Liquid Biopsy: A Tool for Detecting and Monitoring Oral Carcinoma

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Abstract

Oral squamous cell carcinoma is frequently occurring malignant tumour of the H & N (head and neck) region; with a rising oral cancer burden despite attempts of prevention, delay in diagnosis leads to loco-regional spread and poor prognosis. Hence there is an utmost need for biomarkers which could lead to early diagnosis of oral carcinoma. In the quest for innovative and early diagnostic aid, liquid biopsy is evolving as animportant tool in diagnostic oncology. A liquid biopsy also called a dynamic biopsy or fluid phase biopsy is a non-invasive analysis of non-solid biological tissue predominantly blood. Malignant tumours release different biomolecules into body fluids that have the potential to be used as biomarkers for diagnosis, prognosis, and therapy selection. As the clinical application of Circulating tumour DNA (ctDNA), circulating tumour cells (CTCs), & exosomal miRNAs are quite significant they have acknowledged colossal attention have acknowledged gigantic consideration. Evaluation of the circulating biomarkers has made it easy for unique therapeutic methodologies and meticulous medicine. An increasing amount of studies have indicated that application of these biomarkers intended for detecting, therapeutic scheduling, reaction observing and prognosis management. Even though these novel biomarkers can deliver widespread series of conceivable clinical applications yet there arenot any authenticated circulating biomarkers which have been unified into the clinical practice for oral carcinomas. This article is a brief review of the various biomarkers used in liquid biopsy as well as about tumour educated platelets.

Keywords: *circulating tumour DNA (ctDNA), circulating tumour cells (CTCs), Exosomes. Next-generation sequencing, tetraspanins.*

Introduction

Liquid biopsy is a potential revolution in the carcinoma diagnostics which not only helps in detecting the tumour but also monitors the progression of the disease. As a result of the novel biotechnologies and diagnostic potential, it has emphasized the restrictions of present sampling method; out-dated biopsies and minor operating techniques are invasive, indicted with impending problems, occasionally cannot be repeated and executed when clinical circumstances have deteriorated or when a tumour is unreachable. Additionally, the genomic profile of biopsy tissues delivers a tumour profile restricted to a particular period, and may also show the genetic heterogeneity of manytumouralsubclones. These boundaries of the standard technique of biopsy are quite evident in monitoring the evolution of the ailment during follow up. Because of these drawbacks in currentages, the research is being focussed more on the cells that are circulating in the blood which not only helps us in knowing the location of cancer but helps use in monitoring the progression of the disease.Circulating cell-free DNA fragments get separated from apoptotic or necrotic cancer cells are labelled as circulating tumour

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DNA and exosome specifically, these are encapsulated subcellular structures which contain proteins and nucleic acids released by tumour cells.

Liquid biopsy mainly focuses on the segregation of these tumour cells from the peripheral blood smear. The insufficiency of nucleic acid, as well as difficulty in differentiating among normal and tumour nucleic acids, have been overcome by using next-generation sequencing techniques, which helps in precisely detecting the genetic and epigenetic aberrations. It presently offers high specificity, permitting the assemblage of healthy and reproducible data in a simple and non-invasive way by a blood sample. Even now in 21st-century liquid biopsy procedure is not carried out routinely.

Reasons for opting liquid biopsy:

Following are the reasons for considering liquid biopsy over the standard method of biopsies:

1. It aids in understanding the spatial and temporal heterogeneity of cancer.
2. Necessitates less quantity of blood to carry out the investigation
3. It is minimally invasive
4. Helps in quickrecognition of cancer.
5. Actual time observing for therapeuticreactions and resistance could be performed by repeated analysis
6. Shorter turnaround time for genotyping.

Why not tissue biopsy?: It is invasive, lacks representation of the complete variety of malignant clones, multiple sampling is not feasible and despite all these drawbacks, it is still the gold standard in understanding the characteristics of the tumour.

Circulating Tumour Cells (CTCs): There has been tremendous development in isolating the tumour DNA from the heterogeneous components of blood due to the advancements in biotechnological applications since it was first discovered in 1869. Circulating tumour cellsget separated from primary or secondary tumour sites; they circulate in the blood and are accountable for distant metastasis. CTCs are quite rare and occur at a very low frequency like 1CTC per 10^6 -10^7 leukocytes in the initial stage of diseases. CTCs have irregular morphology & they can differ according to cancer type and stage. Circulating tumour cellscan form anaggregate with primarytumour cells or with fibroblasts, leukocytes, endothelial cells or platelets, with more susceptibility to cause distant metastases.

