REVIEW ARTICLE

Circulating Tumor Cells (CTCs) and Red Blood Cells (RBCs) Interactions and Their Potential Clinical Applications: A Scoping Review

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ABSTRACT

Background: Circulating tumor cells (CTCs) are rare tumor cells that spread cancer through the bloodstream. As for red blood cells (RBCs), they play a crucial role in oxygen transport and interaction with cancer cells. CTCs adhere to RBCs, increasing survival and metastasis. Physical interactions and hemodynamic forces influence the distribution and circulation of CTCs, triggering immune responses and activating red blood cells, promoting tumor growth and metastasis. These interactions have significant clinical applications. Previous studies proved that the interactions between RBCs and CTCs were observed. These findings may be a new understanding and development in cancer studies. Therefore, this scoping review aims to identify on how those interactions remain in the body system that may be the metastasis contribution towards CTCs. **Methods:** This study will focus on scoping review that involves a few articles and research papers that have been filtered from online databases such as Scopus, ScienceDirect and PubMed. The process of selecting articles will be followed by Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) 2020 guidelines. Experimental research articles published in English between 2018 until 2023 will be included in this study to be reviewed. **Results:** Throughout the screening process, 7 articles were retrieved as included articles in this paper. Across the study, it showed that RBCs play a different role in the interactions with CTCs that contribute to the cell metastasis and survival. Additionally, a few interactions with different types of cancer cells were reported in a few studies that were clearly unexplored in other studies. These studies used different approach on how to conduct the studies but not to mention that similarly they used *in vitro* as the part of study model. **Conclusion:** From this review, it can be concluded that interactions of RBCs and CTCs were great findings in cancer studies. In the future, continuous study on the interactions may come across with therapeutic therapy study that may help in cancer therapy.

INTRODUCTION

Circulating tumor cells (CTCs) and red blood cells (RBCs) are two cells that play important roles in tumor dispersion and metastasis, eventually contributing to cancer progression and death (Agarwal et al., 2018). CTCs are cancer cells that have shed from the main tumor and entered the circulation, where they might travel to distant organs and form new tumor colonies. Conversely, RBCs may transport oxygen and nutrients to CTCs, allowing them to survive in circulation and increasing their metastatic potential (Lal et al., 2015).

Recent study has revealed that CTCs may interact with RBCs in several ways, including physical adhesion, chemical exchange, and signalling. These interactions have the potential to have a significant influence on CTCs behavior and metastasis. According to Alix Panabières-Pantel (2021), physical adhesion between CTCs and RBCs, for instance, can shield CTCs from immune monitoring and promote their extravasation into secondary tumor sites. In some cases, CTCs may acquire nutrients and oxygen from RBCs, enhancing their survival and potential for metastasis. This exchange can occur through direct contact or via membrane transporters (Zhang et al., 2022). Besides, there was a study mentioned that TGF-β signalling is also involved in the interactions. According to Derynck et al. (2017), TGF-β binding to its receptors on RBCs downregulates their immune function and promotes their activation, further supporting CTCs survival and metastasis. The interactions between CTCs and RBCs are complicated and not entirely understood, but they constitute a prospective target for cancer treatment. It may be able to prevent CTCs metastasis and enhance patient outcomes by interrupting these connections.

The mechanisms underlying the formation of RBC-CTC clusters and their impact on CTC biology still need to be fully understood. Moreover, the role of specific molecular interactions between RBCs and CTCs in promoting tumor cell survival and metastasis remains unclear. Therefore, this study objectives are to provide an overview of the possible interactions between circulating tumor cells and red blood cells and the potential therapeutic clinical manifestation. Besides, this study also conducted to identify the effects of CTCs-RBCs interactions on CTCs survival in the human body. In addition, within the study can be explored the potential of immunotherapies to

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modulate CTCs and RBCs interactions. Hence, identify the problems research gap on the interactions between RBCs and CTCs and the potential clinical applications.

CTCs represent a crucial phase in metastatic cascades, the process which tumors spread throughout the body system. Understanding the relative interactions of CTCs and others cells holds immense advantages for the development of therapeutic approach. This review may contribute to emphasize the interactions of CTCs and RBCs, whereas can develop a novel and more effective cancer therapies.

