. Studies in China were also overrepresented as they have a broader target population. Apart from that, most of the included studies employed observational study design and involved human subjects. This focus on human studies can enhance the relevance and applicability of the findings to clinical settings.

Identifying early-stage biomarkers is essential to improve early detection and treatment of CRC. DNA methylation biomarkers can be utilized in molecular diagnostic blood- and stool-based assays, a non-invasive method feasible in early CRC detection. Employing these samples is more convenient and encourages higher patient compliance. The compilation of findings from 14 studies utilized various biological samples of participants for CRC detection, such as plasma, stool, whole blood, serum, and tissue.

Among the included studies, plasma samples were mostly employed in the identification of DNA methylation biomarkers. These biomarkers include *SMAD3*, *TMEM240*, *WIF1* and *NPY*, *SEPT9* and *SDC2*, *LINC00473*, *SEPT9* and *BMP3,* and *FOXF1*. Higher levels of these methylated genes at the promoter regions have been found in plasma samples from CRC patients compared to healthy individuals, except for the *SMAD3* gene, where a decrease in methylation was detected in 86.6% of plasma CRC patients, as mentioned in Study 4 (Ansar et al., 2020). The hypermethylation and hypomethylation of the studied genes correspond to increased and decreased expression, respectively. Plasma samples consist of cell-free DNA (cfDNA), which can be a promising non-invasive approach for CRC detection. Cell-free DNA refers to the release of DNA fragments into the bloodstream from cancer cells (Canzoniero & Park, 2016). According to Chen et al. (2021), screening tests utilizing plasma rather than whole blood is often suggested since blood cells would introduce an overabundance of genetic material. This could reduce the accuracy of the screening test itself in detecting any changes associated with the disease. However, contradictory to this, whole blood was used as a sample to assess the methylation status of the *SDC2* gene in Study 10, where a substantial difference was identified between CRC and control samples (AUC: 0.85), with 81.5% sensitivity and 68.2% specificity. This suggests that *SDC2* methylation can be a promising CRC biomarker in whole blood samples.

Biomarkers such as *SEPT9* and *SDC2* seem to be the best for early detection in plasma samples because the combination of these biomarkers has been reported in Study 8 to have 81.8% positive methylation in CRC stages 0 to II. In this context, a gene panel is used to detect CRC from the plasma ColoDefense test, resulting in higher sensitivity and specificity of 85.7% and 86.8%, respectively. Also, the AUC value is 0.97, demonstrating better discrimination ability between the CRC and control groups. In comparison, when single gene was used, the resulting sensitivity and specificity were slightly lower, where sensitivity and specificity for *SEPT9* alone were 75.8% and 94.7%, and sensitivity and specificity for *SDC2* alone were 60.4% and 86.8%, respectively. This suggests that combined promoter methylation analysis in a gene panel may increase the accuracy of biomarkers in CRC detection, particularly in early-stage detection compared to single gene analysis.

Other than that, the included studies have also indicated the feasibility of using serum samples for identifying DNA methylation biomarkers. This can be demonstrated by Study 12 where the combination of *GALNT9* and *UPF3A* was utilized and demonstrated good capability in detecting CRC early-stage. The positive methylation in stage I and stage II for the combined gene are high enough which are 87.5% and 100%, respectively. However, when all combined genes from Study 12 were used with the combination of *GALNT9*, *UPF3A*, *WARS*, and *LBD2*, the resulting positive methylation for stage I and stage II was slightly lower. Hence, the used of combined *GALNT9* and *UPF3A* using serum samples has potential in early-stage CRC detection due to its high accuracy in detecting CRC stages I and II.

