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Circulating tumor cells as an emerging tool in cancer therapy

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1. ABSTRACT

Majority of the cancer-related deaths are related to metastasis during which cancer cells invade the surrounding tissues, enter (intravasation) and exit (extravasation) the peripheral circulation and seed distant organs. The **C**irculating **T**umor **C**ells (CTCs) exist in peripheral blood as single cells or as oligoclonal clusters of tumor cells along with platelets and lymphocytes. Detection of CTCs allows characterizing the tumors by their genotype and in predicting the prognosis and response to therapy and explants derived from these cells can be used in drug screening. In this review, we highlight the methods used for isolation and culture of CTCs and their clinical use.

2. INTRODUCTION

Our understanding of the molecular mechanisms involved in tumorigenesis has singificantly improved in the last decade. The

Table 1. Summary of the culture conditions reported for long term CTC culture

Cancer type	No of patients included in the study	No of established CTC lines	Method for isolation of CTC	Culture conditions	Ref
Breast	36	6	CTC-iChip (74) Principle: Hydrodynamic cell sorting Inertial focusing and Magnetophoresis	Ultra low attachment plate; RPMI-1640 medium with EGF (20ng/ml), basic FGF (20ng/ml) and B27 (10ml) in hypoxic condition (4% O_2)	73
Colon	71	1	RosetteSep (CD45 –ve selection)	Medium 1: DMEM/F12 medium with insulin (20 µg/mL), 1% N_2 complement, EGF: 20 ng/mL, L-Glutamine (2mM), FGF2: 10 ng/mL and 2% FBS for 2 weeks in hypoxic condition (2% O_2) Medium 2: RPMI1640 medium with EGF, FGF-2, Insuline-Transferine-Selenium supplement and L-Glutamine in normoxia	75
Prostate	17	1	Ficoll-Paque density gradient separation and RosetteSep (CD45 –ve selection)	DMEM/F12 medium with EGF (50 ng/ml), R-spondin 1 (5% v/v), Noggin (10% v/v), FGF10 (10 ng/ml), FGF2 (1 ng/ml), DHT (0.1nM for CRPC samples, 1nM for hormone-sensitive samples), Nicotinamide (10 mM), A83-01 (0.5 µM), SB202190 (10 µM), Y-27632 (10 µM), B27 additive (1X), N-Acetyl-L-cysteine (1.25 mM), glutamax (2 mM), HEPES (10 mM) and primocin (1:100 v/v)	76
Small cell, Lung	30	3	Ficoll-Paque density gradient separation	RPMI-1640 medium with insulin, IGF-1, transferrin, and selenite	136

genomic data sets from TCGA (The Cancer Genome Atlas) (1) and ICGC (International Cancer Genome Consortium) (2) have a significant impact on the understanding of the biology of tumor cells and thereby leading to the new era of precision medicine. However, with the sophisticated technologies to sequence cancer cells, we narrowed down to the static view of tumor development. But it is well understood that the patient on chemotherapy/ targeted therapy would develop resistance over the course of time (3, 4). Hence, the treatment regimen needs to be continuously monitored during the treatment. Therefore, the current clinical setting demands a rather dynamic view, which would enhance our knowledge on the patient-specific mutational changes for a particular treatment.

Circulating tumor cells (CTCs) are the cancer cells in blood circulation, which shed from primary sites or from metastatic deposits. CTCs exist either as a single cell or as a cluster of cells in the form of microemboli, held together by intercellular junctions. Recent studies suggest that CTCs provide real-time information on the dynamic changes of tumor progression (6-10). The molecular characterization of CTCs would potentially be an important aspect to understand the metastatic cascade in tumor development (11, 12). With the recent technological advancements, several CTCs detection and isolation methods have emerged in the last decade. However, the complexity of their biology in relation to cancer progression and metastasis is still a major challenge towards clinical utility. Only a few studies until today has been reported, where the researchers have successfully cultured CTCs in *ex-vivo* condition for its functional validation (13) (Table 1). In this review, we summarize various techniques and challenges in CTCs isolation, culture methods, CTCs-derived preclinical models and the role of CTCs in clinical research.

