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LIQUID BIOPSY OF INTRACRANIAL NEOPLASMS: REACHING THE UNREACHABLE

V. A. Upadhyay[1], T. I. Trivedi[1*], R. M. Rawal[2]

1. Clinical Carcinogenesis Laboratory, Cancer Biology Department, The Gujarat Cancer & Research Institute, Civil Hospital, Ahmedabad, Gujarat, India.

2. Life Sciences Department, Gujarat University, Ahmedabad, Gujarat, India.

ABSTRACT: Glioma tends to envelop more than 80% of primary malignant tumors amongst all the intracranial neoplasms claiming poor prognosis and lower survival rate. Tumor tissue biopsy is general mode of diagnosis for the disease. However, it encounters certain limitations mainly delayed diagnosis, inadequate sampling and misdiagnosis. Besides, information attained from a single biopsy provides a spatially and temporally limited snap-shot of a tumor which in turn lacks or fails to reflect its heterogeneity. This issue can be circumvented with the recent advent of "Liquid Biopsy "that impart better understanding of the tumor dynamics and augment minimal invasiveness for disease diagnosis together with its serial monitoring. Likely with other malignancy, Glioma tissues also release their molecular information in circulation mainly blood and cerebrospinal fluid. This review majorly summarizes the blood and cerebrospinal fluid based biomarkers predominantly circulating tumor cells, cell free DNA and exosomes that can guide patient management. It covers varied areas from techniques to isolate these markers to their characterization and their further application in field of early diagnosis and treatment.

KEYWORDS: Brain Tumors, Liquid Biopsy, Circulating tumor cells, Cell-Free DNA, Exosomes.

Corresponding Author: Dr. T. I. Trivedi* Ph.D.

Clinical Carcinogeneis Laboratory, Cancer Biology Department, The Gujarat Cancer & Research Institute, Civil Hospital, Ahmedabad, Gujarat, India. Email Address: trupti.trivedi@gcriindia.org.

1.INTRODUCTION

Brain tumors commonly referred to as "intracranial neoplasms", have propensities to devastate illness amongst children and adult, causing a wide range morbidity and mortality with unmet therapeutic needs [1]. Amongst the primary brain tumors, Glioma is the most common type having

an invariable fatal outcome and dismal prognosis [2]. Each year approximately 200000 patients are diagnosed with glioma worldwide. It accounts for 30% of all brain and central nervous system tumors and 80% of all malignant brain tumors. These tumors origin from neuroepithelial tissue i.e. glial or precursor cells and classified on the basis of morphological appearance: astrocytic, oligodendroglial, ependymal and choroid plexus tumors [3]. Such morphological divisions support identification of prognostic and predictive markers for some types of gliomas, whereas molecular subclasses remain increasingly important in the clinical classification and treatment of gliomas [4]. Remedial modalities for gliomas take an account of surgical resection, radiotherapy, and chemotherapy. Combinatorial standard treatment of surgery and temozolomide (TMZ) or procarbazine-lomustine-vincristine (PCV) based chemotherapy, radiotherapy are precautionary used yet, patients still face poor prognosis [5]. However, for the directional treatment, Magnetic resonance imaging is the principal diagnostic checkpoint to suspected brain lesions. Besides, for definitive diagnosis of tumor type and grade, tumor excision or biopsy is pivotal. Moreover, identification of every tumor type along with their respective individual molecular makeup is difficult, in tackling cancer with greater precision. So, tissue extraction or surgery turns out to be an important requirement for further analysis to the disease progression [7]. Tumor tissue biopsy though being standard technique for clinical and investigational studies, major obstructions occurs in its acquisition and utility. Inconveniency transpire from scheduling perspective, thereby increasing cost of patient care and most importantly its invasiveness procedure that might influence improper outcome [8]. Limited access to serial tumor biopsies constitutes a major shortcoming in longitudinal molecular monitoring of targeted treatment [7]. Repeated biopsy itself might increase the risk of the cancer 'seeding' to other sites [9] and the procedure might not be recommended for patients receiving anti-angiogenic treatment [10]. Hence, such approaches if undertaken repeatedly turns out to be risky, invasive and do not always facilitate access to a representative part of the tumor in case the tumors are located in the sensitive areas of the body. As a counterpart to this approach, a less risky and minimally invasive technique should be applied for disease investigation and successive monitoring and that can completely translate the genomic knowledge of the disease. Considering shortcomings of tissue biopsy, a newer approach of "Liquid Biopsy" is explored in various solid malignancies that have a promising potential as a precise treatment selection for individual patient [6].This novel discovered approach of detection with the bio-fluids, is a prominent research interest due to its less invasive property as compared to the traditional tissue biopsy and can be major support in better detection of cancer driver mutations that can expand diagnostic possibilities, studying disease evolution by stratification of genetic profiling and therapy response prediction [15]. Thus in this review we discuss about the three main sampling source considered in Liquid Biopsy: Circulating Tumor Cells, Exosomes, and Circulating Tumor DNA hereby exploring its benefits and challenges in intracranial neoplasms and proposed a workflow for the same (Fig 1).

