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CIRCULATING TUMOR CELLS: AN APPROACH FOR EARLY DETECTION OF CANCER:

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Abstract:

CTCs are the cells which does metastasis and expand rapidly, most challenging Part is their detection because these cells are present in very small amount. Advancement in technologies leading toward perfect detection. Molecular markers plays a leading role in their detection and is in widely use. Not all the metastatic Cells survive but few of them by depending on enzymes released by components of blood, CTCs shows different type of interaction with platlets macrophages , neutrophil etc. Monitoring the genomic instability contributes in early and effective prognosis of related cancer, EMT composition and mesenchymal CTCs are associated in cancer cell progression. Studies suggest that CTCs are interconnected with tumor stage but clinical acceptebility in detection is still not greatly approved.

INTRODUCTION:

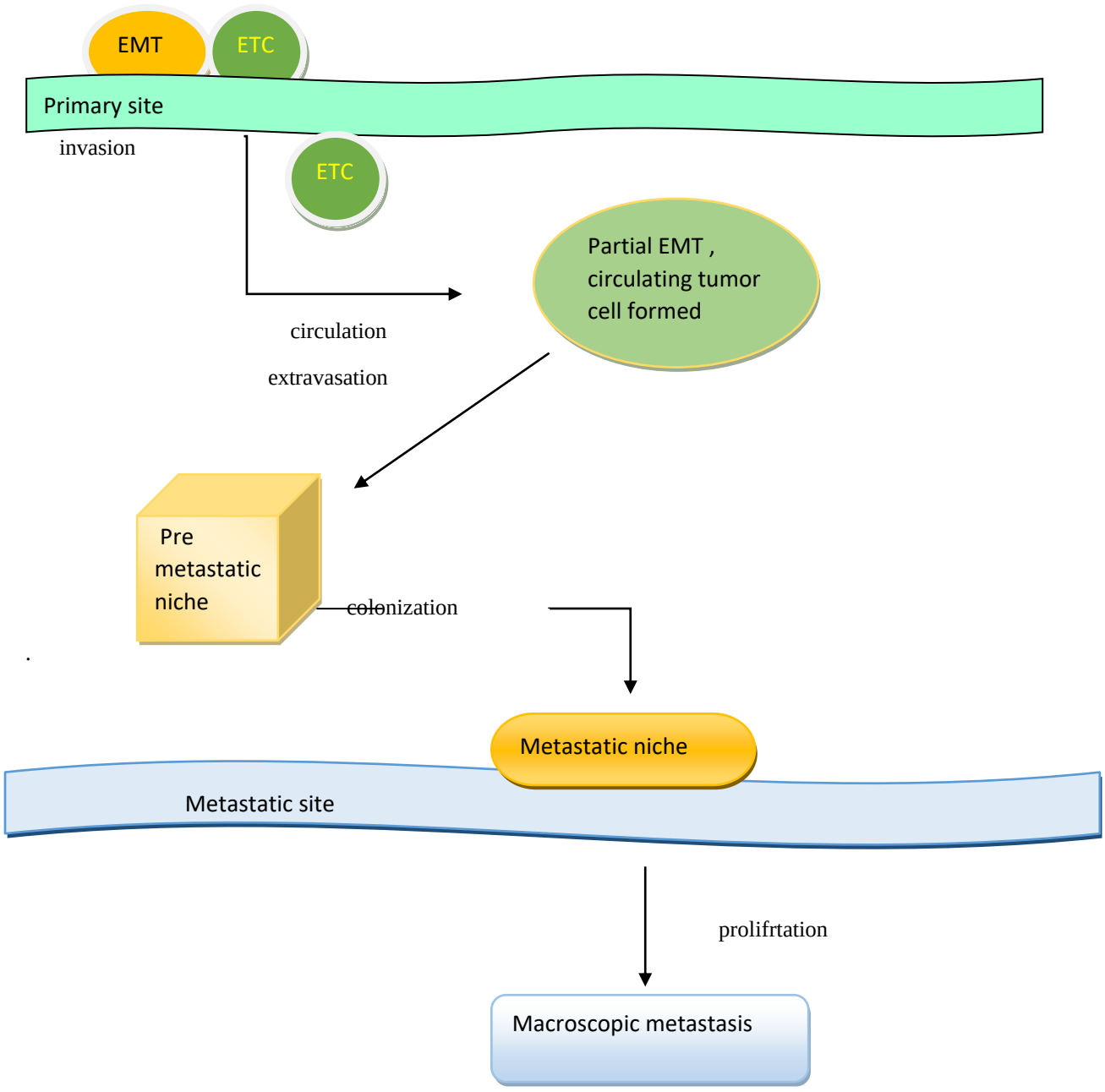
It is very important to detect the presence of cancer at early stage, generally cancer are categorized into two broad categories malignant and benign. There is a less chance for a benign type tumour to be aggressive but the malignant form does metastasis and enhances the survival of cancerous cell but these metastatic forms basically depend on blood stream for their migration from one site to another. The small sloughed off the primary tumour get extravasate and circulate into the blood The spread of cancer cells from its origin behind to different body parts is the major reason for cancer related demise in patients with solid tumours which contain cyst. Metastasis is most death causing and defining speciality of cancer. Major barrier in the improving results of cancer patients is metastasis. The the sloughed off part of primary tumour is generally called CTCs. So, the early detection of these circulating tumour cells leads in great diagnosis and prognosis for the cancer patients. Epithelial mesenchymal transition (EMT) helps the primary tumour to break and fascilitate their movement in blood stream.

