Mini review

Tissue-free non-invasive diagnostic methodology for brain tumour: Present scenario and future direction

Anirban Ghosh[1], Shubhamitra Chaudhuri[2]

[1]Cell Development and Immunobiology Laboratory (CDIL), Department of Zoology, School of Sciences, Netaji Subhas Open University, Kolkata, West Bengal, India

[2]Department of Neurosurgery, Bangur Institute of Neurosciences, Institute of Post Graduate Medical Education and Research (IPGME&R), Kolkata, West Bengal, India

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Corresponding author: **Anirban Ghosh.** Email: anirbanghosh@wbnsou.ac.in, aghosh06@gmail.com

ABSTRACT

Intracranial neoplasia is characterized by their various forms and functions including gliomas which are hard to detect properly, monitor and treat in any therapeutic regime. Present day MRI based techniques act efficiently to detect and acquire the spatial information but fall apart to gather sufficient biological attributes of the lesion to monitor and treat accordingly. In contrast, invasive biopsy is difficult within the cranium and poses a serious threat to lead metastasis. Therefore, a prominent parallel initiative has been undertaken throughout the global community to find out potential diagnostic protocols to diagnose and monitor brain tumour in a non-invasive way. Like other cancers, liquid biopsy by obtaining cellular and molecular components from the brain tumour, either from fluid-filled CNS ventricles or CSF, or leaching out into the peripheral biofluids are under constant scrutiny for finding out different molecular signatures of neoplastic growth applying innovative biomedical methodologies and instrumentations. At the same time, a new domain of research applying computer aided methods of image analysis has opened up to assist the process more potently. In this short review, we tried to show the glimpses of these newer areas and approaches of brain tumour diagnosis which may revolutionize the future of brain tumour diagnosis. Also, we hint at some potential routes to acquire biomolecular information on the brain and how higher order integration of data processing from biological and radiological fronts may be the future of these diagnostics.

Keywords: Brain tumour; computer-aided diagnosis; liquid biopsy; biomarkers; deep-learning; AI.

INTRODUCTION

Detection and diagnosis of brain tumour is now mostly dependent on complete resection of contrast enriching tumour mass in magnetic resonance imaging (MRI) that lack sensitivity and specificity. In many occasions such diagnosis fails in definite detection of types and phases of the tumour principally because of positional unsuitability and technical shortcoming of the MRI for the tumours sitting within the cranium. Also needle aspiration based cytological detection remains an inappropriate option for brain cancer diagnosis. Practical experience shows that MRI suspects of a specific brain tumour are detected differently in post-surgical biopsy. This uncertainty poses a challenge in employing chemotherapy prior to tissue biopsy after surgical intervention. Also, post-surgical management of patients, monitoring of invasiveness and recurrence and follow-up medication become difficult as brain tumour lacks any potent non-invasive or minimally invasive reliable diagnostics. But surgical intervention within cranium for definite diagnosis and recurring invasive intervention for determining disease progression and evolution to adopt precise treatment regime is practically and technically impossible, and may increase the invasiveness and growth of the tumour mass in the brain.

Non-invasive approach: Radiological

Brain Tumour remains always a challenge to the neuro oncologists and neurosurgeons for the difficulty in diagnosis and detection of the tumour type and nature due to its anatomical position and complex tissue organization. They are typically diagnosed symptomatically at primary level till date with headache and dizziness, nausea, seizure, focal neurologic impairments, personality changes etc where imaging techniques are employed or followed to detect the lesions (1). Now a variety of imaging techniques in combination with their variants are used to suspect the position and localization of brain tumours, evaluate oedema, haemorrhage and hydrocephalus lesions. The commonly employed techniques are computed tomography (CT), magnetic resonance imaging (MRI), functional MRI (f-MRI), magnetic resonance spectroscopy (MRS), positron emission tomography (PET) or single-photon emission computed tomography (SPECT) for detecting brain tumour position, size, shape with other physical features and to predict internal physicochemical attributes, which are secondarily used to basically make the educated guess about the types and grades of the tumour (1, 2).

