Molecular Therapy
Oncology
Clinical Perspective

Potential of sonobiopsy as a novel diagnosis tool for brain cancer

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Brain tumors have a poor prognosis. Early, accurate diagnosis and treatment are crucial. Although brain surgical biopsy can provide an accurate diagnosis, it is highly invasive and risky and is not suitable for follow-up examination. Blood-based liquid biopsies have a low detection rate of tumor biomarkers and limited evaluation ability due to the existence of the blood-brain barrier (BBB). The BBB is composed of brain capillary endothelial cells through tight junctions, which prevents the release of brain tumor markers to the human peripheral circulation, making it more difficult to diagnose, predict prognosis, and evaluate therapeutic response through brain tumor markers than other tumors. Focused ultrasound (FUS)-enabled liquid biopsy (sonobiopsy) is an emerging technique using FUS to promote the release of tumor markers into the circulatory system and cerebrospinal fluid, thus facilitating tumor detection. The feasibility and safety data from both animal models and clinical trials support sonobiopsy as a great potential in the diagnosis of brain diseases.

INTRODUCTION

Brain cancer has a poor prognosis and can lead to poor quality of life due to damage to the brain's neurological function.[1] An early and accurate diagnosis of brain tumors is essential to improve treatment outcomes. Although several imaging modalities are available to diagnose the disease, a biopsy is still required for a definitive diagnosis. However, surgical biopsies are invasive and pose significant clinical risks, such as destruction of brain tissue and impairment of brain function. Moreover, the performance of repeat biopsies to track disease progression and treatment response is generally not feasible.[2]

Blood-based liquid biopsies are increasingly being used as a non-invasive diagnostic method, but there are limitations associated with their use for assessing brain tumors. First of all, the BBB acts as a protective barrier within the brain, eventually restricting the release of large molecules, such as DNA and RNA, from brain tumors into the peripheral circulation.[3,4] In addition, any tumor markers released into the blood tend to have a short half-life and low expression levels, making their effective detection challenging using conventional blood tests. Moreover, brain tumors exhibit heterogeneity, so their molecular profiles can vary between different areas within the same lesion. Therefore, further research is required to develop techniques that could be used to facilitate the spatial detection and analysis of blood-based brain cancer biomarkers.

BBB

The BBB is a characteristic of the brain that makes brain tissue different from other parts of the human body, which is mainly composed of brain capillary endothelial cells through tight junctions.[5] The BBB can strictly control the transport of substances to the central nervous system (CNS), provide an appropriate environment for maintaining normal nervous function, and limit the entry of toxins, pathogens, and the body's own immune system to protect the CNS from injury and disease.[6] However, the BBB also brings challenges to brain tumor biopsy by preventing the release of brain tumor markers to the human peripheral circulation,[7] making it more difficult to diagnose, predict prognosis, and evaluate therapeutic response through brain tumor markers than other tumors.

BRAIN TUMOR BIOMARKERS

As defined by the National Cancer Institute, a biomarker is "a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease." It is often used in the detection and diagnosis of many diseases, especially tumors. In clinical trials, biomarkers can be used for a variety of assessment of patients, including estimating the risk of disease, distinguishing benign and malignant tumors, identifying different malignant tumors, predicting treatment response, judging the prognosis of patients with cancer, monitoring disease status, monitoring relapse, and so on,[8] which covers almost the whole process of tumor prevention, detection, treatment, and prognosis. Cancer biomarkers are found in tumor tissue, serum, and other body fluids and contain a variety of molecules, including DNA, mRNA, enzymes, metabolites, transcription factors, and cell surface receptors.[9]

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Table 1. Brain tumors biomarkers and their significance