3. CTCs ISOLATION METHODS AND CHALLENGES

3.1. Methods for isolation of CTCs

Isolation of CTCs from the blood enables better characterization of the representative cells which accounts for the cancer progression and response to treatment. The rarity of CTCs in peripheral blood demands enrichment, which increases the probability to capture these cells for further genomic and functional characterization. As

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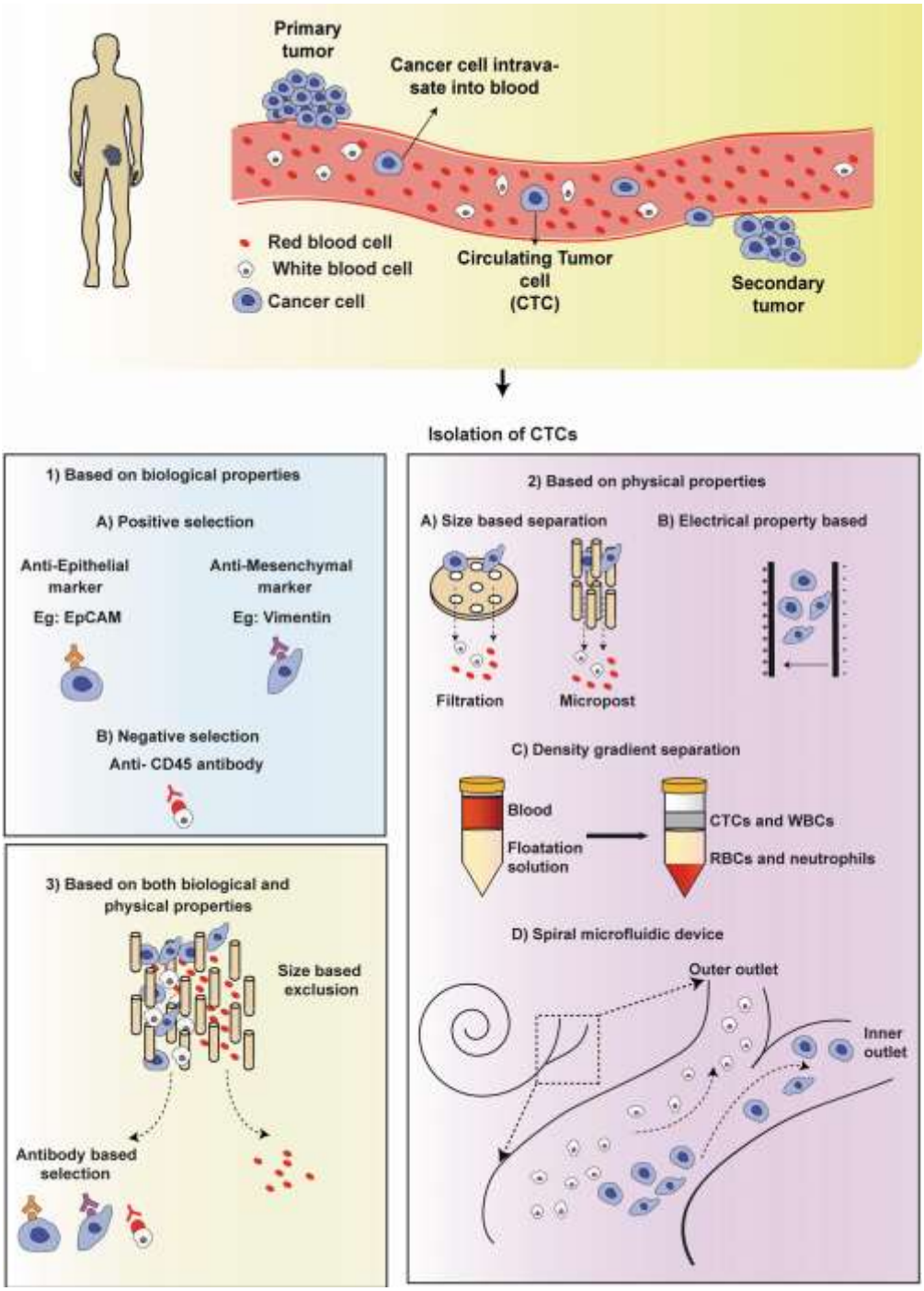

Figure 1. Current isolation techniques for CTCs.

there is no standard protocol for the isolation of CTCs, many strategies were developed in recent years. These techniques are either based on the physical or biological properties of CTCs (Figure 1). The isolation of CTCs by its biological properties relies on specific surface markers that are detected

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Table 2. Comparison of size and cell number of various cells in the blood and CTCs

Cell type	Size (µm)	Cell number (per ml)	References
Erythrocytes	6 - 8	4 - 6.2 billion	137-140
Neutrophils	15 - 17	1.8 - 7.7 million	
Basophils	10 - 14	0.25 - 1 million	
Eosinophil	12 - 15	0.05 - 0.4 million	
Monocyte	15 - 20	0.1 - 0.8 million	
Lymphocyte	6 - 15	1 - 4 million	
Platelets	2 - 3	0.1 - 0.4 billion	
CTCs	12 - 25	1-10	

by antibodies. CTCs can also be enriched on the basis of physical properties through the filtration-based method; a custom made microfluidic devices; centrifugation on ficoll density gradient and also based on dielectrophoresis.