MATERIALS AND METHODS

Study Design

This study mainly will conduct scoping review as a study design. Scoping reviews are a technique used to systematically map the evidence across a variety of study designs in a field (O'Brien et al., 2016). They aim to map the breadth and depth of research on a particular topic, identify gaps in the literature, and inform future research directions. Scoping reviews are a valuable tool for synthesizing research and informing practice, particularly for exploring new topics or providing an overview of rapidly developing fields. They are particularly useful for exploratory research and identifying areas that warrant further investigation.

The study aims to systematically map the existing literature on the interactions between CTCs and RBCs. This helps to provide a comprehensive overview of the current state of research in this area. By reviewing a wide range of studies, the scoping review identifies gaps in the current understanding of CTCs and RBCs interactions. This highlights areas where further research is needed, such as the specific molecular mechanisms involved and the potential therapeutic applications. The scoping review serves as a preliminary step to assess the volume and nature of the available evidence on CTCs and RBCs interactions. This helps to determine whether a full systematic review is feasible and warranted.

Search Strategies

The scientific papers that studied those topics were obtained through a list of reliable publishers such as Scopus, PubMed, Science Direct, Springer Link and Research Gate search engines. The main topic focused on the possible interactions between circulating tumor cells (CTCs) and red blood cells (RBCs) and the potential clinical applications to enhance cancer therapies. In order to assist the research process, some highlighted keywords that will be utilized in this research study would be "Circulating Tumor Cells", "Red Blood Cells" "Interactions", "Clinical Applications", "mechanisms" and other relevant keywords to assist the research process. The word "AND" was placed between the keywords to narrow the search while the term "OR" widen the exploration. The collected journal or articles were filtered using the Arksey and O'Malley Method Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA, 2020) Guideline. PRISMA 2020 Guidelines is significant as it benefits to ensure the quality of the review, it allows the readers to access the strengths and weaknesses of the articles.

Selection Criteria

There are some criteria set for the review finding (Table 1). These criteria help to ensure that the review is focused on high-quality review. The study must be experimental and journal-based to provide relevant ideas and evidence on the topic. The language restriction ensures that the articles can easily understand the studies, while the date restriction within 2017 onwards ensures that the review is up-to-date. The focus on CTCs and RBCs are obviously relevant to the topic of the review, and the inclusion of studies on the interactions between these two cell types broadens the scope of the review to include the latest research in this area. Finally, the requirement that the studies be available as full-text articles ensures that the reviewers can access all of the necessary information to properly evaluate them.

Table 1: Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
● Research articles that published in English. ● Full text articles. ● Published between 2018 until 2023. ● Experimental papers	● Articles do not meet the objectives of study. ● Book chapter, review paper, systematic review or discussion paper.

Data Extraction

Data been extracted and analysed from screened articles that involved information on author names, publication year, type of interaction, model of study, cancer cells involved, and mechanism approach in the study. This data was tabulated to provide an overview on how the discussion will be concluded on interaction of RBCs and CTCs.

RESULTS AND DISCUSSION

Based on the screening of articles, a number of 2134 potential articles were resulted from selected databases including Scopus, Science Direct and PubMed. Duplicated articles were recorded with similar titles, in resulting 261 articles been removed and screened out. Then, the 1873 articles were screened by titles and abstracts. From the number of articles, 1839 articles were removed as these articles were not meet the discussion on this study. The remaining of the articles were screened out on the availability either it is full text access and open access. All the articles were retrieved. Remaining 34 articles were screened for inclusion and exclusion criteria. 25 articles were removed as it does not meet the main objectives of the discussion, and 2 articles were excluded because the articles were review paper. Finally, 7 articles were included for data extraction on this study. The flow of the screening process has been illustrated using PRISMA 2020 Flow Diagram as Figure 1 shows all phases of screening and elimination process.