Presently the most utilized technique to diagnose brain tumour is different variants of MRI which are dependent on measuring proton spin deviation of constituent water molecules of tissue while implied

under strong external magnetic field and the data processed through complex computer programming to develop the prospective 3D image of tissue by scanning the tissue plains (3, 4). But MRI lacks sensitivity and specificity which fails in definite detection of types and phases of the tumour principally because of positional unsuitability and technical shortcoming of the MRI for the tumours sitting within the cranium. To enhance the detection efficacy and accuracy, multidimensional approaches have been taken. Different T1-weighted and T2-weighted capture with fluid attenuated inversion recovery methods (T2 FLAIR) are now in regular practice. Use of different contrast agents are intended to aid such diagnostic methods like Gadolinium based contrast enhancing of target tissue in Gd-DTPA MRI which showed promises to detect abnormal cell density, necrotic patches, vascularization and blood-brain-barrier modifications (5). Innovative techniques using cellular markers expressing on tumour cells or in that microenvironment loaded in liposomes with gadolinium (like CD105-Gd-SLs) or use of other nano-carriers like super magnetic-iron-nanoparticles (SPIOs) are several approaches to enhance the detection efficacy in MRI (6, 7).

Present direction of radiological diagnosis

Rapid enhancement in MRI and other imaging techniques analysis are now inclining more on computer-based automation processes because interpretation of MRI images by technicians for brain tumour detection and classification is a slow and manual error prone process. Presently, different algorithms are developing for this computer-aided diagnosis (CAD) where data-scientists and programmers are playing crucial roles. Their approaches are not only dependent on MRI, but they are intended to cross verify robust data sets from multiple diagnostic sources like electroencephalogram (EEG), computed tomography (CT), single-photon emission computed tomography (SPCT), positron emission tomography (PET), magnetic resonance spectroscopy (MRS) (8). In this process different data science characterized descriptive features, machine learning and deep learning tools and classification approaches like support vector machine (SVM) and various neural network applications, namely, MPNN, PNN, CNN, DRN, DCIGN, DBN and ANN are now used (9-11). Though these methods offer enhanced accuracy, improved speed and reduced noise in brain tumour detection, classification and area segmentation, employing multifaceted tools, huge databases, robust systems and algorithms to reach the accuracy level is yet not a realistic and workable option in regular practice. Also needle aspiration based cytological detection remains an inappropriate option for brain cancer diagnosis. Practical experience shows that MRI suspects of a specific brain tumour detected differently in post-surgical biopsy (Fig.1).