3.1.1. Isolation of CTCs by biological properties

Antibody-based enrichment techniques can be broadly classified into positive and negative selection. In positive selection, epithelial markers such as EpCAM and cytokeratin are used for CTCs isolation, while in negative selection CD45 is used to deplete leukocytes. Till date, the CellSearch® system is the only FDA approved system to enrich CTCs. It is designed to isolate CTCs of epithelial origin (EpCAM +ve, cytokeratin (CK 8, 18 and 19) +ve and CD45 –ve) and has been clinically tested in breast (14), prostate (15) and colorectal (16) cancer patients. MagSweeper is another patented device, which uses magnetic beads functionalized with EpCAM antibody and has been reported clinically in the breast (17) and prostate (18) cancer patients. EPHESIA CTCs chip (19) and CTCs-chip (20) are two microfluidic-based devices, which capture CTCs by using specific antibodies. Moreover, CTCs can also be captured *in vivo* directly from the blood vessels of cancer patients. GILUPI CellCollector® used EpCAM functionalized medical wire, which is inserted into the cubital vein for 30 minutes which screens 1.5-3 liters of blood (21). It has been used to isolate CTCs from lung (22-25), breast (22, 26), prostate (27-30), neuroendocrine (31), head and neck (32) cancer patients.

3.1.2. Isolation of CTCs by physical properties

3.1.2.1. Size exclusion

The difference in thesize of CTCs from normal blood cells is reported in many cancers such as breast (33, 34) and prostate (35) (Table 2). Thus, the size-based exclusion of CTCs allows label-free enrichment by filtering out the cells of a certain size. It will allow the isolation of CTCs without any antibodies; hence the isolation of CTCs is not biased based on their EMT status. The size-based selection of CTCs is proven to be effective in non-small cell lung carcinoma (36). ScreenCell® is a commercially available filtration system which isolates, quantify and analyze the CTCs by allowing blood to pass through a microporous filter (37-39).

Membrane microfilter, which consists of a semipermeable membrane having a pore size of 8 µm is also suitable for CTCs isolation (40). The deposition of trapped cells in the filter can later inhibit the filtration process. This was overcome by the introduction of 3D filters (41), in which two filters are separated by a space. The upper layer has a pore size of 9 µm and the lower filter has a pore size of 8 µm. The smaller cells like RBCs and WBCs can transverse through the gap, whereas the CTCs will get trapped on the top layer. The relatively low flow rate of the 3D filters is overcome by using 2D membrane filters with slots. Higher viability and recovery rate are reported for 2D membrane slot filters. Spiral microfluidic device allows label-free size-based isolation of viable CTCs using hydrodynamic force. The spiral system could recover more than 85% of CTCs across multiple cancer cell lines (42).

3.1.2.2. Density-based centrifugation

Size and density-based isolation of CTCs can be achieved by using density gradient solutions such as sucrose gradient or Ficoll. The separation of the cellular constituents will achieve upon centrifugation of the blood sample relative to the density of suspension fluid. Subjecting to centrifugation, cells of different types will pass through the density gradient at different rates and

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will be settled in different layers. The cells such as RBCs, neutrophils will settle at the bottom layer whereas CTCs, plasma and mononuclear cells will settle on the top layer. Low cost and requirement of minimum equipment enable this method cheaper than others. The limitation of this method is the possibility to lose the cells of interest. Low recovery of cells has reported in Ficoll-Hypaque based density gradient separation in breast cancer (43). Percoll density gradient, which is based on silica particle suspension offers more efficiency in separating mononuclear cells, neutrophils, and platelets (46). However in another study by Chang *et al.*, contradicts the efficiency of Percoll to the Ficoll (47). Nevertheless, the pitfalls for the density gradient based enrichment are applicable for both Ficoll and Percoll.

3.1.2.3. Electrical property-based separation

The membrane potential of a cell is maintained by the combined activity of ion channels and transporters. It is shown that the electrical and dielectric properties such as membrane capacitance, membrane resistance, cytoplasmic conductivity, and permittivity are different for cancer cells and normal cells (48). The difference in these properties of the normal and cancer cells causes differential movement of cells under the non-uniform electric field. The differential movement of the particle under non-uniform electric is referred to as dielectrophoresis (DEP). DEP devices with various geometry have been used for the isolation of cancer cells from normal cells. CTCs isolation using the difference in dielectric property has been reported in oral cancer (49, 50), breast cancer (51), colon cancer (52) and prostate cancer cells (53). The major pitfall for this type of separation is the exponential decay of the electric field owing to the increase in distance from the electrode.