CTCs-EMT:

CTCs enhances the clinical application in cancer screening and treatment .EpCAM CTC based detection technique widely used for breast cancer, prostrate cancer and pancreatic colorectal, As the EpCAM are found to be positive it indicates the presence of relative cancer. No doubt EpCAM positive ctc predict early metastasis and poor survival of the patient. EpCAM shows less expression for neurogenic cancer. Some cases suggest that the amount of negative CTCs is higher than the positive cells in Non small lungs cancer (NSLCs) patients[1]. However the efficiency for the detection of EpCAM positive CTCs can be Increase by using Epithelial and Mesenchymal cancer marker and by marker independent detection method [2]. CTCs varies patient to patient due to different therapeutic response.Epithelial Mesenchymal Transition (EMT) responsible for invasion and metastasis of CTCs by exhibiting molecular alteration , EMT -related transcription factor(EMT-RTR) belong to SNAIL, TWIST and ZEB , families [3]. EMT related molecule are cytoplasmic nuclear proteins which are currently used for molecular based technologies for CTC identification[4]. Earlier- cadhering twist and vimentin were in demand [5]. FISH(flow cytometry sorting immmuno straightening and fluroscence situ hybridization) staining were traditionally used in CTC detection techniques[2]. Different types of CTC biomarkers are present such as human epidermal growth factor receptor 2 (HER-2), estrogen receptor (mainly in female), prostrate specific membrane antigen (PSMA), survivin and folate receptor. For a melanomic cell CTCs (M-CTCs) detection, the markers are HMW-MAA[6-8], MART- 1 and MAGE A3.

A patient with NSCLC EGFR and KRAS mutation plays an important guiding role for treatment while receiving EGFR Tyrosin Kinase inhibitor and anti-EGFR therapy[2], for a patiet with pancreatic ductal adeno carcinoma(PDAC). There is 90%

chance of KRAS mutation ,Kulemann et al observed that dispute rate of KRAS mutation in PDAC tumor and CTC s was 42%[9] process in which the epithelial tumour cells loses bonding between adjacent cells and aquire mesenchymal and invasive properties.Tumour cells get dissociated by EMT activation and enters in the blood circulation, the circulation leads the travelling of CTCs to distant site. Then after CTC undergo a MET(reverse process) and multiply/ expand to form macro-metastases [10-12].Using mouse model it was observed that epithelial type CTC with the mesenchymal transition had the strongest lung metastasis formation capacity whereas limited metastatic activities are shown nby mesenchymal type CTCs. Bioinformatic analysis of seven set of genes chips by Guan et al showed that main change in EMT composition and mesenchymal CTCs were closely observed such as cell adhesion , EMT and apoptosis . In breast cancer JNK signalling include shear stress and leads poor patient survival.[13]. Baseline presence of CTCs makes the poor prognosis in the NSCLC ptients. Analysing EMT-CTC subtype and interaction between peripheral blood mono nuclear cell will be helpful in designing better anti metastatic treatments.[14].



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fig: Different stages involves in CTCs formation and metastasis: tumor cell invasion at the primary site, partial metastatic tumor cell formed and extravasate by circulation , colonization leads the attachment at metastatic site.