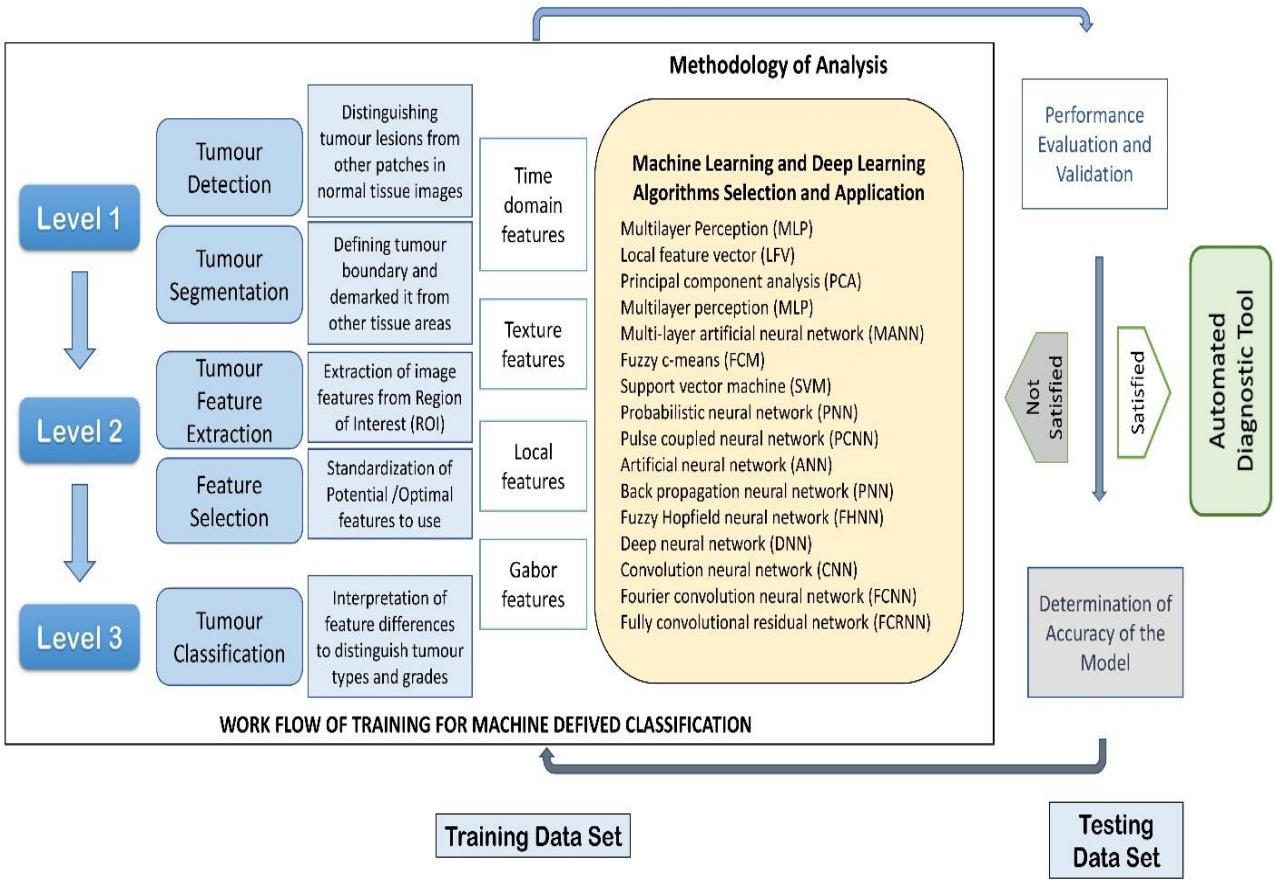

Fig. 1: Schematic representation of complete workflow of image-based machine-learning or deep-learning methodologies and techniques employed to understand the diagnostic features and define tumour categories by computer-aided protocols.

Shortfall of radiological diagnosis and complementary way forward

This uncertainty poses a challenge in employing chemotherapy prior to tissue biopsy after surgical intervention. Also, post-surgical management of patients, monitoring of invasiveness and recurrence and follow-up medication become difficult as brain tumour lacks any potent non-invasive or minimally invasive reliable diagnostics. But surgical intervention within cranium for definite diagnosis and recurring invasive intervention for determining disease progression and evolution to adopt precise treatment regime is practically and technically impossible, and may increase the invasiveness and growth of the tumour mass in the brain. Therefore, some non- or minimally invasive diagnostic approaches with radio-magnetic imaging techniques are required to increase the detection accuracy for brain tumours. Mostly, in designing such diagnostic protocols, multiple analytes are in trial where cell-free bio-fluid based multi-layered analysis may be considered. Presently, other intense areas of research to achieve significantly higher accuracy in diagnosis of brain tumour types, progressive stages and nature, are various approaches of liquid biopsy assays which may also be utilized for brain tumour management.