3.2. CTCs as a prognostic and predictive marker

CTCs play a vital role in metastatic cascade and hence can be exploited as a promising prognostic marker. The enumeration of CTCs has been correlated with poor prognosis; early relapse; progression-free and overall survival; recurrence; the advancement of the disease and the response to treatment in various cancer types. Identification of CTCs in the circulatory system has been associated with poor overall survival in breast and metastatic prostate cancers (54, 55). A meta-analysis of non-metastatic colorectal cancer patients has demonstrated that the presence of CTCs is associated with aggressive disease progression and poor survival (56). The enumeration of CTCs from the peripheral circulatory system has been related to the progression-free and overall survival of patients with breast (54, 57-59), non- small cell lung (60, 61), squamous cell carcinoma of head and neck (62), colorectal (63) and prostate cancers (64). The CTCs count in patients with bone-metastatic castration-resistant-prostate cancer (mCRPC) treated with docetaxelwas reported to be used as a predictor of overall survival (65). In breast cancer patients the advancement in the TNM stage is positively correlated to the number of CTCs detected (66, 67). Moreover, CTCs from lung cancer patients has been related to histological as well as TNM staging (61). In non-small cell lung cancer patients, the post-operative identification of CTCs using magnetic bead enrichment and laser scanning cytometry (MAINTRAC®), was related to tumor relapse (68). The presence of CTCs in the peripheral circulation of esophageal cancer patients has been related to poor prognosis, disease progression, and poor overall survival, thus suggesting it as a potential tool to improve the prognosis of patients (69).

Though CTCs have been widely suggested as a non-invasive prognostic marker for many cancers, it has not been widely exploited in clinics. As CTCs counts vary with the patients, determining a standard baseline of CTCs count is difficult. Though there are a number of available methods for CTCs isolation, choosing the right method is the primary challenge (70). The difficulty in evaluating the sensitivity and reproducibility for the identification of CTCs using current methods also limits the use of CTCs as biomarkers. The cost-effectiveness of the current methods also creates a bottleneck in the clinical utility of CTCs.

3.3. Current challenges

Even though monitoring of CTCs can serve as a potential diagnostic, prognostic and predictive

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tool in various cancer types, it is not widely used in clinics. Although there are many techniques for CTCs isolation, very few methods are cost-effective and commercially feasible for clinical use. The lack of standardization of the methods also makes it difficult to choose among them. For instance, the CellSearch system has been approved by the FDA for CTCs detection in metastatic breast, prostate, and colon cancer patients, but not in metastatic lung cancer patients (70). The isolation based on biological properties relies on the epithelial markers of CTCs. Owing to intra- and inter-tumor heterogeneity, the separation based on the specific antibody is not an optimal approach. The alteration of transcription pattern during the epithelial to mesenchymal transition (EMT) also downregulates the epithelial markers, which possibly result in missing out of CTCs in invasive cancers. Robust and more sensitive assays have to be developed for CTCs isolation and further to be tested in clinics.

It is worthwhile to note that the isolation of CTCs by any enrichment strategies would inevitably lead to cell loss. For instance, enrichment of CTCs based on the epithelial markers will fail to capture those CTCs, which are partially mesenchymal and vice-versa. Consequently, few research groups have tried short-term culturing of CTCs without prior enrichment (71, 72). In spite of several advanced techniques to isolate CTCs, there is still an urgent need to develop a highly reliable method.

4. *EX VIVO* CTC CULTURE

4.1. The necessity for CTC culture

The low number of CTCs is the primary challenge to isolate CTCs from the peripheral blood. In spite of the recent advances in single-cell analysis, only a few techniques maintain cell viability (15, 16). Hence further functional characterization of the isolated CTCs remains a challenge. The ability to expand CTCs in *ex vivo* condition could yield a reasonable number of cells for further validation and characterization. In the past decade, researchers delved into various possible methods to expand CTCs in the cell culture conditions, which could enable a wide range of clinical applications including early detection, the evolution of tumor genotypes during disease progression and to predict response to a given treatment (Figure 2).