Genomics of CTCs:

Genomic level specially gene mutation in CTC were also performed such as breast cancer prostate cancer and mutational dispute between corresponding tumor and CTC were often found [15-19] Such combination of tumor cells and CTC mutation testing will lead the prognosis more effectively. RNA sequencing of single cell CTC indicate the activation of Wnt signalling in androgen resistant patient[21].Paoletti et al results suggest that CTCs were the sub clonal cells of tumor .Lambro et al declared that CNA of CTCs were interpatient and could have the chance of missing in bulk biopsy analysis[22]. Magbanua et al worked on metastatic breast cancer and the result is found that 65% of patients having low proliferation of CTCs and 35% of

patients having high proliferation of CTC. Cheng et al [23]performed the single celled trascriptome analysis on 666 CTCs in patients and found that CTC within a patient are heterogenous with regards to EMT and MET like stages. There is the 8.6 times chances of re-occurrence in those patients who is having the neutrophin to leucocyte ratio>3.[6]

Interaction of CTCs with blood environment

Action associated with neutrophill:

The majority of CTCs dies due to Anoikis (A process in which CTCs do not assemble properly due to programmed cell death). Small amount of tight interaction was observed with neutrophils and macrophages, myeloid derived suppressor cells(MDSCS) or cancer assotiated fibroblast (CAF). Binding of ctcs and neutrophill is directed by cell-cell junction and may require cell adhesion molecule. Assotiation of CTCs with these blood components help them to escape the immune system by suppressing the activation of peripheral leukocyte and promotes their survival. If a patient found with increased number of neutrophil in the blood circulation it will be the sign of poor prognosis [24-25]. Indirectly neutrophil promotes metastasis. The DNA histone complex and proteins released by activated neutrophil forms NET (neutrophil extracellular trap). NET were able to catch CTCs and metastatis [26-27].

Interaction of CTCs with Macrophages:

Tumour assotiated macrophages(TAMs) contributes to metastatic progression with primary tumour, spread and extravation. Hamilton et.al. cultured mononuclear cells with CTCs and tried to investigate CTCs. Macrophage interaction. He found that CTCs able to induce monocyte differentiation to TAMs and secrete oesteopontin, MMP9, Chitinase, and platlet factor to promote leukocyte requirement, invasion and migration TAM enhances the protective cell formation and confers resistance to the shear stress . From the blood of a patient with multiple cancer such as melanoma,PDAC[28], melanoma, breast cancer and colorectal cancer[29], M2 like a macrophage phenotype CD163 as well as epithelial marker is identified. Gast. Et.al. revealed that fusion hybrids increase tumour heterogenicity and metastatic behavior leads worse surval among NSCLC.

Interaction with platlets

Attachment of CTCs with platlets and adherence of CTCs to endothelial wall is mediated by aII6B3 and P-selectin. Activation and requirement of platlets supports the survival of CTCs as well as their proliferation.Procoagulants and prothhrombotic released by p platlets helps in CTCs expansion[30-31] TGF beta accelerates EMT in CTCs [32]. Platlets help CTCs to escape from NK cell attack by following mechanism:-

I. MCH-1 transferred to the surface of tumour cell; and thus Nk cells are unable to recognize the tumor cell, [33] thus MCH-1 supressing drugs may leads to the cancer treatment.

ii. NKG2D (lowering down) which disturb antitumour cytotoxicity[34-35]

iii. By surface shielding

iv. TNF related ligands which is derived from induced glucocorticoids of platlets activats GITR in NK cells and lowerind down their Cytotoxicity.[36].

In one study it is found that the interaction of the intergins alfa6 beta1 and receptor of platlets with disintergin and metalloprotease as it is necessary for extravation of cancerous cells[37]

CTCs Enrichment and Isolation:

It's a great challenge to isolate small portion of CTCs from numerous blood cell of patient. Nanoscale material and novel microfluid chip hoping to provide a useful framework of CTCs related technologies . Three main strategies of Ctcs includes capture and enrichment, detection and identification and finally release. Capture and enrichment is interaction of material with CTCs by physical interaction orb antigen antibody interaction, fluroscence spectrometry, , fluroscence microscopy flow cytometry and electrical impedence (Raman scattering)

Enrichment based CTC detection technologies

The minority of CTC in blood can be enhanced by two method:

I. Negative Enrichment - This technique apprehends the healthy blood cells and removes the target cells (CTCs)
II. Positive Enrichment - This technique works completely opposite to negative enrichment as it takes CTCs captive while detaching hematological cells.