Biomarkers	Significance
Circulating tumor cell	diagnosis, monitor treatment response, monitor the degree of disease progression, prognose[34–36]
Circulating free DNA	diagnosis, real-time assessment of treatment effectiveness and post-treatment monitoring[37,38]
Circulating cell-free microRNAs	diagnosis and monitoring response to treatment, recurrence, prognosis, predict[39–44]
Circulating proteins	diagnosis, recognition of disease recurrence, monitoring response to treatment and predictive biomarker[45–48]
Circulating extracellular vesicles	diagnosis, monitoring and prognosis response to treatment[49–52]
Mutations in the v-Raf murine sarcoma viral oncogene homolog B (BRAF) gene	prognostic markers[53,54]
O-6 methylguanine-DNA methyltransferase (MGMT)	prognostic and predictive biomarker[28,55,56]
Telomerase reverse transcriptase (TERT)	monitoring response to treatment, prognostic and predictive biomarker[57–61]
CD133	prognostic biomarker[62,63]
Isocitrate dehydrogenase (IDH)	prognostic biomarker[30,64,65]
Loss of heterozygosity (LOH)	prognostic and predictive biomarker[33,66,67]
Epidermal growth factor receptor (EGFR)	treatment resistance, efficacy of therapeutic drugs, diagnosis and prognostic biomarker[68,69]
Glial fibrillary acidic protein (GFAP)	diagnosis and prognostic biomarker[32,70]
Tumor protein 53 (Tp53)	prognostic biomarker[31,71]

The role of tumor markers has been verified and applied in a variety of cancers, such as gastric cancer,[10,11] colorectal cancer,[12–15] prostate cancer,[16–18] pancreatic cancer,[19,20] breast cancer,[21] bladder cancer,[22] and so on. In brain tumors, there are also a number of tumor markers (extracellular vesicles,[23,24] circulating tumor cells,[25] oncometabolites,[26] circulating tumor DNA (ctDNA), circulating cell-free microRNAs,[27] O6-methylguanine-DNA methyltransferase,[28] epidermal growth factor receptor (EGFR),[29] isocitrate dehydrogenase (IDH1),[30] tumor protein TP53,[31] glial fibrillary acidic protein (GFAP),[32] loss of heterozygosity[33]) that can reflect disease progression, treatment response, and prognosis.

Detection of these tumor markers is often performed by biopsy, but brain tumors are challenging to detect due to objective limitations such as the presence of the skull and the BBB, as well as the fragility of the CNS. Table 1 lists some biomarkers for the brain and their significance.

FUS-ENABLED LIQUID BIOPSY

Focused ultrasound (FUS)-enabled liquid biopsy (sonobiopsy) is an emerging technique using FUS to promote the release of tumor markers into the circulatory system and cerebrospinal fluid, thus facilitating tumor detection.[72] FUS involves using high-intensity ultrasound waves precisely targeted to a specific focal point within the body. The energy from the ultrasound waves causes the formation and subsequent collapse of tiny bubbles, which generates localized mechanical forces, known as acoustic cavitation, for diagnostic and therapeutic purposes.[73,74] Modified microbubbles for certain purposes are normally utilized to enhance the acoustic cavitation.

For a long time, brain tumors were generally detected by imaging (such as MRI and computed tomography), followed by surgical excision and tissue biopsy before final confirmation. However, resection of brain tumors has surgical risks[75] and complications such as bleeding, nerve damage, and infection.[76,77] Blood-based biopsy techniques can obtain highly relevant tumor information while being non-invasive, rapid, and inexpensive.[78] It is used for the diagnosis, molecular characterization, and monitoring of brain cancer by detecting circulating tumor-derived biomarkers, such as DNA, RNA, extracellular vesicles, and proteins shed into the blood circulation by tumors.[79–83] Different from traditional ultrasound, FUS usually uses concave transducers, lenses, or phased arrays to focus ultrasound to a focal tissue,[84] thus generating high energy density there.[85] FUS can cause a cavitation effect, where, when the ultrasonic intensity is sufficient, the bubble continues to increase with the expansion period of the ultrasonic wave, reaching an unstable size and then violently collapsing, resulting in various biological and thermal effects for diagnostic and therapeutic purposes.[73,74,86] FUS combined with intravenous microbubble injection can transiently and reversibly open tight junctions through ultrasound cavitation and increase the permeability of the BBB, thereby promoting the release of brain tumor biomarkers into the circulatory system (see the sonobiopsy diagram in Figure 1).