4.2. *Ex vivo* CTC culture

There are numerous studies published on the isolation of CTCs from cancer patients, but only a few research groups succeeded in culturing the CTCs in *ex vivo* conditions. This reflects the limited success in the attempt to culture and expands CTCs. There is only a limited study, which has been successful in establishing CTCs lines for long-term cultures (summarized in Table 1). One of the pioneer's study to expand CTCs in *ex vivo* condition was published by Yu *et al.*, in which they were successful in establishing CTCs lines from six ER+ve breast cancer patients (73). They have used CTCs-iChip, a microfluidic device to enrich CTCs (74). Interestingly, these CTCs lines could survive for more than 6 months in cell culture conditions. Similarly, in another study by Cayrefourcq *et al.*, one permanent CTCs line was established from a colon cancer patient. This established CTCs line was able to survive *ex vivo* for more than a year and yet, exhibited the characteristics of tumor cells from the origin (75). A CTCs line established from advanced-stage prostate cancer patient exhibited to form organoids (76). Two other CTCs lines were established from SCLC patients, exhibits both spheroidal and adherent morphologies were reported to survive for more than 4 months. The above studies illustrate the plausibility of culturing and expanding CTCs *ex vivo* to facilitate the progress towards personalized therapy (77). However, the success rate in establishing a permanent CTCs line is very low, as the number of CTCs isolated from blood is very meager and also is biased towards the advanced stage of cancer.

4.3. Short term CTCs culture method

Even though cancer cells have a proliferative advantage (78), the success rate of establishing a permanent CTCs line remains a big challenge. However, several studies used isolated CTCs to culture for short-term (3-14 days). It has been reported by Khoo *et al.*, that the short-term CTCs culture could predict the patient's response to the treatment. In this study, the nucleated cell fraction

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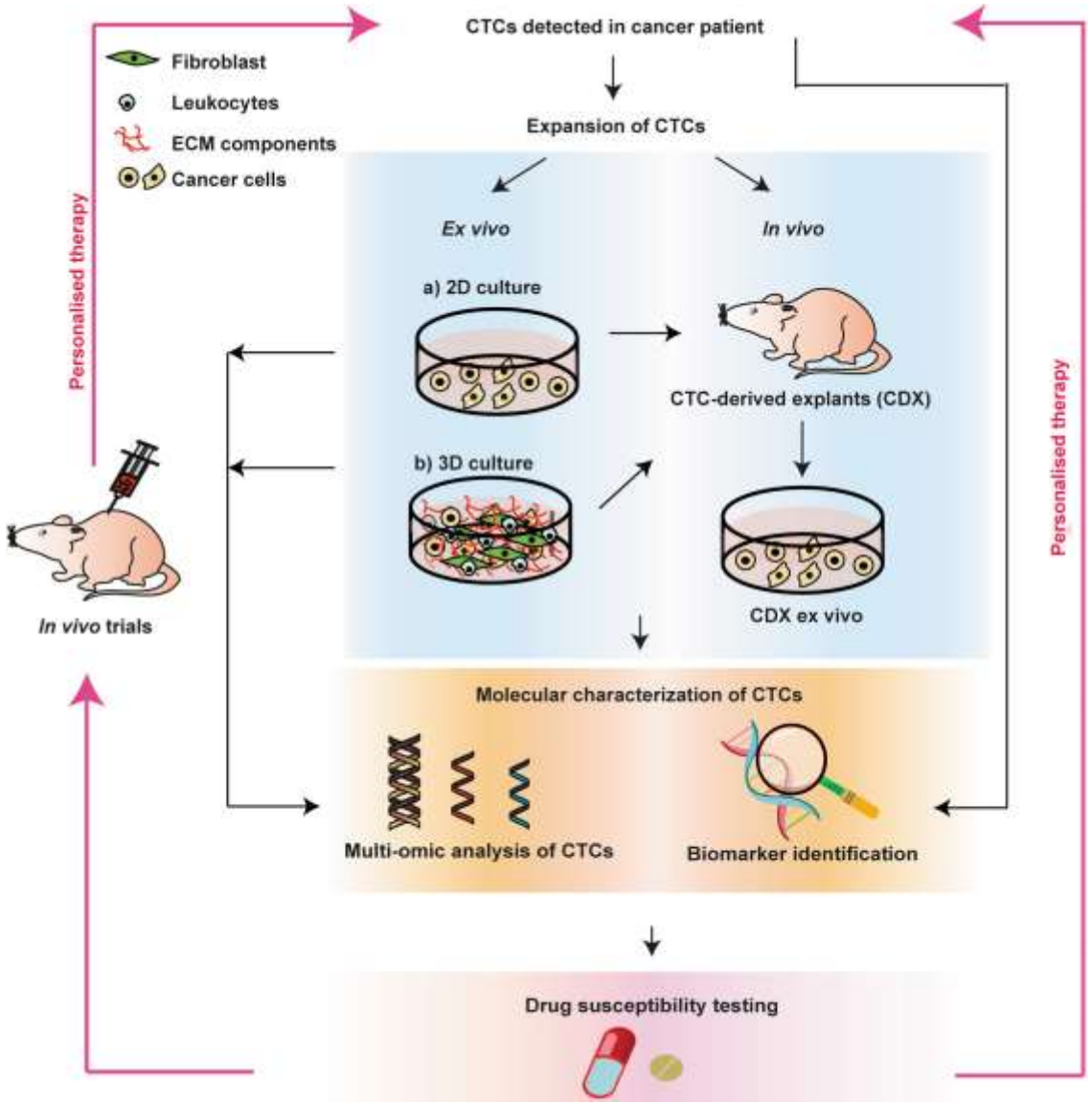