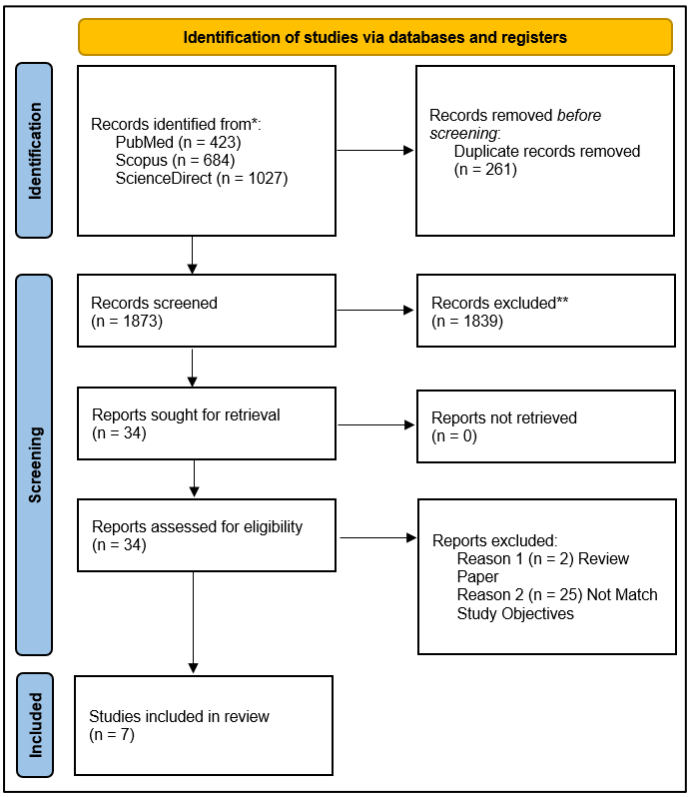

Figure 1: PRISMA flow diagram

Mechanism of Approach on CTCs-RBCs Interaction

Circulating Tumor Cells (CTCs) is type of secondary tumor cells that initiate into metastasize of tumor cells throughout body system. Interactions between other cells such as cell adhesion, signalling pathways and molecules exchange contribute to its proliferation and survival in body system. It was observed that RBCs do interact with CTCs that can contribute to its survival and proliferation as well as other cells. These interactions can be shown in Table 2 that conclude the interaction throughout the study. From the articles, most of the study findings resulting on cell adhesion and migration as one of the interactions that can be seen on towards CTCs and RBCs. Meanwhile, protein profile such as Lysosome-Associated Membrane Protein 2 (LAMP2) were observed on RBCs that associated with breast cancer as the hallmark biomarkers in the breast cancer. Throughout the study, it can be seen that there are a few interactions of RBCs and CTCs been concluded and it comes with different of approachable mechanism to illustrate on how the interactions occur in the body system.

Firstly, Liang et al, (2020) used an approach of bioinformatic tools which is LinkedOmics database to analyse the co-expression genes related to Erythrocyte Membrane Protein Band 4.1 Like 1 (EPB41L1) in Kidney Renal Cell Carcinoma (KIRC). LinkedOmics is a platform that allows biologists and clinicians to access, analyse, and compare cancer multi-omics data inside and across tumour types. The LinkedOmics database combines multi-omics and clinical data for 32 cancer types and 11,158 patients from the Cancer Genome Atlas (TCGA) project. This database reveals that their involvement in cell adhesion pathways through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were analysed.

Besides that, Pereira-Veiga et al., (2023) used an approach of proteomic profile to analyze the protein composition of RBCs in cancer patients. In this study, RBCs were observed contain LAMP2 and Purine Nucleoside Phosphorylase (PNP) that associated with breast cancer metastasis. LAMP2 can be found on the surface of RBCs membrane aside with other glycoproteins. This LAMP2 were reported that associated in breast cancer (BC) metastasis which is provide a proper binding that can provide help to BC from oxidative damage and their survival. Besides LAMP2, PNP also reported express on RBCs that associated on BC for its survival and proliferation. Additionally, through this approach of study it had been reported that amino acids also played roles in the interaction between CTCs and RBCs that provide a decent nutrient for metabolism of tumor cells for its survival and proliferation.