Liquid biopsy assays for detecting cancer is thought to be the future of cancer diagnosis and monitoring. Over two decades of continued search with hope and despair to reach the clinical application we are still with limited use of this method in only some of the cancers like lung, breast, prostate cancer and melanoma (12). The method is evolving fast and different approaches, biological information and technical developments are enriching the performance of the protocol (13). Tissue biopsy requires invasive interventions with lots of anatomical limitations, restrictions in repetitive process, fear of damage of unaffected tissue areas and high probability of metastatic trigger of tumour mass. Such a method, though, provides us the most accurate bioclinical information about the tumour, but it is incapable of real-time monitoring of disease progression and drug response. In contrast, bio-fluid dependent biopsy may compensate for most of the requirements (14).

Liquid biopsy: Speaking a lot without touch

The major target components for liquid biopsy are various, which include circulating tumour cells (CTC), cell free DNA (cfDNA), circulating tumour DNA (ctDNA), tumour associated miRNA, circulating marker proteins or peptides, exosome or extracellular vesicle (EV) derived DNA/RNA and proteins. The CTC may be considered as the direct messenger of tumour, particularly, metastatic aka advanced stages of tumour which may possibly be identified in blood (B-CTCs), in lymph fluid, afferent or efferent ducts of lymph nodes (L-CTCs) in nearby strategic positions of tumour mass or in CSF in case of brain tumours (csf-CTCs) (15). CTCs may have the potential to be counted as the dynamic diagnostic tool as they increase or decrease with the increase or decrease of tumour burden which is already documented for breast cancer and pancreatic cancer (16, 17). In most of the approaches, CTC analysis is accompanied with ct-cfDNA, analysis of exosome derived DNA, miRNA or proteins (EXOs) and this combined approach is the present reality of tumour liquid biopsy research. In cancer, apoptotic and necrotic cells release circulating cell-free DNA fractions designated as cfDNA of which a subset is the ct-DNA representing a small portion of total cfDNA. Keeping in mind the very small proportion of these DNA circulating in body fluid, targeted approaches are undertaken in contrast to whole genome or whole exon sequencing (WGS or WES). Other PCR based technologies like BEAMing (beads, emulsion, amplification and magneting) and droplet digital PCR (ddPCR) are showing high range of sensitivity (18, 19). To date, similar to the conventional biopsies, liquid biopsy with CTCs, ctDNA, miRNA and EXOs are the research hotspots to develop most efficient and reliable non-invasive diagnostic tool not only to detect, but to diagnose and monitor the dynamic state of tumour in development or regression under any clinical or therapeutic regime and management (Fig. 2).

Liquid biopsy in brain tumour: Peep into the cranium

Though there is still a major lack of standardization of methods, this non-invasive biopsy may become especially useful for brain tumours. Brain tumours possess a major difference for its anatomic location inhabiting a closed compartment with some sort of cellular and molecular restriction due to the presence of blood-brain-barrier. Yet, recent observations surprisingly found the presence of circulating brain tumour cells in the blood stream as well as CSF (20). In connection, the presence of circulating tumour derived DNA and RNA and proteins, tumour associated exosomes are reported with varied specificity and sensitivity in brain tumours. Eibl and Schneemann, 2021, elaborately mentioned about nearly two decades of study where over 50 different ctDNA markers were investigated either for methylation specific PCR or LOH or mutational studies where samples were obtained from different types of glioma. Recent studies recognized that more miRNAs show up or downregulation with tumour (21). Among the plethora of miRNA identified with brain tumours few are showing their prominence like miR-10, miR-21, miR-124, miR-210, RUN6-1 and are used in diagnostic purposes at the experimental level (22,23). However, the miRNA data is not conclusive enough to categorize and classify the brain tumour types, grades and for clinical subtyping (Fig.3).