3.1 **Negative Enrichment**

Lara et al in 2004 was the first developer of this technology. This technique includes the following steps i.e. Lysis of red cells, leukocytes stained via inmunomagnets with an anti-CD45PE is followed by the sorting using QMS (Quadrupole magnetic cell sorter). The final step includes the cell count and analysis through filtration and visual counting, cell counter.

GASI (Geometrically activated surface interaction) was developed by Hyun et al to increase the efficiency of negative enrichment.

The possible contiguity between antigens on the cells & antibodies on the channel surface is due to the herringbone structure.

Another microfluidic process was performed by Sajay et al. The depletion of immunomagnets employs the removal of CD45 - positive WBCs which is completed by RBCs depletion & isolation of target cells via filter membrane

Merits of CTCs detection through Negative Enrichment includes:

(i) They do not depend on biomarker expression of target CTCs

(ii) Collection of CTCs is done in an intact manner i.e. molecular & cellular level of analysis.

(iii) Automation is easy for the same.

Limitations

(i) The purity of CTCs is compromised i.e. CTCs are rare in comparison to hematological cells which leads to the purity of CTCs <4%.

Classical CTCs related technologies based on physical properties: physical separation method by difference between size density electrical properties and deformability. TRACK-ETCH Type membrane is an approach for CTCs isolation by size but it has low efficiency hence pressure regulating system, the flexible microspring array device is an improved method with 90% efficiency in 76% of the sample (near 196). A density dependent technique allow RBC and WBC to be filtered. Apostream [39] microfluidic chamber that introduced with dielectric electrophoresis techniques. Primary and cancer causing tumour mass moves through out the body cast tumour cells at different stages in which its benign form is converted malignant form of cancer & there is a humongous proof found on this the extensively used CTC detection are.

1. Immune magnetic separation these methods uses trapping agent-labelled magnetic beads to either identification of positive CTC's utilizing cell surface marker anti-CD45 deplete WBC in negative[40-41]

2. Flow cytometry :it is basically a technology used to examine the subpopulation of cells[42]. An upgraded method called an ensemble decision aliquot was introduced as the flow – based procedure was not able to provide CTC's morphological unformats an to link up the gold standard set by pathologist

: **Microfluidic chips** : it was introduced to attain higher capture efficiency of CTC's[44-46]

: **CTC filters** : CTC filters is used for accumulation of CTC according to their sires [47]

Nanostructure embedded microchips for detection isolation and characterization of circulating tumour cells.

CTC's or circulating tumour cells spread the tumour from the anatomical site where tumour progression begins and divides to yield a cancerous mass to other organs or parts of the body via blood [48-49]

: CTC's requires specific type of biopsy for detection i.e, tumour liquid biopsy it's a different level of challenge to detect CTC's in blood because CTC's concentration in blood is very low as compared to haematological cells[51]

Nano Velcro in CTCs detection

Nano Velcro cell-affinity substrates is a unique concept of detecting CTC in humans which was pioneered by a team at UCLA .its working include CTC capture agent which is coated with nanostructure substrates that immobiliseCTC's with great efficiency.[52-53]

Evolution of nano Velcro chips has been captured via 3 distinct generation .the 1st generation nano Velcro chip includes SINS(silicon nonovire substrate)and a cover of microfluidic chotic mixer created by CTC enumeration.[54-55]

The 2nd generation nano Velcro chip was designed focusing single-CTC isolation which constitue a transparent polymer nanosubstrate the isolation is followed by genotyping of single CTC to identify its role as tumour[56-57]. The possible changes applied on SINS by grafting thermoresponsive polymer brushes describes the 3rd generation of nano Velcro chips[58] ,the attraction in the accessbility of capture agent on SINS by a confirmationally changing the temperature on polymer brushes helps to capture and release CTC's at 37 and 4*C respectively [58] .

The nanostructrual components of cells including cell-surface structure and extra-cellular matrix are the basic fate of the cell as they control the mandatory behaviour of the cell. Hence, the evolution of nano Velcro CTC as says is to relaise those roles and built a useful tool for CTC's detection ,isolation and attributes [59-60]
Patient with cancer and acute diseases were examined by testing their blood samples it was noticed that in 26 sample first generation chips their was higher sensitivity and elevated dynamic range in CTC enumeration. It was seen that first gen Nano Velcro chips unfold the chances

I) Firstly for more precise inuigilation of severity of disease
II) Secondly it takes core to detect CTC at an pre stage of disease

Betterment in first generation nano Velcro CTC chips has led to device configuration with compact device footprint in this process filtration of CTC according to size.