Apart from that, Zhu et al., (2018) used an approach by engineered RBCs with Folate receptor and Magnetic Nanoparticles (MNPs) on their surface in artificial blood samples. This approach reported to engineer RBCs to enhance its adherence to CTCs. In result, it could rapidly adhere RBCs to CTCs which lead to a better adhesion that provide cell survival and proliferation. However, this

approach was purposely been used to allows them to effectively capture cancer cells in the bloodstream.

Meanwhile, Roychowdury et al., (2023) used an approach of the advanced APR method to stimulate cancer cell transport while maintaining a local region of RBCs. Each cell is modeled as a fluid-filled membrane with both elasticity and bending stiffness, using a Lagrangian surface mesh composed of triangular elements. The authors created algorithms to solve problems including connecting regions with different viscosity, keeping hematocrit within the window, and shifting the window while retaining the dynamics of the CTCs and neighbouring RBCs. The study focuses on the fluid dynamics and biomechanical elements of how CTCs move in the bloodstream, particularly the effects of RBCs on CTCs trajectory. The authors aim to understand how the presence of RBCs influences the movement of CTCs and how these interactions can be accurately modeled computationally.

Table 2: Evidence on RBCs-CTCs Interactions

Author (Publication Year)	Type of Interactions	Model of study design	Cancer cells	Mechanism approach
Liang et al. 2020	Cell adhesion and migration	In vitro	Human KIRC cell line 786-O	Linkedomics database was used to analyze co-expression genes related to EPB41L1 in KIRC, revealing their involvement in cell adhesion pathways through GO and KEGG pathway analysis.
Pereira-Veiga et al. 2023	Protein profile that associated with BC metastasis	In vitro	Human breast cancer	Proteomic approaches to analyze the protein composition of red blood cells in cancer patients, particularly those with breast cancer.
Zhu et al. 2018	Cell adhesion	In vitro	Human breast carcinoma cell line MCF-7 & colorectal cancer cell line HCT116	The engineered RBCs, with folate receptor (FA) and magnetic nanoparticles (MNPs) on their surface, could rapidly adhere to CTCs in artificial blood samples.
Roychowdury et al. 2023	Cells motion and trajectory	In vitro	Human brain cancer & Human Breast Cancer	The advanced APR method to simulate cancer cell transport over a mm-scale distance while maintaining a local region of RBCs.
Pepona et al. 2020	Cell adhesion	In vitro	Murine mammary gland carcinoma cells (4T1, ATCC)	3D simulations of fluid flow and CTCs transport in the reconstructed and idealized bifurcated vessel.
Wang et al. 2021	Cell margination and adhesion	In vitro	-	3D simulation on the behaviors of the tumor cells in a real microvascular network.
Tan et al. 2019	Cell adhesion and trajectory	In vitro	Human prostate cancer cell (PC-3 cell)	A coupled fluid-solid interaction model was used to study cancer cell transport and adhesion in microfluidic devices.

Pepona et al., (2020) used an approach of numerical simulations by applying Three-dimensional (3D) simulations of fluid flow to transport CTCs in the reconstructed vessel. The researchers used 3D numerical simulations to determine how hemodynamic parameters influence the locations of tumour cell arrest and adhesion. The model utilised in the study included numerical simulations to explore the behaviour of CTCs and their interactions with the local hydrodynamics. Fluid flow and CTCs transport simulations in the reconstructed and idealised bifurcated vessel were carried out with an in-house massively parallel computational fluid dynamics solver.

Furthermore, Wang et al., (2021) used an approach of 3D simulation to generate a realistic microvascular network. The study used a hybrid method that combines smoothed dissipative particle dynamics and the immersion boundary

method (SDPD-IBM) to mimic tumour cell behaviour in the presence of RBCs in a microvascular network constructed from rat mesentery. The research focused on the effects of RBCs on the margination and adhesion dynamics of tumour cells within the microvascular network to characterise the interactions between tumour cells and RBCs.