Figure 2. Current methodologies for expansion of CTCs and clinical applications of CTCs.

after the RBC lysis is directly cultured in hypoxic condition (1% O_2), hence there was no prior enrichment of cells. The CTCs in the majority of the samples were proliferative till day 14, but on day 21, the number of CTCs declined, suggesting it's poor survival capability in *ex vivo* conditions. Interestingly it has been reported that even though in some samples, there were no CK+ve cells in the day 0 of the culture, they could see some CK+ve cells in the subsequent days, which clearly illustrates that short term CTCs culture could be a better choice for CTCs research (71). The short term CTCs culture were also reported to be used in the bladder (79), mesothelioma (80) and esophageal cancer (81), where CTCs were isolated by MetaCell® (a size based enrichment method to filter the peripheral blood cells through a polycarbonate membrane). Short term CTCs cultures were also established using a three- dimensional co-culture system with CAF (Cancer-Associated Fibroblast) and ECM proteins. Using this model Zhang *et al.*, expanded CTCs from 14 of 19 early-stage lung cancer patients

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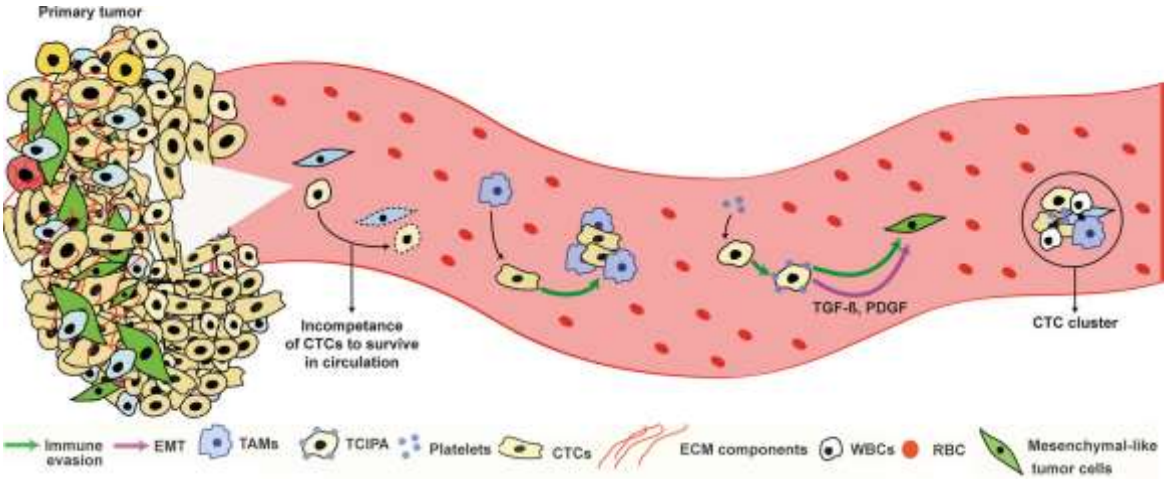

Figure 3. CTCs and their microenvironment in the vasculature.

and further found that the cultured cells carried a mutation in TP53 gene similar to the matched primary tumor cells (82). As discussed above, the short-term CTCs cultures may not have the pure CTCs population. However, the number of cells amplified in a short period of time can mitigate the use of single-cell studies and also demonstrates the possibility of functional validation of cells.

5. CTCs CLUSTERS IN TUMOR METASTASIS

5.1. Circulating Tumor Cells and its microenvironment

The cells from the solid primary tumor shed into the bloodstream, however, only a few cells can withstand the hemodynamic forces, the effects of fluid shear and overcome the immune elimination (83). The subpopulation of CTCs, which are Metastatic-Initiating cells (MICs) should necessarily possess the intrinsic ability to congenially interact with blood cells to evade the immune mechanisms thereby colonizing at the distant site. Therefore, it will be captivating to study the microenvironment of tumor cells in circulation, which could aid as a clue to the mechanisms through which CTCs evades the immune response and provoke metastasis.