Accuracy is one of the advantages of sonobiospy. Due to cranial interference in the brain and the fragility of neural tissue, the focus of energy can help BBB opening (BBBO) in the target region of the brain tumor to open accurately. Studies have shown that FUS can accurately trigger BBBO in the hippocampus and entorhinal cortex of patients with Alzheimer's disease,[87] and the accuracy of the neuronavigational FUS for targeted brain tumor regions has been described above.[73,88,89]

The most important advantage of sonobiospy is its repeatability, which is closely related to the characteristics of brain tumor. Brain

tumors are highly heterogeneous and develop dynamically.[90] Different tumor biomarkers will be released into the circulatory system according to the development stage of brain tumors, response to treatment, occurrence of recurrence, etc. Multiple longitudinal diagnosis is extremely important, as it can track the progression of brain tumors, judge the prognosis, and detect recurrence in time. By focusing the ultrasound on different parts of the tumor at different time points in tumor development, sonobiospy can overcome the temporal and spatial heterogeneity of brain tumors.

ANIMALS STUDIES

FUS-mediated fluid biopsies of the nervous system have been validated in animal models, such as Alzheimer's disease.[91] Here, we discuss some advances in animal studies on sonobiospy of brain tumors. In healthy porcine models, FUS significantly enhanced the plasma concentration of brain-specific biomarkers GFAP and myelin basic protein (MBP).[92] Lifei Zhu et al. used FUS in combination with microvesicles to locally release the mRNA of mouse glioblastoma tumor into the blood for liquid biopsy. EGFP mRNA is usually an undetectable tumor-specific marker in the blood. After FUS treatment, EGFP mRNA in circulating blood was significantly increased. This was demonstrated in both the orthotopic human glioma xenograft model (U87) and the orthotopic mouse glioma xenograft model (GL261).[76] This study shows that the FUS-mediated BBBO can enhance brain-to-blood trafficking, which may provide a support technique for non-invasive and region-specific liquid biopsy of brain tumors. In terms of sensitivity, there is exciting research. Christopher P. Pacia et al. showed that in a mouse glioblastoma (GBM) model, sonobiopsy increased the detection sensitivity of EGFRvIII from 7.14% to 64.71% and telomerase reverse transcriptase (TERT) C228T from 14.29% to 45.83%. In the porcine GBM model, it also increased the diagnostic sensitivity of EGFRvIII from 28.57% to 100% and TERT C228T from 42.86% to 71.43%.[1] This suggests that sonobiopsy can significantly enhance the detection of brain-tumor-specific mutations both in the mouse GBM model and a pig GBM model.

CLINICAL TRIALS

Based on previous animal studies, clinical trials of sonobiopsy have also been conducted. In a prospective single-arm, open-label trial, 9 patients with glioblastoma underwent transcranial MR-guided FUS (MRgFUS), and the results showed increases in plasma cell-free DNA (cfDNA) (2.6-fold), neuron-derived extracellular vesicles (3.2-fold), and brain-specific proteins (1.4-fold). An increase in cfDNA-mutant copies of IDH1 was also detected in one of the patients. This prospective study suggests that MRgFUS can enhance the signal of brain-derived biomarkers, which has the potential to support fluid biopsy of the brain.[4] In 2023, Jinyun Yuan et al. reported the first prospective trial of sonobiopsy in patients with high-grade glioma with neuronavigation. The analysis of blood samples before and after FUS treatment showed that the sonobiopsy enriched plasma ctDNA, especially the TERT mutation ctDNA level, with a 5.6-fold increase. This is commendable work, which provides data support for the clinical transformation of non-invasive diagnosis of brain tumors.[93]