The key dissimilarities are their outsized size up to 20-30 μm, mechanical plasticity, and dielectric mobility properties in comparison to the blood cells. Successful techniques of isolation are membrane filtration, density gradient stratification dielectric mobility, photo-acoustic & microfluidic separation.[1, 2]. The principal documented function of CTCs is in prognosis; in advancing tumours, scientists have found that the total number of CTCs in a 7.5ml blood sample is expressively related by the prognosis, with a precise cut off value. Cristofanilli et al reported "in metastatic breast cancer the patients with more than 5 CTCs in 7.5ml blood have shorter progression-free survival and overall survival compared with patients with a lesser count".[6] CTCs significantly provide information related to the epigenetic changes occurring in tumour cells. DNA methylation in liquid biopsy has proved to be a prospective biomarker for staging, prognosis.[7]

Circulating Tumour DNA: Mandel and Metais (1948) gave their principal experimental confirmation of cfDNA in blood.It took nearly 3 decades for its application in carcinomas when Leon et al proved that the circulating tumour DNA levels have increased in the pancreatic cancer patients.Till date, various mechanisms regarding how the ctDNAis entering the blood have been hypothesized. In physiological circumstances, circulating free DNA are separated from the apoptotic and necrotic cells by phagocytosis by macrophages and scavenger cells [9,8] in addition tothey can be released by the living cells. In a healthy human being the concentration of plasma cfDNA ranges from < 10ng/ml to> 100ng/ml with a half-life of between 0.16hrs and 2.5hrs. Normal physiological situations such as inflammation, trauma, or exhaustive exercise can also lead to the release of cfDNA but the DNA which are released due to tumour tend to have large fragments as there occurs irregular digestion leading to the formation of DNA fragments with large kilobasepairs.

Exosomes: Exosomes are nano-sized vesicles discharged by cells and evident in the body fluids, such as plasma, urine, saliva or ascites. The se are dissimilar from the extracellular vesicles which usually get separated directly from the cell membrane, likemicrovesicles (50-1000nm in length)apoptotic bodies, exosomes are the last product of the recycling endosomal pathways and originate from intrinsic budding of the plasma

membrane[10]. However, they were formerly regarded as cellular discarded products it is now documenteda key role where they act in intercellular communication reliant on the cargo of functional molecules from donor to distant cells. Many studies have shown that these cells are involved in cancer progression and metstatization. After getting separated from cancer these cells encourage EMT and affect propagation, relocation and attack of tumour cells as well as are supportive to the angiogenesis and institute an immunosuppressive milieu[16].

Exosomes are evolving as an original chemo-resistance mechanism, chieflyreliant on drug liberation throughvesicle budding, nullification of antibody-based drugs, and exosomes arbitratedtransfer of micro RNAs (miRNA).[15]Exosomes containa lipid bilayer which encompassesboth trans-membrane and non-membrane proteins, as well as noncoding RNAs, mRNAs, and either ss or ds DNA[14]. Concerning proteomic analyses, Exosomes are categorised through a preservedset of proteins autonomous of their cellular origins, such as CD63, CD81, and CD9 tetraspanins.[10] Nevertheless, the protein configuration of exosomes coarselybear a resemblance to originating cells, thereforesignifying an exosomal cell-type or tissue-specific signature.[12] It has been established that definite RNA transcripts are augmented up to 100 times in exosomes paralleled by the donor cells, therefore backing up active packaging.[11] Various procedures are established to resourcefully gatherexosomes from bodily fluids. The present protocols follow the vesicle detachment concerning the biophysical characteristics, which includes the dimension, morphology and density, whereas others are based on the immune-affinity captured.[16,3] Till now the regularly followed protocol adopts series of centrifugations with an increase in speed to eliminate cellular debris and greater plasma membrane-derived vesicles, following this procedure is the sedimentation of EXOs by ultracentrifugation.

Tumour educated platelets: This is the newer concept that is being considered in liquid biopsy. Platelets are not educated by the tumour but they form the first cell type that interacts with a tumour which leads to change in their mRNA profile. Since platelets are available abundantly in the blood it does not need high sensitisation techniques to isolate them in comparison to the circulating tumour DNA or exosomes.

In the last decade, there was much appreciation for the involvement of platelets to the immune system of the body in health and certain diseases including cancer too.[4] They are regarded as "scanning soldiers" of the immune system, in that way detecting the existence of bacteria inflowing the bloodstream, lymphocyte cross-communication, and then regulating immune cell extravasation [14]. In cancer patients, the platelets function on tumour resident cancer cells as well as the tumour cells that have entered the bloodstream. Initially, platelets generate an environment supporting the neovascularisation by providing the tumour with various pro-angiogenic factors like VEGF, PDGF and b-FGF and stimulates expression of the factors[15]. Then, platelets reduce the apoptosis of tumour cell and anoikis and induce the switch of epithelial mesenchyme in tumour cell with direct physical interaction and discharges of TGFb molecules[17].Consequently, a significant contribution to the metastatic process is done by the capacity of platelets to safeguard tumour cells in motion from the normal immune response.