Besides plasma, serum and whole blood, stool offers a valuable medium in CRC detection due to the natural shedding of cancer cells into the colonic lumen. Based on the findings, several methylated genes were found in stool samples, namely *SDC2*, *CLIP4*, *KCNQ5* and *C9orf50*. The shedding of the tumor cells into the stool occurs before the invasion of blood vessels during CRC development (Ahlquist et al., 2012). Also, the concentration of ctDNA in stool samples is much higher than in plasma due to the dispersion of ctDNA throughout the total blood volume when it is introduced into the circulation (Cao et al., 2021). This results in higher sensitivity for detecting abnormal DNA methylation, making stool feasible for early detection

of CRC. The differences in the performance of methylated genes between different samples can be observed in the *SDC2* gene between Studies 3 and 8. Using stool samples, *SDC2* identified malignancy with higher sensitivity (83.8%) and specificity (98.0%), and AUC of 0.95 in detecting all CRC stages compared to plasma samples in Study 8 with slightly lower sensitivity (60.4%) and specificity (86.8%), and AUC of 0.80.

SDC2 is the most reported methylated gene in stool samples, as reported in Studies 2 and 3. Based on the results, both studies revealed a higher sensitivity and specificity to detect early-stage CRC, but Study 2 outperforms Study 3 in this case. Owing to this, the sensitivity of *SDC2* to detect stages 0 to II in Study 2 is 89.1%, meanwhile, for Study 3 is 87.0%. Additionally, the specificity of this biomarker in both studies is significantly greater, ranging from 90.2% to 98.0%, with an AUC of 0.90 to 0.95. This demonstrates that *SDC2* is a feasible biomarker with the potential to be a single precise biomarker in early CRC detection using stool samples.

Other than that, according to the findings in Study 7, *KCNQ5* and *C9orf50* can be considered as promising biomarkers in identifying early-stage CRC in stool samples. This is due to the high positive methylation in detecting stages 0, I, and II among CRC patients. For *KCNQ5*, the positive methylation in detecting stages 0, I, and II is 84.4%, 82.8% and 69.5%, respectively. Meanwhile, for *C9orf50*, the positive methylation in detecting stages 0, I and II is slightly higher than *KCNQ5* which is 90.6%, 87.9% and 84.7%, respectively. The study also highlighted that methylation of *C9orf50* alone is high enough rather than a combination of *KCNQ5* and *C9orf50* because *C9orf50* alone exhibits higher sensitivity and specificity to detect all stages, making it a good candidate for a single biomarker. Furthermore, the AUC value of *C9orf50* alone (0.90) is excellent in distinguishing CRC from non-CRC patients.

Besides *SDC2*, *KCNQ5* and *C9orf50*, *CLIP4* shows potential in early-stage CRC detection, as depicted in findings from Study 9. The reason for this is that it can identify stage I with an accuracy of 96.2% and stage II with an accuracy of 83.1%. All stool biomarkers from Studies 2, 3, 7, and 9 demonstrated immense potential in early-stage CRC detection. Among these biomarkers, *CLIP4* has the greatest accuracy with the highest sensitivity and AUC values of 90.3% and 0.96, respectively, making it a valuable biomarker with a strong ability for disease detection.

Overall, it can be concluded that *SDC2*, *KCNQ5*, *C9orf50*, *CLIP4*, the combination of *SEPT9* and *SDC2*, and the combination of *GALNT9* and *UPF3A* (from Studies 2, 3, 7, 8, 9, and 12) show promise as reliable DNA methylation biomarkers for early-stage CRC detection. Pooled data revealed that these biomarkers perform well, with specificity over 80% and sensitivity over 70%, and an AUC range of 0.85 to 0.97, indicating strong discriminatory ability. On the other hand, Studies 1, 4, 5, 6, 10, 11, 13, and 14 lacked information regarding early-stage detection status, hence it cannot be confirmed if the biomarkers from these studies are potential for early detection. However, certain biomarkers from these studies demonstrated good accuracy and could be further validated for their potential in early-stage CRC detection.