Genetic markers like MGMT promoter, p16, p73, PTEN, DAPK, p15INK4B, p14ARF methylation, or

mutation of TP53, EGFR, IDH, PIK3CA, TERT, ATRX, H3F3A, ATM and some other genes followed by mutation study of genome or large gene panels powered by NG gene sequencing ultimately showed no conclusive markers, but the mutational importance of handful of genes which are now mostly recognized in WHO classification of brain tumours (24). Mair and Mouliere, summarized single nucleotide variance and other genomic alterations in glioma plasma samples where different methodologies like PCR-BEAming of target genes, targeted sequencing of 54-gene panels or capture sequencing of 68-gene panels or personalized sequencing by INVAR method could not cross the success limit of detection level of about 50%. Also, detection sensitivity of specific cfDNA is challenging, especially for CNS tumours, due to their lower reachability in plasma. Dependence on epigenetic alteration of bio-fluid samples possesses some technical drawbacks for both bisulfite conversion and bisulfite-free methods, whereas exosome protein markers and miRNAs are not yet sufficiently standardized (25). Many of the liquid biopsy studies for brain tumour depends on CSF collected through lumbar puncture and rarely through cisternal puncture (reported to be more effective) showed higher efficacy of detecting cf-ctDNA/RNA and EV derived DNA/RNA matched with tumour tissue. Miller *et al.*, 2019, claimed that about 50% of their diffuse glioma patient CSF samples were detected with ctDNA and notice mutations in IDH1 and 2, EGFR, co-deletion of 1p/19q along with TP53, TERT, ATRX mutations in cluster or exclusively, and the observed sequential accumulation of mutation over the course of progression of glioma (26). However, a recent study using specific personalized capture panels developed on matched tumour biopsies showed high capture value of cfDNA for glioma, even in plasma and urine (27), but for this the invasive tumour biopsy data was required.