Although the mechanisms by which CTCs survive in the hostile microenvironment of blood circulation is not well understood, it has been well understood that the interactions of CTCs with platelets and platelet-derived cytokines could assist CTCs in the metastatic cascade. The intrinsic ability of tumor cells in the vasculature to form aggregates with platelets through its cell surface receptors is termed as Tumor cell-induced platelet aggregation (TCIPA) (Figure 3) (84). TXA2, thrombin, ADP and tumor-associated proteinases are reported to promote the formation of TCIPA (85-88). In TCIPA, the platelets are attached to CTCs by the formation of the GPIIb-IIIa-fibrinogen bridge (85, 89). Moreover, the CTCs surrounded by platelets serve as a shield to conceal against NK cell-mediated tumor cell lysis (90-92). Additionally, the platelets are reported to confer major histocompatibility complex (MHC) to CTCs, which subverts the immune surveillance (93). Furthermore, the platelets in TCIPA stimulate the release of α-granules, which mainly consist of platelet-derived growth factor (PDGF) and TGF-β at several-fold higher than non-tumorous cells (94), which promotes CTCs to undergo EMT (Epithelial-Mesenchymal Transition). TGF-β derived from platelets could activate NF-κB and TGFβ/Smad pathways in tumor cells, which promotes transdifferentiation of CTCs into a highly invasive mesenchymal-like phenotype (95). TGF-β is also reported to activate β-catenin and STAT3, which upregulates PDGF in hepatocellular carcinoma (96, 97).

It has been reported that CTCs could differentiate monocytes to macrophages which

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expresses markers for TAM (Tumor-associated macrophage) (98, 99). Hamilton *et al*., have a cocultured CTCs line with PBMCs. The macrophages present in the culture revealed the overexpression of complement factor D (CFD)/ adipsin and vitamin D-BP (VDBP) and increased secretion of OPN (Osteopontin), lipocalin-2 (LCN2), CHI3L1, uPAR, MIP-1, and GDF-15/MIC-1. These results conclude that the CTCs through TAMs can evade the immune system, thereby gaining survival advantage in the treacherous journey through the vasculature (Figure 3). Interestingly the presence of CTCs in breast cancer patients was found to have significantly increased expression of CD95 (FAS) on the cell surface peripheral T-helper cells (100). The interaction of CD95L from the CTCs on interaction with cell surface CD95 of T-helper cells could assist the apoptosis of the helper T cells. This mechanism could also aid the immune evasion of CTCs.

5.2. CTC clusters formation and cancer metastasis

CTCs have been reported in the blood either single cell or in the form of clusters. The cells in the bloodstream have several fates: it could go in apoptosis or anoikis or could be eliminated by the immune system. However, CTCs clusters, have an advantage to escape (101) and extravasate successfully to reach the distant organs and involve in micro- or macro-metastasis. A study by Aceto *et al*., experimentally demonstrated that the metastatic initiating potential of CTCs clusters is 23 to 50 fold more than single CTCs (102). Also, RNA sequencing at the single-cell level resulted in the upregulation of plakoglobin expression in the CTCs clusters. Moreover, knockdown of plakoglobin in mouse models resulted in decreased CTCs cluster formation and reduced the level of lung metastasis. Also, it has been validated that the level of plakoglobin resulted in poor prognosis in the breast cancer patient. Hence plakoglobin dependent cellular junctions play a vital role in the formation of CTCs clusters and thus contributing to the metastasis.

However, it is also inquisitive to dwell into how CTCs clusters, a multicellular aggregate travel through the narrow capillary vessels to colonize the distant organs. It is astounding to notice that the CTCs clusters in a microfluidic device, readily formed a reversibly single-file chain-like structure, which could make the CTCs clusters to travel through narrow passages in the capillary by reducing the hydrodynamic resistance. This was also demonstrated *in vivo* in the capillaries of zebrafish, where the CTCs clusters exhibited similar reorganization as observed in the microfluidic device (103). Hence, it could be fair enough to conclude that the survival advantage coupled with the ability to deform into capillary vessels bestow CTCs clusters to extravasate and reach to the distant organs more efficiently than single CTCs.

5.3. Correlation of CTC clusters in blood with poor prognosis

Although majority of the CTCs isolated from the blood exist as single cells, however CTCs were also reported to be present as an oligoclonal clusters of 2 or more tumor cells along with the aggregation of platelets, lymphocytes in the vasculature of cancer patients, along with endothelial cells and tumor-derived fibroblast (11, 95, 101, 104-111). The clusters of CTCs which possess three or more distinct nuclei are termed as circulating tumor microemboli (CTM) (112, 113). These clusters are formed as an aggregation of oligoclonal tumor cells in the primary site and together enters into the bloodstream (102). CTCs clusters in breast (114, 115), melanoma (116), renal (117), colorectal (118), pancreas (115), prostate (109, 115, 119) and lung cancers (109, 115, 120) have been reported. Further, studies have been shown a direct association of the presence of CTC clusters with the poor prognosis in the lung (121-123) and breast (102, 124, 125) cancer patients.