Apart from that, the evidence on the role of DNA methylation biomarkers was gathered and analysed from the selected studies. Findings from Studies 1, 5, 6, 11, and 14 indicate that DNA methylation-based biomarkers contribute to multiple aspects of CRC progression by influencing key processes in tumor development, such as proliferation, migration, cell transformation, metastasis, and angiogenesis.

DNA methylation is a crucial mechanism that is strongly associated with the expression of tumor suppressor genes. Alterations in DNA methylation patterns can lead to the silencing of these genes, thereby contributing to tumorigenesis (Jin et al., 2011). Study 1 highlighted the role of *SM22α* as a tumor suppressor. It has been reported that *SM22α* can inhibit cell proliferation and invasion, promote cell death, and potentially prevent metastasis of CRC cells. When this gene is aberrantly methylated, its tumor-suppressing functions are disrupted, which can promote CRC tumorigenesis. The study reported the downregulation of *SM22α* in CRC tissue rather than in the adjacent normal tissue of the CRC patient (Liu et al., 2018). Therefore, this study highlights the role of *SM22α*, indicating that its downregulation can enhance cell proliferation and metastasis in CRC.

Besides that, *WIF1* is a tumor suppressor gene mentioned in Study 6. The downregulation of this gene due to hypermethylation has been associated with promoting CRC tumorigenesis. According to the study, the repression of the *WIF1* gene results in the activation of Wnt signalling pathway, which is known to be crucial in cell transformation and cancer progression (Overs et al., 2021). Hence, this underlines the role of *WIF1* gene in cell transformation which potentially contributes to CRC progression.

Other than that, *TMEM240* is a gene reported to have a role in CRC tumorigenesis by influencing CRC cell growth and migration. Study 5 demonstrated that *TMEM240* may repress cell proliferation. This was revealed when overexpression of *TMEM240* suppressed the development of DLD-1 cells, which are known as CRC cells (Chang et al., 2020). Conversely, when *TMEM240* is silenced, the growth

of CRC cells increases, and the cells actively proliferate. Additionally, increased expression of *TMEM240* has been reported to suppress the migration of CRC cells. Therefore, alterations of *TMEM240* which causes its silencing, can have a substantial impact on the development of CRC in terms of cell growth and migration.

Furthermore, *LINC00473* which is known as long noncoding RNA, was found to be downregulated in CRC, as reported in Study 11. This gene can suppress cell proliferation and prevent the colonies formation by accumulating endogenous miR574-5p or miR15b-5p (Ruiz-Bañobre et al., 2022). However, when *LINC00473* is downregulated, its tumor suppressor capabilities which can promote cell apoptosis, are reduced. Hence, it is explained that *LINC00473* plays a significant role in CRC cell initiation and progression.

On the other hand, *FOXF1* is a crucial element in CRC progression as its increased expression is linked to the angiogenesis process in CRC, as stated in Study 14. It has been implicated that overexpression of the *FOXF1* gene results in increased epithelial-mesenchymal transition (EMT) gene signatures (Dastafkan et al., 2023). This underscores its role in promoting metastasis through EMT induction, making it a significant element in CRC progression. The findings on the role of DNA methylation-based biomarkers in CRC progression can imply the need for further interventions to enhance the diagnosis of CRC.

CONCLUSION

In conclusion, this systematic review underscores the potential of DNA methylation-based biomarkers in early-stage colorectal cancer (CRC) detection. Several promising biomarkers, including *SDC2*, *KCNQ5*, *C9orf50*, *CLIP4*, a combination of *SEPT9* and *SDC2*, and a combination of *GALNT9* and *UPF3A*, have been identified for their potential in early-stage CRC detection. Since these biomarkers exhibit high performance in terms of sensitivity, specificity, and AUC value, and can be identified in a non-invasive method, these findings support the use of DNA methylation biomarkers as effective tools for CRC detection. Beyond detection, DNA methylation biomarkers are also implicated in key aspects of CRC tumorigenesis such as cell proliferation, migration, transformation, metastasis, and angiogenesis. Understanding these roles can provide crucial insights into the early molecular events that lead to CRC. This knowledge can drive the development of highly sensitive and specific screening tools, improving the diagnosis of CRC.