According to ClinicalTrials.gov, two clinical trials involving the ultrasound biopsy of brain tumors are currently recruiting (ClinicalTrials.gov: NCT04940507 and NCT05383872). The BRAINFUL trial (ClinicalTrials.gov: NCT04940507) plans to combine liquid biopsy with high-intensity FUS to enhance the release of ctDNA into the circulation and improve the sensitivity and specificity of biopsy for brain tumors to evaluate the utility of MRgFUS in improving ctDNA abundance in brain tumors and enhance non-invasive detection of brain tumor methylation signatures while understanding the changes in ctDNA over time. There is also a prospective, multi-center, pivotal clinical trial focused on the safety and efficacy of targeted BBB disruption for biopsy in subjects with suspected glioblastoma brain tumors (ClinicalTrials.gov: NCT05383872). We look forward to the results of these ongoing clinical trials. Both studies have in common that MRgFUS technology will be used, and the combination with liquid biopsy is expected to lead to fundamental advances in the diagnosis and monitoring of brain tumors.[77]

ACCURACY AND SAFETY

In the past, sonobiopsy was usually guided by MRI. However, due to its high cost, complex operation, and long imaging time, its wide application in clinical practice was limited. The focused energy of sonobiopsy requires high-precision guidance to open the BBB in the target area. The current main approach is neuronavigation, a computer-assisted and interactive stereotaxic method that can position the instrument during surgery according to neuroradiological images obtained before surgery, allowing online feedback of positioning and intraoperative changes, thereby ensuring accuracy and safety.[94–96] A preliminary study in healthy swine shows that neuronavigation can successfully direct focal beams to open the BBB with accuracy comparable to neurosurgical stereotactic surgery (2.3 ± 0.9 mm).[88] Neuronavigational FUS successfully induced BBBO in the basal ganglia and cerebral cortex in adult macaques[89] and was consistent with the accuracy reported in the neuronavigation-guided surgery in humans.[94] Lu Xu et al. developed a neuronavigation-guided sonobiopsy device and characterized its targeting accuracy *in vivo*, *in vitro*, and *in silico*. The results showed that the device successfully induced BBBO in pigs with an accuracy of 3.3 ± 1.4 mm, and the accuracy of the computer numerical simulation was 5.5 ± 4.9 mm.[73] Therefore, it can focus precisely in the target area of the brain, which lays a foundation for its clinical application.

Considering the fragility of the nervous system, the safety of the diagnosis of brain tumors is the primary consideration; we need to reduce or even eliminate the damage caused by diagnosis. First, multiple studies have shown that BBBO induced by FUS is reversible,[97–99] and it can be completely closed within 6–24 h.[100,101]

Moreover, FUS-mediated BBBO is non-invasive. Previous studies have shown that BBBO, which allows drug permeation, does not cause significant acute damage to endothelial or neuronal cells.[102] In healthy pig models, sonobiopsy not only enhanced the release of brain-specific biomarkers but also did not have significant brain tissue damage according to histological analysis, gross pathological