Moreover, Tan et al., (2019) used an approach of numerical simulations which a coupled fluid solid interaction model was used to study cancer cell transport and adhesion in microfluidic devices. The study evaluated the impact of cell size and ligand density on cell adherence to cylinder surfaces. The cells showed transient rolling behaviour in the collective cell trajectory study. The paper learned the collision consequences of RBCs on cancer cells, specifically the impact on CTCs trajectories. The study's numerical simulations sought to assess the binding behaviour of CTCs under a variety of situations, including varied molecule densities and the presence of RBCs.

Research Design Related

Across the review study, it was clearly shown that most of the methods involved *in vitro* methods to conduct the study in cell culture. However, each study using a different method as an approached to their studies. According to Tan et al. (2019) and Pepona et al. (2020), their studies show that numerical simulations method been used to illustrated the three-dimensional microfluidic condition of vessels that equals to the human body system. The idea was to provide the real microvascular condition on how does the CTCs were transported in the vessels. On the other hands, Roychowdury et al. (2023) simulated different methods such as Advanced Physics Refinement (APR) method and Smoothed Dissipative Particle Dynamics and Immersed Boundary Method (SDPD-IBM) to provide the ideas on how does the interactions between CTCs and RBCs are applied as the microvascular network condition. Bioinformatics tool, as an example Linkedomics database been used to express genes that related with the interactions of both cells. Additionally, engineered method also been used by Zhu et al. (2018) to engineered the RBCs as the model of study to understand the interaction on both cells.

Cancer Cells Related

Four studies which are by Pereira-Veiga et al., (2023), Zhu et al., (2018), Roychowdury et al., (2023) and Pepona et al., (2020) set breast cancer cells as the selection model cells for the study. Breast cancer cell lines widely been used in any research studies related with cancer and tumor. This situation provides significant benefits on research studies as what been reported by Raghavendra et al., (2020), that breast cancer cells share similar biological microenvironment with others cancerous cells and sometimes also having a same gene expression such BRCA1 and BRCA2 genes that also expressed on ovarian cancers. These similar conditions can provide insights into other cancer cells as well. In contrast, according to Tan et al. (2019), the cancer cells been used were prostate cancer cell lines and Liang et al. (2020) kidney renal clear carcinoma cell (KIRC) been used as the model of their studies.

This review provides a few understanding on how interactions of CTCs and RBCs were illustrated. In future a few key aspects should be compromise to enhance the study in the field of interactions between CTCs and RBCs. Firstly, most of the studies were conducted in vitro model of study. This model of study is limited as it does not consider the complexity of a live thing. In vitro studies often employ isolated cells or tissues that do not interact with other cells and tissues in the same manner that they would in the body. This can make it challenging to extrapolate in vitro study results to what would happen in a full organism. Therefore, future researcher can consider of in vivo model of study as it considers the real scenario of body system without change the setting of the real vascular system. Besides, the review come across a few interactions that been observed in the study which were not deeply discussed because of limitation on the study such as LAMP2 and PNP functions in RBCs that influenced CTCs metastasis. However, with these findings it will be more interesting if the future researchers continue on the recent study of PNP and LAMP2 to discover it functions in the interactions of RBCs and CTCs in the future. Additionally, using a different approach on cancer cells may also consider as a new understanding on the interactions. This is because a clear understanding of each cancer cells need to be considered as it has different physiological conditions that need to be discovered.

CONCLUSION

This study emphasized that CTCs had an interaction with RBCs in order to survive and proliferate in body system. The review on other studies concurred that there are a few interactions on RBCs and CTCs that contribute to CTCs metastasis. In addition, the researchers had illustrated a few methods that proved on the contribution of the interactions of RBCs and CTCs in the metastasis of CTCs. The objectives of the study to identify the interactions of CTCs and RBCs have been achieved. The studies are prone only highlighting the interactions between CTCs and RBCs, which are not specifically observed on the therapeutic approach. This may be a limitation for the studies because

it needs to be developed to achieve the objectives on the potential therapeutic. In future, this study can expand to develop a promise therapeutic approach as cancer therapy. More research and studies are necessary in order to provide a full understand on the interactions of RBCs and CTCs. The development of technology should be taken as an aid to improvise the studies in the future.

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