ACKNOWLEDGEMENT

The authors would like to thank the lecturers at the Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia for the feedback on search strategy. All authors reviewed and contributed towards revising the final manuscript for important intellectual content. This research was not funded by any grant.

REFERENCES

Ahlquist, D. A., Taylor, W. R., Mahoney, D. W., Zou, H., Domanico, M., Thibodeau, S. N., Boardman, L. A., Berger, B. M., & Lidgard, G. P. (2012). The stool DNA test is more accurate than the plasma Septin 9 test in detecting colorectal neoplasia. *Clinical Gastroenterology and Hepatology, 10*(3), 272-277.e1. https://doi.org/10.1016/j.cgh.2011.10.008

Ahmed, F. and Ahmed, N. (2017). MicroRNAs as molecular markers for colon cancer diagnostic screening in stool and blood. *Medical Research and Innovations, 1*(2). https://doi.org/10.15761/mri.1000108

Ansar, M., Wang, C. J., Wang, Y. H., Shen, T. H., Hung, C. S., Chang, S. C., & Lin, R. K. (2020). SMAD3 hypomethylation as a biomarker for early prediction of colorectal cancer. *International Journal of Molecular Sciences, 21*(19), 7395. https://doi.org/10.3390/ijms21197395

Canzoniero, J. V., & Park, B. H. (2016). Use of cell free DNA in breast oncology. *Biochimica Et Biophysica Acta (BBA) - Reviews on Cancer, 1865*(2), 266–274. https://doi.org/10.1016/j.bbcan.2016.03.006

Cao, Y., Zhao, G., Cao, Y., Chen, Z., Liu, X., Yuan, M., Yang, J., Wang, X., Ma, Y., Liu, Z., Xiong, S., Zheng, M., & Fei, S. (2021). Feasibility of methylated *CLIP4* in stool for early detection of colorectal cancer: a training study in Chinese population. *Frontiers in Oncology, 11*. https://doi.org/10.3389/fonc.2021.647066

Cao, Y., Zhao, G., Yuan, M., Liu, X., Ma, Y., Cao, Y., Miao, B., Zhao, S., Li, D., Xiong, S., Zheng, M., & Fei, S. (2021). *KCNQ5* and *C9orf50* methylation in stool DNA for early detection of colorectal cancer. *Frontiers in Oncology, 10*. https://doi.org/10.3389/fonc.2020.621295

Chang, S. C., Liew, P. L., Ansar, M., Lin, S. Y., Wang, S. C., Hung, C. S., Chen, J. Y., Jain, S., & Lin, R. K. (2020). Hypermethylation and decreased expression of *TMEM240* are potential early-onset biomarkers for colorectal cancer detection, poor prognosis, and early

recurrence prediction. *Clinical Epigenetics, 12*(1). https://doi.org/10.1186/s13148-020-00855-z

Chen, R., Pagano, I., Sun, Y., Murakami, K., Goodison, S., Vairavan, R., … & Furuya, H. (2022). A diagnostic gene expression signature for bladder cancer can stratify cases into prescribed molecular subtypes and predict outcome. *Diagnostics, 12*(8), 1801. https://doi.org/10.3390/diagnostics12081801

Chen, Z., Zhao, G., Wang, K., Wang, X., Ma, Y., Xiong, S., Zheng, M., & Fei, S. (2021). Blood leukocytes methylation levels analysis indicate methylated plasma test is a promising tool for colorectal cancer early detection. *Journal of Cancer, 12*(12), 3678–3685. https://doi.org/10.7150/jca.57114

Crowe, M. (2015). Crowe Critical Appraisal Tool (v1.4). Conchra. https://conchra.com.au/2015/12/08/crowe-critical-appraisal-tool-v1-4/