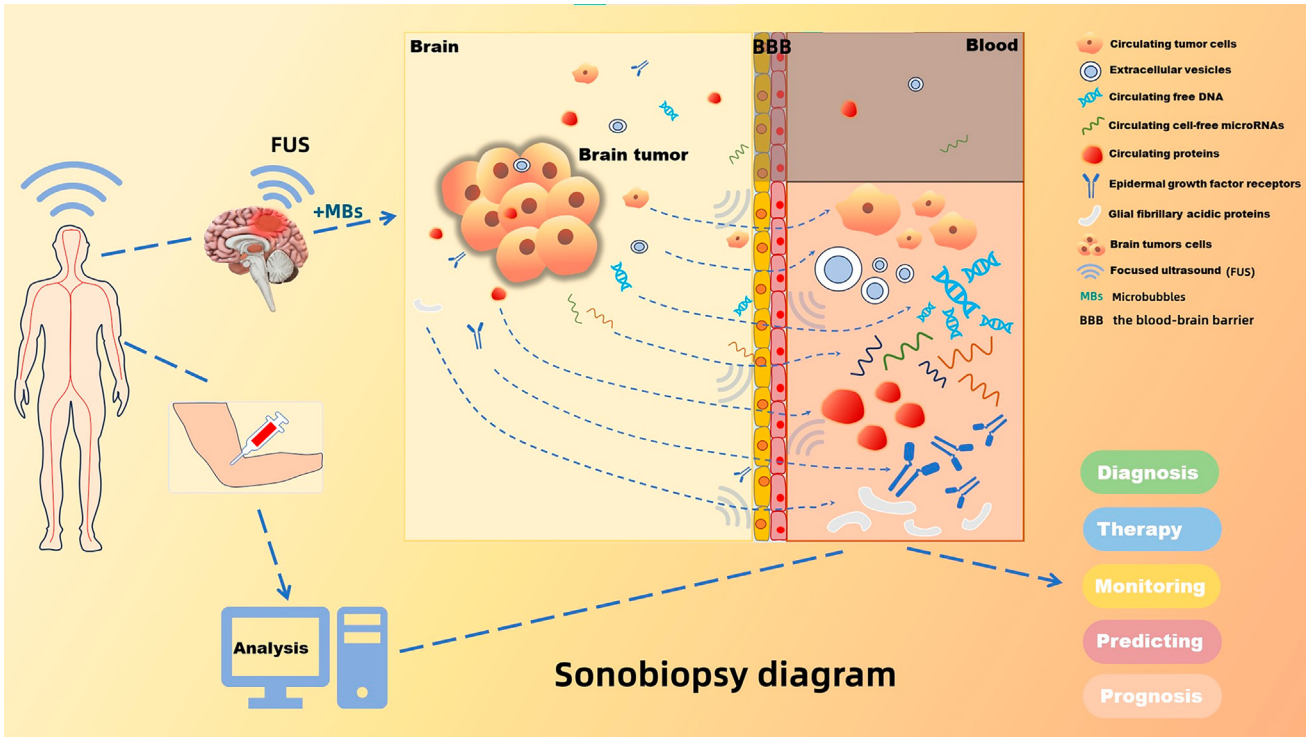

Figure 1. Sonobiopsy diagram

Under the irradiation of focused ultrasound, the blood-brain barrier (BBB) was temporarily and reversibly opened and the release of brain tumor markers into the peripheral circulation increased to promote tumor detection.

assessment, MRI, and FUS cavitation monitoring.[92] In animal experiments on the sonobiopsy of brain tumors, there is no significant off-target tissue damage in FUS-induced microhemorrhage or the number of apoptotic cells in a mouse GBM model. It also shows no significant tissue damage in a pig GBM model according to histological analysis.[1] However, in another mouse glioblastoma sonobiopsy experiment, brain sections of H&E showed microhemorrhages, especially in the tumor periphery, and the microhemorrhage's density was related to the intensity of the sound pressure used, with no significant difference between the intensity of 0.59 MPa and that of the control group. In addition, no off-target injury of the surrounding brain tissue was found in the examination of brain images.[2] In the rhesus macaques BBBO experiment, which is closer to humans, neither histological nor neurobehavioral traits were not affected.[103]

Histological analysis of human surgical resection of the tumor after ultrasound biopsy also confirmed the safety of the procedure. In the first prospective human trial using neuronavigation-guided FUS to detect high-grade gliomas,[93] the brain tumors dissected 1.7 h after FUS intervention were observed by staining sections, and there was no change of tissue damage. In addition, transcriptome analysis showed that sonobiopsy did not induce a significant inflammatory immune response in a short time. In a human clinical trial of neuronavigation-guided FUS, NaviFUS-induced BBBO was shown to be safe and tolerable for all patients in the trial without immunological response 7 days after FUS treatment.[104]

Currently, a clinical trial (ClinicalTrials.gov: NCT04692324) sponsored by the Mayo Clinic is underway to establish a biomarker database of cerebrospinal fluid for brain tumors while evaluating the possibility of continuous sampling of cerebrospinal fluid for longitudinal biomarker evaluation. This may be beneficial to enrich and improve the safety of sonobiopsy.