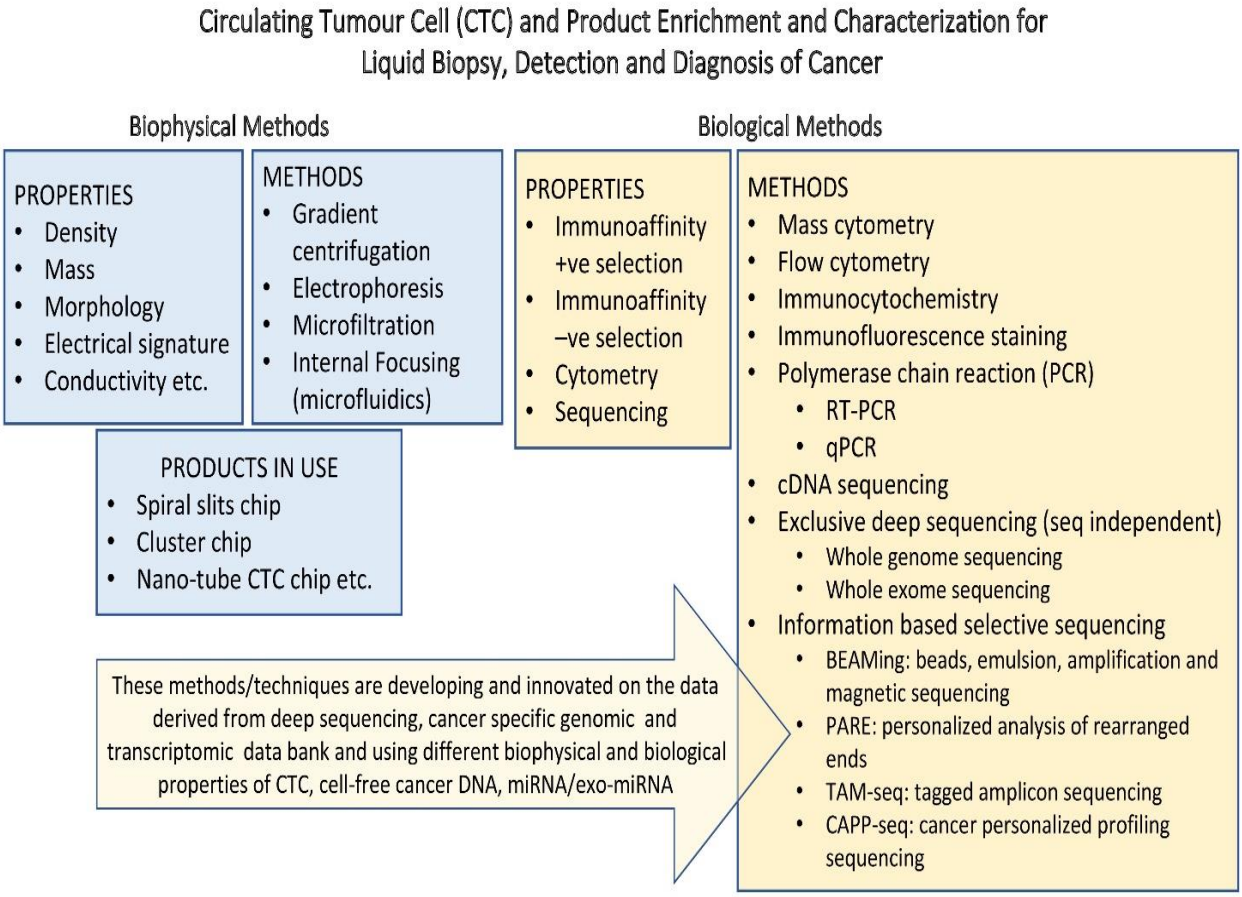

Fig. 2: Schematic representation of liquid biopsy methods, components and their enrichment techniques to detect the cellular and molecular signatures of cancer. Innovative molecular biological, biomedical techniques, protocols, instrumentation and their applications are developing faster to search and optimize newer molecular diagnostic methodology with more information to diagnose and monitor cancer for better management and treatment.