Dastafkan, Z., Rezvani, N., & Amini, S. (2023). Diagnostic value of *FOXF1* gene promoter-methylated DNA in the plasma samples of patients with colorectal cancer. the International Journal of *Biological Markers, 38*(3–4), 194–202. https://doi.org/10.1177/03936155231207109

Doubeni, C. A., Corley, D. A., Quinn, V. P., Jensen, C. D., Zauber, A. G., Goodman, M., Johnson, J. R., Mehta, S. J., Becerra, T. A., Zhao, W. K., Schottinger, J., Doria-Rose, V. P., Levin, T. R., Weiss, N. S., & Fletcher, R. H. (2016). Effectiveness of screening colonoscopy in reducing the risk of death from right and left colon cancer: a large community-based study. *Gut, 67*(2), 291–298. https://doi.org/10.1136/gutjnl-2016-312712

Gallardo-Gómez, M., Rodríguez-Girondo, M., Planell, N., Moran, S., Bujanda, L., Etxart, A., Castells, A., Balaguer, F., Jover, R., Esteller, M., Cubiella, J., Gómez-Cabrero, D., & De Chiara, L. (2023). Serum methylation of *GALNT9*, *UPF3A*, WARS, and LDB2 as noninvasive biomarkers for the early detection of colorectal cancer and advanced adenomas. *Clinical Epigenetics, 15*(1). https://doi.org/10.1186/s13148-023-01570-1

Han, B., Zheng, R., Zeng, H., Wang, S., Sun, K., Chen, R., Li, L., Wei, W., & He, J. (2024). Cancer incidence and mortality in China, 2022. *Journal of the National Cancer Center*. https://doi.org/10.1016/j.jncc.2024.01.006

Han, Y. D., Oh, T. J., Chung, T. H., Jang, H. W., Kim, Y. N., An, S., & Kim, N. K. (2019). Early detection of colorectal cancer based on presence of methylated syndecan-2 (*SDC2*) in stool DNA. *Clinical Epigenetics, 11*(1). https://doi.org/10.1186/s13148-019-0642-0

Jin, B., Li, Y., & Robertson, K. D. (2011). DNA methylation: superior or subordinate in the epigenetic hierarchy? *Genes & Cancer, 2*(6), 607–617. https://doi.org/10.1177/1947601910393957

Lima, A. B., Reis, M. B. D., Matsushita, M., Reis, M. T. D., De Oliveira, M. A., Reis, R. M., & Guimarães, D. P. (2023). Combined *SEPT9* and BMP3 methylation in plasma for colorectal cancer early detection and screening in a Brazilian population. *Cancer Medicine, 12*(15), 15854–15867. https://doi.org/10.1002/cam4.6224

Liu, Y., Wei, E., Zhao, J., Kong, D., & Li, B. (2018). Downregulation of *SM22α* protein by hypermethylation of its promoter in colorectal cancer. *Oncology Letters*. https://doi.org/10.3892/ol.2018.8350

Locke, W. J., Guanzon, D., Ma, C., Liew, Y. J., Duesing, K. R., Fung, K. Y., & Ross, J. P. (2019). DNA methylation cancer biomarkers: translation to the clinic. *Frontiers in Genetics, 10*. https://doi.org/10.3389/fgene.2019.01150

Miller, K. D., Nogueira, L., Mariotto, A. B., Rowland, J. H., Yabroff, K. R., Alfano, C. M., Jemal, A., Kramer, J. L., & Siegel, R. L. (2019). Cancer treatment and survivorship statistics, 2019. CA: *A Cancer Journal for Clinicians, 69*(5), 363–385. https://doi.org/10.3322/caac.21565

Ministry of Health Malaysia. (2021). *National Strategic Plan for Colorectal Cancer 2021-2025*. Ministry of Health Malaysia. https://www.moh.gov.my/moh/resources/Penerbitan/Rujukan/NCD/Kanser/National_Strategic_Plan_for_Colorectal_Cancer_(NSPCRC)_2021-2025.pdf