EPCAM : CTC trapping

The anti -EPCAM is most familiar CTC trapping agent used for epithelial origin solid tumours. The reason behind their extensive used is mainly because of poor stability and high cost of antibody and dissem-ination and translation of CTC based diagnostics can be limited low resource environment

although this restriction can be fixed by changing anti-EPCAM with aptamers which was prepared by a process wherelydive cells are used to select aptamers for target recognition and this very process is known as CELL SELEX which help in accurate target of cancer cells[62-63]

A 549 non-small cell lung cancer (NSCLC) cell produces 2 single stranded DNA aptamer via cell- selex patients with prostate cancer and acute disease blood samples were tested and first gen chips showed higher sensitivity and elevated range in CTC [64]Betterment of first gen nano-Velcro CTC chips has act led to device configuration with compact device footprint at efficient cost filtration of CTC acc. to size significant so that cancer patient gets benefitted.

Second generation nano Velcro-LMD-technology

Molecular analysis of CTC's was found successful because of the second generation nano Velcro-LMD-technology. This generation basically focuses on single CTC isolation by coupting LMD with transparent nano substrates with a covering of poly (lactic-10-glycolic acid) (PLGA)substrate.

Takes place at efficient cost significant number of cancer patient gets unfitted . in astudy jointly conducted by urooncology teams at ucla hospital & cedras -sunai medical centre in this study 40 pro state cancer were admitted out of this 40 patient 32 has metastatic disease(define) & 8 patient has benign disease(define)

And CTC's was recognized in all 40 patients.

PLGA- chip preparation

Preparation of PLGA nano Velcro substrates includes a slide of LMD which was engraved by PLGA nanofibers. Then PLGA is covalently bonded to streptavidin. The bioliur anti bodiesmakes it possible to identify CTCS and help them to isolate single CTCS.[66-67]
The 2nd generation technique was first applied on detection of a signature oucogne to isolate single melanoma . BRAF v600e has been visualised in 60% of melanoma anti-CD146 was the capture agent for melanoma CTCS.

Nano Velcro- LMD technology

LMD was modified to LCM(laser capture microdissection) because of the substantial loss in cell during collection due to static charge LCM working included IR laser which was used to melt LCM cap, ultimately dropping "a sticky finger "for adheraition on laser dissection nano Velcro substrate . this process helps in CTC s isolation of prostate cancer for detection of broader mutations . prostate cancer average life is approximately 10-15 years .the enhancement of 2nd generation nano Velcro substrate will lead to understand tumour heterogeneity & clonal evolution.

Nano- Velcro chip: 3rd Generation

The 3rd generation nano Velcro was designed to eradicate the issues faced in 2nd generation (I .e) poor viability of CTC's isolation ,more labour consuming process . the molecular and functional analysis of CTC's are high on demand & the importance of CTC's isolation with less technical utility , greater quality and efficiency was covered by the 3rd generation nano Velcro . the blueprint of 3rd generation nano Velcro constitute thermoreponsine polymer brushes which were implanted on SINS to capture CTC's at 37' C and release them at 4'C.

A biotin group is applied on polymer brushes for conjugation with anti-EPCAM .at 37'C the anti EP-CAM captures the CTC's while at the confirmational change in temperature at 4'c allows the CTC's release via anti EP-CAM. The goal is to rapidly purify CTC's from blood to set the stage for understanding & exploring the wide range of application of CTC's[68]

CONCLUSION:

Interaction of CTCs with blood cells lead their survival in circulating microenvironment. Anti metastatic therapy can be targeted by CTCs. Cell search system serving a new way for analysing CTCs for diagnosis and treatment. EMT is an important characteristics of metastatic tumor cells.However mesenchymal marker based detection technologies loosing their efficiency because of false positivity due to tumor associated fibroblasts and endothelial characters. Different molecular markers posses by different types of cancer. Nanotechnology based detection technologies and microfluidic based CTCs detection technologies is on high demand in recent years, However these technologies need large scale clinical approval. The limiting amount of DNA, RNA and protein content of CTCs is a major obstacles in genometranscriptome, epigenome and proteome properties. Cluster of CTCs are more efficient in interaction with other blood component, if these cluster combine with specific biomarker for strategically detecting CTCs and Interaction with peripheral blood cell would improve monitoring power of CTCs and clinical applications.

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