Then, there is the formation of a cell fibrin platelet aggregation encircling the CTCs which provides mechanical protection, and it transmits major histocompatibility complex class I to CTCs, which results in supplementary safeguardin contradiction of natural killer cells. Current lab-on-a-chip microfluidic methodexaminationdiscovered the occurrence of animpending CTC sub-population with heightened platelet coverage and perhaps platelet-mediated defence. On activation, platelets can discharge numerous growth and pro-angiogenic issues[3], and after these issues are unconfined at a metastatic niche, a tumour-stimulating growth microenvironment is generated[4] Therefore, platelets are an essential constituent of the tumour microenvironment and are deliberated asignificantfeature of oncology as they contribute to tumour initiation, tumour progression, and therapy response. Attention has been rehabilitated in cancer avoidance facilitated by daily use of aspirin, a provocative topic spanning many years. Aspirin canconstraintumour-intrinsic cyclooxygenase activity, which mayregulate intracellular signalling ways and the local inflammatory response, and platelet-resident cyclooxygenase activity, which can affect procedures such as platelet activation and aggregation.

Challenges: Since it is still an emerging study there many obstacles that we need to overcome for this technique to be used routinely. Following are the challenges that we face presently:

1. We still lack in recovering huge amounts of analytical samples from the liquid biopsy.
2. Detecting genes translocation and RNA expression data is a puzzling task.
3. We cannot identify those early-stage cases.
4. To recognise the fundamental modifications that recognise or resistant tumour cell clones.
5. Justification and reproducibility of molecular and computational data.
6. Dispersion and application of liquid biopsy on anenormous scale as repetitive analysis.
7. The decrease in mutational/expressional data complexity.

Future Aspects:

1. High throughput assaying such as multiplex digital PCR and expression array.
2. Advanced genomic method having higher sensitivity to recognise any mutations occurring in matched ctDNA and tumour tissues
3. Enhanced understanding of dynamics of the biology of CTCs, exosomes and ctDNA release.
4. Calibration and logical authentication of techniques for liquid biopsy
5. Overview of cheaper biotechnology and computational software.
6. Using this software to implement the databases for a gigantic amount of data.

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References

1. Li P, Mao Z, Peng Z, et al. Acoustic separation of circulating tumor cells. Proc Natl Acad Sci US A 2015; 112: 4970–4975
2. Zheng S, Lin HK, Lu B, et al. 3D microfilterdevice for viable circulating tumor cell (CTC)enrichment from blood. Biomed Microdevices2011; 13: 203–213.
3. Buder A, Tomuta C and Filipits M. The potential of liquid biopsies. CurrOpin Oncol2016; 28: 130–134.
4. Wang J, Chang S, Li G and Sun Y. Applicationof liquid biopsy in precision medicine:opportunities and challenges. Front Med 2017;11: 522–527.
5. Le Gal K, Ibrahim MX, Wiel C, et al.Antioxidants can increase melanoma metastasisin mice. Sci Transl Med 2015; 7: 308re8.
6. Logozzi M, De Milito A, Lugini L, et al. Highlevels of exosomes expressing CD63 andcaveolin-1 in plasma of melanoma patients.PLoS One 2009; 4: e5219.
7. Cristofanilli M, Budd GT, Ellis MJ, et al.Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N EnglJ Med 2004; 351: 781–791
8. Pixberg CF, Schulz WA, Stoecklein NHand Neves RP. Characterization of DNAmethylation in circulating tumor cells. Genes2015; 6: 1053–1075.
9. Jahr S, Hentze H, Englisch S, et al. DNAfragments in the blood plasma of cancerpatients: quantitations and evidence for theirorigin from apoptotic and necrotic cells. CancerRes 2001; 61: 1659–1665.
10. Stroun M, Lyautey J, Lederrey C, et al. Aboutthe possible origin and mechanism of circulatingDNA apoptosis and active DNA release. ClinChim Acta 2001; 313: 139–142.
11. Thery C, Zitvogel L and Amigorena S.Exosomes: composition, biogenesis andfunction. Nat Rev Immunol 2002; 2: 569–579.
12. Skog J, Wurdinger T, van Rijn S, et al.Glioblastoma microvesicles transport RNAand proteins that promote tumour growthand provide diagnostic biomarkers. Nat CellBiol2008; 10: 1470–1476.
13. Nilsson J, Skog J, Nordstrand A, et al. Prostatecancer-derived urine exosomes: a novelapproach to biomarkers for prostate cancer. Br JCancer 2009; 100: 1603–1607
14. Thery C, Zitvogel L and Amigorena S.Exosomes: composition, biogenesis andfunction. Nat Rev Immunol 2002; 2: 569–579.
15. Thakur BK, Zhang H, Becker A, et al. Double-strandedDNA in exosomes: a novel biomarkerin cancer detection. Cell Res 2014; 24: 766–769.

16. Giallombardo M, Chacartegui Borras J, Castiglia M, et al. Exosomal miRNA analysis innon-small cell lung cancer (NSCLC) patients'plasma through qPCR: a feasible liquid biopsytool. J Vis Exp 2016; 27;(111):53900

17. Tucci M, Mannavola F, Passarelli A, StucciLS, Cives M and Silvestris F. Exosomesin melanoma: a role in tumor progression, metastasis and impaired immune systemactivity. Oncotarget 2018; 9: 20826–20837