Overs, A., Flammang, M., Hervouet, E., Bermont, L., Pretet, J. L., Christophe, B., & Selmani, Z. (2021). The detection of specific hypermethylated *WIF1* and NPY genes in circulating DNA by crystal digital PCRTM is a powerful new tool for colorectal cancer diagnosis and screening. *BMC Cancer, 21*(1). https://doi.org/10.1186/s12885-021-08816-2

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., . . . Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Systematic*

Reviews, *10*(1). https://doi.org/10.1186/s13643-021-01626-4

Pontone, S., Lauriola, M., Palma, R., Panetta, C., Tomai, M., & Baker, R. (2022). Do difficulties in emotional processing predict procedure pain and shape the patient's colonoscopy experience?. *BMJ Open, 12*(2), e050544. https://doi.org/10.1136/bmjopen-2021-050544

Ruiz-Bañobre, J., Rodriguez-Casanova, A., Costa-Fraga, N., Bao-Caamano, A., Alvarez-Castro, A., Carreras-Presas, M., Brozos-Vazquez, E., Vidal-Insua, Y., Vazquez-Rivera, F., Candamio-Folgar, S., Mosquera-Presedo, M., Lago-Lestón, R. M., Muinelo-Romay, L., Vázquez-Bueno, J. N., Sanz-Pamplona, R., Moreno, V., Goel, A., Castillo, L., Martin, A. C., . . . Díaz-Lagares, A. (2022). Noninvasive early detection of colorectal cancer by hypermethylation of the *LINC00473* promoter in plasma cell-free DNA. *Clinical Epigenetics, 14*(1). https://doi.org/10.1186/s13148-022-01302-x

The American Cancer Society. (2024). Colorectal Cancer Statistics | How Common Is Colorectal Cancer? American Cancer Society. https://www.cancer.org/cancer/types/colon-rectal-cancer/about/key-statistics.html

Thomsen, M., Rasmussen, M., Njor, S., & Mikkelsen, E. (2018). Demographic and comorbidity predictors of adherence to diagnostic colonoscopy in the Danish Colorectal Cancer Screening Program: a nationwide cross-sectional study. *Clinical Epidemiology*, Volume 10, 1733-1742. https://doi.org/10.2147/clep.s176923

Wang, J., Liu, S., Wang, H., Zheng, L., Zhou, C., Li, G., Huang, R., Wang, H., Li, C., Fan, X., Fu, X., Wang, X., Guo, H., Guan, J., Sun, Y., Song, X., Li, Z., Mu, D., Sun, J., . . . Zou, H. (2020). Robust performance of a novel stool DNA test of methylated *SDC2* for colorectal cancer detection: a multicenter clinical study. *Clinical Epigenetics, 12*(1). https://doi.org/10.1186/s13148-020-00954-x

World Health Organization (2020). Colorectal cancer Source: Globocan 2020. https://gco.iarc.fr/today/

World Health Organization. (2023). Colorectal Cancer. World Health Organization (WHO). https://www.who.int/news-room/fact-sheets/detail/colorectal-cancer

Wu, D., Guangpeng, Z., Jin, P., Zhu, J., Li, S., Qi, W., … & Qian, J. (2016). Detection of colorectal cancer using a simplified *SEPT9* gene methylation assay is a reliable method for opportunistic screening. *The Journal of Molecular Diagnostics, 18*(4), 535-545. https://doi.org/10.1016/j.jmoldx.2016.02.005

Ye, J., Zhang, J., & Ding, W. (2024). DNA methylation modulates epigenetic regulation in colorectal cancer diagnosis, prognosis and precision medicine. *Exploration of Targeted Anti-tumor Therapy, 5*(1), 34–53. https://doi.org/10.37349/etat.2024.00203