Circulating Tumor Cells (CTC's)

Tumor is a collection of millions and billions of cells enclosing genetic mutation that signals them to replicate and invade the local nearby tissue. With the proliferation, the cells also disengage from the edges of the tumors and enter the circulation [30]. These cells remain loose in circulation where they can cluster together and can even enter or evade the other tissues. Thus it can be said that CTC's can informate the complete tumor biology which can be a key to cancer diagnosis or treatment. However, CTC's detection in bloodstream of cancer patients has resulted in better understanding of the biology of solid cancer and the related circulating progeny. Various epithelial cancers like lung, colon, and breast, prostrate have presented the expression of CTC's. This ideates that circulating progeny of cells are found not only in the phase of disseminated malignancy but also at every phase of cancer development [21]. Moreover according to seed and soil hypothesis, CTC's has seeds to drive the possibility of distant metastasis [22] and hence can be called as harbinger of metastasis. Sometimes these circulating cells disseminate at the same i.e. they self seed at the same primary tumor making the cancer more aggressive and difficult to handle. Hence, these CTC's can potentially serve as a liquid biopsy marker that reveals important information on therapeutic targets and/or resistance mechanisms, which might help in future to stratify patients for targeted therapies [23].Numerous studies related to CTC's have been done and potential efforts are made to detect them. Nevertheless this turns out to be technically challenging due to lack of enough quantity of CTC's in the blood and their biological heterogeneity [24]. Because there are low amounts of CTC's in bloodstream, CTC enrichment is required. Varied methods have been used to isolate and detect CTC's from the sample that are inclusive of both based on physical characteristics i.e. size, density together with other methods based on their biological properties like immunomagnetic separation, microscopically, invasion assay and others [31] EpCAM based detection and mRNA/reverse transcription polymerase chain reaction (RT-PCR) assays have also been potentially used to detect CTC's [25]. The special microenvironment of brain limits the access to glioma cells in circulation which made a major limitation to CTC's studies in brain tumors. However the dogma was broken by various studies that could potentially identify CTC's in intracranial neoplasm. CTC's have been identified in the peripheral blood of the Glioblastoma patients by immunostaining of the mononuclear cells with antibodies against GFAP [26]. The study has also linked the relation of CTC's identification and EGFR amplification that showed that every single cell of the tumor tissue showed heterogeneous pattern of EGFR amplification but putative CTCs from the same patients frequently presented with high EGFR gene amplification. Thus, EGFR signalling might support the release and/or survival of CTCs in the circulation, and antibodies against EGFR could become suitable tools to capture CTCs in future studies [26]. Macarthur et al in 2014 illustrated a method to identify CTCs in glioma patients based on telomerase activity that showed an elevated expression in all tumor cells except normal cells. This method could be stated as highly sensitive and specific

because telomerase based detection was epithelial cell independent [28]. Comparatively an uncomplicated and less complicated method was employed by Sullivan et al that used CTC-iChip to detect the presence of CTC's in Glioblastoma patients. However they could detect CTCs in at least one blood specimen from 13 of 33 patients [29].Various other techniques have been used in different studies to identify CTC's in bio fluids of Glioma patients. Ge et al in 2015, effectively detected tumor forming CTC's in the peripheral blood and cerebrospinal fluid of the glioma patients that measured polyploidy of chromosome 8 examination by CEP8-FISH in 10 brain glioma specimens [27].