CHALLENGES

Although FUS has shown great potential in the diagnosis and treatment of brain tumors, its technological development remains challenging. Firstly, most studies have been conducted on animal models. Therefore, more clinical investigations are warranted to compare the sensitivity and specificity of liquid-based sonobiopsies with traditional tissue biopsies.[3] Secondly, future clinical research should also evaluate the impact of clinical factors such as disease type and patient age on the opening of the BBB. Thirdly, the skull can significantly attenuate the ultrasound beam. New imaging schemes must be developed to account for cranial anatomical variations between different individuals. Fourthly, more research is required to determine the optimal FUS parameters to promote the release of tumor biomarkers without damaging brain tissue and improve the diagnostic techniques for detecting brain tumor biomarkers in the blood. In addition,

further studies are needed to evaluate the potential impact of repeated FUS on brain tissue damage so as to determine the optimal time interval for each FUS intervention. Finally, transient BBBO could facilitate the entry of toxic blood components into the brain, which may potentially damage the CNS. Therefore, further research is required to evaluate the potential adverse effects of this technology.

IMPROVEMENTS

The improvement direction of the sonobiopsy of brain tumor is mainly focused on improving the performance of ultrasonic instruments, developing new ultrasound contrast agents, and developing an intelligent assistant diagnosis system. In the future, with the continuous progress of science and technology, we expect to develop higher-performance ultrasonic diagnosis and treatment equipment that can intelligently select treatment parameters according to the characteristics of patients. At the same time, in the process of promoting the BBBO by FUS, the molecular markers of brain tumors are identified and tracked synchronously so as to evaluate the situation of brain tumors real time and accurately. At the same time, the research and development of new ultrasound contrast agents will further improve the sensitivity, specificity, and effectiveness of ultrasound biopsy and provide doctors with a more reliable basis for diagnosis. In addition, the development of an intelligent assistant diagnosis system will greatly improve the efficiency and accuracy of ultrasound biopsy and make the diagnosis process more rapid and accurate.

PROSPECTS

The sonobiopsy of brain tumor has a broad development prospect. With the continuous improvement and optimization of technology, it will play a vital role in the early screening and diagnosis of brain tumors, helping doctors to detect and treat potential tumor lesions in time. In addition, the personalized diagnosis and treatment characteristics of sonobiopsy will meet the needs of different patients and achieve a more accurate treatment plan. Interdisciplinary cooperation will promote the common development of medical imaging, oncology, neuroscience, and other disciplines and jointly solve problems in the diagnosis and treatment of brain tumors. With the continuous expansion of the clinical application of sonobiopsy, it will also play a greater role in neurotumor surgery, tumor treatment evaluation, and other fields to provide patients with more comprehensive and high-quality medical information.

CONCLUSION

To conclude, brain sonography has great potential for diagnosing and treating brain tumors. As a cutting-edge technology in the stage of clinical transformation, this technique presents several technical challenges that arise due to tumor heterogeneity, variations in the response of different cellular components to ultrasound waves, and cranial anatomical variations. In addition, ultrasonic power, irradiation time, probe size, etc., need to be further explored and optimized. Therefore, more clinical studies are required to address these challenges and facilitate the effective and safe implementation of this technique into routine clinical practice.

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AUTHOR CONTRIBUTIONS

All authors read and approved the manuscript. Conceptualization and design, X.Z. and L.Y.; writing – original draft preparation, L.Y., K.F., and Q.L.; writing – review and editing, X.Z. and L.L.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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