Yuan, L. (2024). DNA Methylation patterns as biomarkers for cancer diagnosis and prognosis. *Biology and Medicine*, *16*(7). https://doi.org/10.35248/0974-8369.24.16.707

Zhang, Y., Wang, Y., Zhang, B., Li, P., & Zhao, Y. (2023). Methods and biomarkers for early detection, prediction, and diagnosis of colorectal cancer. *Biomedicine & Pharmacotherapy*, 163, 114786. https://doi.org/10.1016/j.biopha.2023.114786

SUPPLEMENTARY DATA

Annexure 1: Characteristics of Included Studies

Study No	Author and Publication Year	Journal of Publications	Title of Study	Study Design	Study Population	Country
1.	Liu, Y. et al. (2018)	*Oncology Letters*	Downregulation of *SM22α* protein by hypermethylation of its promoter in colorectal cancer	-	78	China
2.	Han, Y. D. et al. (2019)	*Clinical Epigenetics*	Early detection of colorectal cancer based on presence of methylated syndecan-2 (*SDC2*) in stool DNA	Retrospective case and prospective control study	585	South Korea
3.	Wang, J. et al. (2020)	*Clinical Epigenetics*	Robust performance of a novel stool DNA test of methylated *SDC2* for colorectal cancer detection: a multicenter clinical study	Multicenter clinical study	1110	China
4.	Ansar, M. et al. (2020)	*International Journal of Molecular Sciences*	*SMAD3* hypomethylation as a biomarker for early prediction of colorectal cancer	-	548	China
5.	Chang, S. et al. (2020)	*Clinical Epigenetics*	Hypermethylation and decreased expression of *TMEM240* are potential early-onset biomarkers for colorectal cancer detection, poor prognosis, and early recurrence prediction	Case-control study	556	China
6.	Overs, A. et al. (2021)	*BMC Cancer*	The detection of specific hypermethylated *WIF1* and *NPY* genes in circulating DNA by crystal digital PCR™ is a powerful new tool for colorectal cancer diagnosis and screening	Cohort study	45	France
7.	Cao, Y. et al. (2021)	*Frontiers in Oncology*	*KCNQ5* and *C9orf50* methylation in stool DNA for early detection of colorectal cancer	-	460	China

Annexure 1: (Cont.)

8.	Chen, Z. et al. (2021)	*Journal of Cancer*	Blood leukocytes methylation levels analysis indicate methylated plasma test is a promising tool for colorectal cancer early detection	Validation cohort study	213	China
9.	Cao, Y. et al. (2021)	*Frontiers in Oncology*	Feasibility of methylated *CLIP4* in stool for early detection of colorectal cancer: a training study in chinese population	Case-control study	321	China
10.	Siri, G. et al. (2022)	*Journal of Cancer Research and Therapeutics*	Analysis of *SDC2* gene promoter methylation in whole blood for noninvasive early detection of colorectal cancer	Case-control study	130	Iran
11.	Ruiz-Bañobre, J. et al. (2022)	*Clinical Epigenetics*	Noninvasive early detection of colorectal cancer by hypermethylation of the *LINC00473* promoter in plasma cell-free DNA	Retrospective cohort study	868	Spain
12.	Gallardo-Gómez, M. et al. (2023)	*Clinical Epigenetics*	Serum methylation of *GALNT9*, *UPF3A*, *WARS*, and LDB2 as noninvasive biomarkers for the early detection of colorectal cancer and advanced adenomas	Multicenter cohort study	433	Spain
13.	Lima, A. B. et al. (2023)	*Cancer Medicine*	Combined *SEPT9* and *BMP3* methylation in plasma for colorectal cancer early detection and screening in a Brazilian population	-	262	Brazil
14.	Dastafkan, Z. et al. (2023)	*The International Journal of Biological Markers*	Diagnostic value of *FOXF1* gene promoter-methylated DNA in the plasma samples of patients with colorectal cancer	Case-control study	100	Iran