5.4. CTC clusters and drug susceptibility

CTCs clusters are shown to be more resistant to chemotherapeutic drugs than single CTCs (126). Drug susceptibility testing on CTCs clusters would be an advantageous strategy to screen the appropriate drug from various drug combinations *in vivo*, which could then be used for personalized therapy. In the study by Khoo *et al*., demonstrated that drug response was monitored by the serial sampling of CTCs clusters using an integrated microfluidic device, where CTCs were cultured without prior enrichment (72). Moreover, the

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IC_{50} value of one of the cultures obtained from the post-treatment time point was higher than the corresponding pre-treatment sample, suggesting the possible onset of resistance to the treatment. In another study from the same group reflected the persistence of CTCs clusters in the culture not only reflects the therapeutic resistance but also inversely correlates with the overall survival of breast cancer patients (71). In another study by Yu *et al*., demonstrated that drug susceptibility testing on established CTCs cell lines and predicted the sensitivity and resistance of various chemotherapeutic drug and drug combinations (73). Also, they could identify a few drug targets, which were mutated in CTCs but not in the primary tumor. However, there is an urgent need to establish a more robust and sensitive assay, which could be translated into the clinics.

6. ESTABLISHMENT OF CTC-DERIVED EXPLANTS (CDX) MODELS AS A TOOL FOR PRECLINICAL DRUG TESTING

Before the advent of liquid biopsy, patient-derived xenograft (PDX) models were widely in use to predict personalized therapy (127-131). However, the development of PDX models has several technical and clinical constraints (132, 133). For the first time, Hodgkinson *et al*. demonstrated the tumorigenicity of the enriched CTCs from SCLC patient by successfully generating CTCs derived explants (134). The CTCs isolated from both chemosensitive and chemorefractory SCLC patients gave rise to tumors in NSG mice. Moreover, these CDX models exhibited a similar response to etoposide and platinum treatment in comparison with the corresponding donor patients.

Similarly, out of the six established CTCs lines from breast cancer patients, Yu *et al*. succeeded to generate three CDX models, which exhibited similar histological features with the tumor samples from the corresponding patients (73). Likewise, Cayrefourcq *et al*., have successfully tested the tumor-initiating potential of a CTCs line established from a colon cancer patient in a CDX model (75). The tumor formed in the mice was found to be more than 10% of their body weight by ~40 days. Therefore, it would be logical to conclude that the CDX models could be in complementary with PDX models. In addition, the CDX model could be developed for those patients, who may not have undergone surgery or biopsy. Furthermore, the xenografts, formed in the CDX are derived from a small subset of cells, which inherently have invasive capabilities, thereby closely mimicking patient tumors. However, the biggest challenge is to generate a CDX model as the success rate is low and the duration to establish and to screen for suitable drug takes more time (135).

7. CONCLUSION

All the preclinical models can assist researchers in understanding the basic mechanisms involved in tumor progression and to come up with a suitable strategy to treat the disease. However, in the era of personalized medicine, liquid biopsy is the more advanced strategy, which can be adapted and conducive to use in the current clinical setting. Besides CTCs, the liquid biopsy also encompasses ctDNA, which provides an alternative way to explore the genomic evolution of tumors in a patient-specific manner. In the future, CTCs and ctDNA could synergistically be used for tailoring personalized medicine.

Nonetheless, the pre-eminence of isolating and expanding CTCs *ex vivo* and *in vivo* could screen various drug combinations and turn up with the best suitable drug or drug combinations, to the continuously evolving tumors during the course of treatment. However, the currently established methods to isolate CTCs are not reliable in all cases. Moreover, the intrinsic short lifetime of CTCs also hampers the possibility of amplifying the cells *ex vivo* from all clinical cases. An improved and efficient way for CTCs isolation and optimization of culture conditions to expand the CTCs population will be of tremendous value for both clinicians and researchers. Thus, understating the role of CTCs in tumorigenesis will be indispensable for cancer management.

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Abbreviations: CTCs: Circulating Tumor cells, RBC: Red Blood Cell, ECM: Extracellular matrix, CAF: Cancer Associated Fibroblast, MICs: Metastatic-Inititating cells, TCIPA: Tumor cell-induced Platelet Aggregation , MHC: Major Histocompatibility Complex, PDGF: Platelet-derived Growth Factor , EMT: Epithelial-Mesenchymal Transition, TAM: Tumor Associated Macrophage, CDX: CTC-derived Explants , ctDNA: Circulating tumor DNA

Key Words: Circulating tumor cells, CTC, CTC clusters, CTC culture, CTC derived explants, Review

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