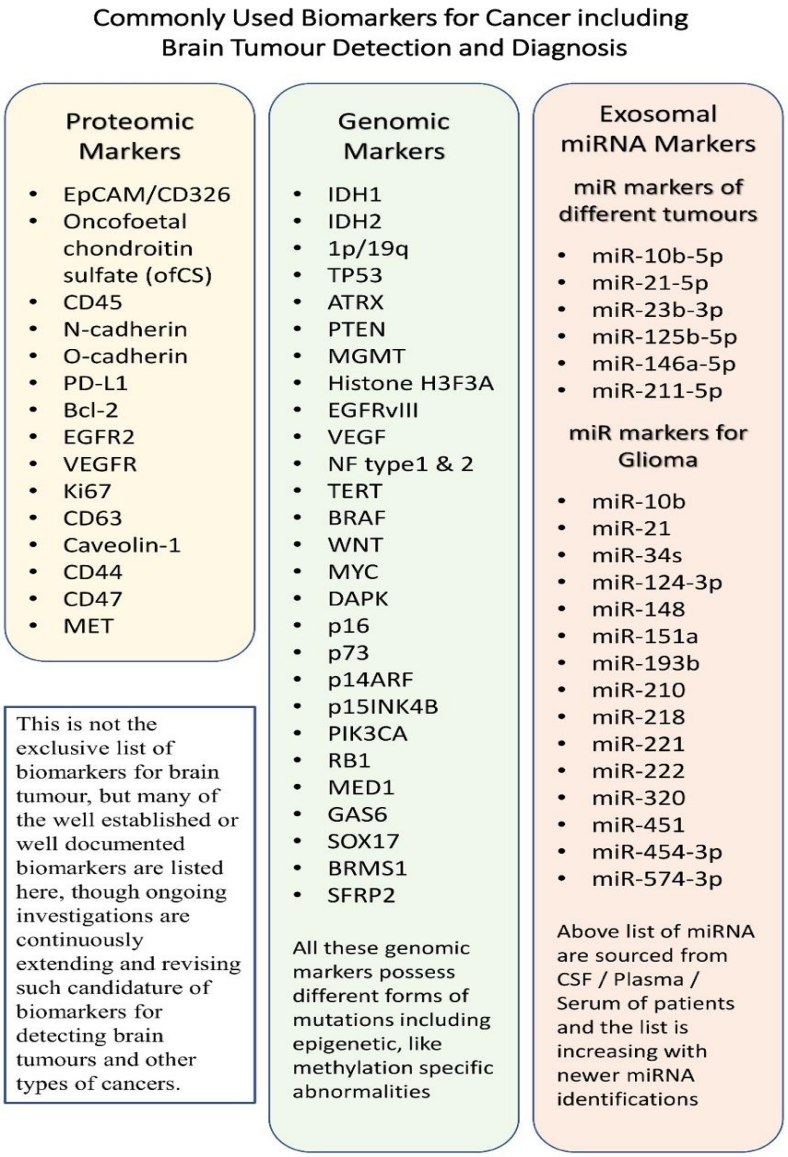

Fig. 3: Different types of biomarkers – some of them are already in use for molecular diagnostics and many others have shown potential to become biomarkers in case to case basis for different types of brain tumours.

Fig.3 has enlisted many of the well documented biomarkers from proteomic levels including different cell-surface and intracellular proteins, array of genes identified with mutations in the ORF regions or promoter regions as well as alteration of promoter methylation patterns and an array of microRNA and the list is ever-increasing. At present, with identifying novel biomarkers, it is our biggest challenge to figure out the most suitable or effective biomarker combination as per the characteristics of the population to formulate an efficient diagnostic and prognostic tool which can aid the non-invasive diagnosis for brain tumour with reliable accuracy and efficiency.

Agents with potential: A less exploited zone for spying

In the scenario of liquid biopsy of cancers, very few initiatives have been taken so far, rather investigators are keener to understand basic biological phenomena of brain tumour and its clinical significance (28). However, a major area for brain tumour detection in the periphery may arise from nearby lymph gland and lymph, which is still ignored for primary brain tumour detection. It is already reported that a significant amount of brain tumour migration and metastasis occurs through cervical lymph nodes. CSF and interstitial fluids drain from CNS carrying the components of CNS extracellular and cellular components along with immune cells which encounter tumour tissue and microenvironment in the brain (29). Meningeal lymphatics and cervical lymphatics are the nearest systems which harbour cellular and cell-free components of brain, interacting antigen presenting cells with expected tumour associated macrophages and lymphocytes, and these are the routes which brain tumour cells heavily engage in metastasis (30). Recent studies demonstrated that lymph node based liquid biopsy assay may produce information on presence and metastatic status of T1 colorectal cancer and such node based liquid biopsy is capable of detecting circulating cancer stem cells (31). Yet, such an area is unexplored in studying brain tumours and their diagnoses.

The future non-invasive BT diagnosis: Higher order integration of approaches

Now the entire diagnostic regime for cancer is under a rapid transformation phase where liquid biopsy of cancer with the involvement of advanced flow cytometric and fluidics application, next-generation sequencing technologies and chip-based arrays (32) are playing the vital role in detecting cancer type and stage by using the massive biological information. Such approaches are under vigorous trial for brain tumour depending on the basic biological data from genomic, transcriptomic and proteomic findings. Despite these efforts, some potential areas are still remaining less investigated to exploit further to get closer to the accuracy, like the lymphatic traces of cancer and immune cells mentioned earlier, which may have a chance to play crucially in collecting biological data or features from liquid phase for the brain tumour. On the other hand, the advancement of non-invasive imaging techniques, primarily MRI, CT and their variants and next-generation computer-aided diagnosis (CAD) are used and interpreted in a new dimension by analysing the data through machine learning, deep learning and artificial intelligence (AI) dependent applications. Proper assembly of data from these different approaches and interpretation of that gigantic volume of information to extract selected features and characteristics are now going to be a machine dependent systemic approach. We are still lagging behind to identify this cross-disciplinary need and initiate our act upon this area. When all these lacunae will be addressed properly or sufficiently, sooner or later, we may expect a complete automation of the whole diagnostic process of all cancers including brain tumours where suggestions on surgical and chemotherapeutic interventions and management will be the diagnostic outcome.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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