Exosomes

Tumorous cells communicate with its microenvironment indirectly influencing the neighbouring cells. Molecules encapsulated in the extracellular vesicles are generally taken up during the communicative process [32]. Extracellular vesicles are the small sized 30-2000 nm cell secreted vesicles by both the normal and the tumor cells. They are involved in multiple biological functions that include cell-cell communication, its remodelling, intonation of tumor micro-environment and immune-modulation [11].These vesicles contain the genomic, epigenomic as well as the proteomic information of the cell of origin that can potentially decode the molecular characteristics of neoplasm that can the treatment response. Thus their developed a growing interest in EV's as a potential biomarker to study disease burden and therapeutic response in patients. Studies with extracellular vesicles have been done in case of Lung cancers, Liver cancers, Metastasis and many others. Extracellular vesicles are generally further classified on basis of size and site of origin. One such kind of extracellular vesicle is "Exosomes" that are originated from multi-vesicular bodies that act as intracellular signalling devices. Exosomes are nano-sized sacs that range in size from 30 nm to 100 nm and posses density of 1.13–1.19 g/mL that can be separated by sucrose-density gradient sedimentation by ultra-centrifugation.[33, 34] These are release under varied pathological and physiological conditions which can be modulated externally by ligand cognition or induced stress condition [35]. Exosomes that are release from both normal and malignant cells promote tumorigenesis, apoptosis as well as the development of chemo-resistance. These act as cargo that develops a pre-metastatic niche at the distant organs via vascularisation and induction of endothelial and stromal cell differentiation [36]. Exosomal studies have been carried out in malignancies that have greater potential to metastasize such as Breast, Lung, and Liver. In terms of central nervous system, intractability of many brain tumors for clinical purpose and their distinct biology that embeds to the unique microenvironment of the central nervous system (CNS) with their cellular interactions within, exosomes can be a latent sample type [12]. Routinely used biomarkers have already been potentially identified in exosomal RNA including mutant form of EGFR (EGFRvIII), IDH1 and miR-21 [14]. Molecular studies have shown that these vesicles released from glioblastoma patients potentially stimulates angiogenesis in terms of certain proteolytic enzymes

(gelatinases and plasminogen activators), pro-angiogenic growth factors (VEGF and TGFβ), and the promoting-angiogenic CXCR4 chemokine receptor that can attribute to its proliferative, motility and tube forming capacity [13]. It has been established that exosomes mediate the transfer of histones, oncogenic species (EGFRvIII), non-coding RNA (miRNA) and tumor suppressors (PTEN) in glioma cells [15]. Meta-analysis of the exosomal markers include Alix, TSG101, CD9, CD63, CD81 and CD151 and as observed, many of them were ranked in the top 10% of genes that were differentially expressed between tumor and normal tissue [15]. Exosomal EGFR wildtype expression levels in patient CSF has also been linked to chemotherapeutic response and can potentially act as a marker for drug sensitivity in GBM patients, as EGFR over-expression is present in up to 70% of GBM cases. The results showed that the levels of miR-21 target genes of PTEN, RECK and PDCD4 were up-regulated at protein levels [14]. In pre-clinical models, EVs isolated from glioblastoma cell lines contain tumor-specific mRNA and miRNAs.

Circulating Tumor DNA/Cell Free DNA

Circulating tumor DNA is generally the tumor fetched fragmented DNA dissociated from the cells different from the cell free DNA circulating freely in the bloodstream and not necessarily of the tumor origination. Plasma of malignant tumors contains circulating tumor DNA shed in circulation which can potentially demonstrate mutations and tumor burden. This can afford the opportunity to diagnose, monitor recurrence, and measure therapy response solely through non-invasive blood draw [16]. Studies in varied malignancy have shown a remarkable change in the levels of circulating free DNA (cf-DNA) depicting therapy response and changes associated with recurrence of the disease [17,18,19]. This may be informative in studying genetic alterations involved in disease progression and its genesis. In case of brain tumors, this can turn out to be an effective method of diagnosis avoiding tissue biopsies. Earlier studies have worked on detecting MGMT methylation in the plasma of glioblastoma patients receiving TMZ by real-time PCR [20]. Tumor-derived cf-DNA has been even earlier detected in the CSF of patients with primary CNS malignancy and CNS metastases from solid Tumors including melanoma, lung and breast cancer using next generating sequencing and the digital PCR (DigPCR) platform. However, not more studies have been done in the field of Liquid Biopsy from blood for the intra-cranial tumors, by the fact that blood has no exposure to the brain tumors due to the blood brain barriers. Moreover, cerebrospinal fluid assessment from the CNS, for the cell free DNA studies can be more reliable source for marker based studies. The blood-based cell free DNA obtained can be a more reluctant marker to study blood-brain barrier damages.

2.CONCLUSION

The bio fluid based markers i.e. cell free nucleic acids, circulating tumor cells and extracellular vesicles together have never been correlated for the marker based study in intracranial malignancies. The earlier prototype of tissue biopsy for disease measuring, can now be intervene by introducing

the “Liquid Biopsy” as a part of routine diagnostics and therapy monitoring via a safer and less-invasive way of sampling. In case of intracranial neoplasms, the peripheral blood is less used since the primary drainage in brain is cerebrospinal fluid due to presence of blood brain barrier. However, studies have shown effective role of cerebrospinal fluid in glioma diagnosis for the reason that CSF directly showers the intracranial segment of the body. CTC identification and Cell free DNA measurement from CSF have shown promising results for biomarker identification as compared to those in the serum and/or plasma of the same patients. CTC and extracellular vesicles together can be effectively used as biomarkers because it reveals the complete knowledge about the intra-tumoral heterogeneity. Eventually, deeper understanding of the origins of cell free-DNA, CTCs, and EVs turns out to be important aspect, considering that all three appear to arise through separate mechanisms. It seems likely that these sample sources may complement each other as each of them is able to provide unique information about tumor burden, genetics, and biology. Even with their current limitations, circulating biomarkers have shown tremendous clinical and research potential in numerous cancer types, including glioma. A correlative study involving serum and cerebrospinal fluid as surrogate sampling source for disease monitoring, in otherwise difficult to access tumors may be clinically an advantage in patient management. Furthermore, these sample sources can be an enormous resource for molecular landscaping of tumor type, for study of prognostic markers and treatment modalities.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPLIMENTARY FILES

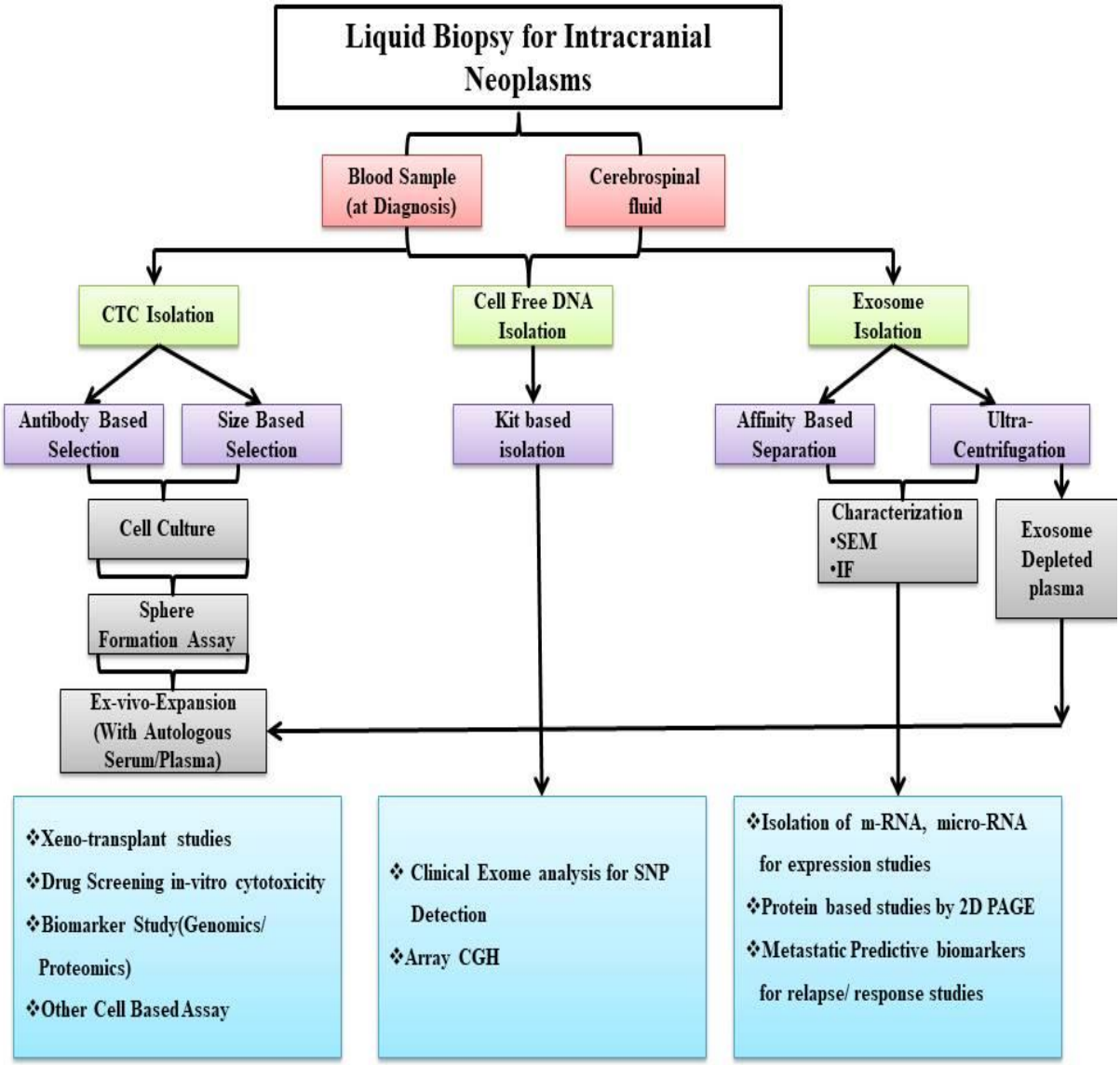

Fig:1 Hypothetical Study design with Liquid Biopsy samples of Intracranial Neoplasms