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A Systematic Mapping Analysis on the Detection of Tumor Cells Targeted by Enzymes through the Cerebrospinal Fluid

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Abstract

Although neural tissue cancers are frequently regarded as terminal, the precise origins and molecular or cellular mechanisms underlying these conditions remain elusive. Despite existing knowledge, the progression patterns and secondary effects associated with different brain tumors have not yet been fully mapped out. Cerebrospinal fluid (CSF) leakage remains a prevalent diagnostic indicator in tumor detection, and in this context, enzymes associated with brain cancer were analyzed. The primary objective of this investigation was to explore how CSF is involved in brain cancer processes and its relationship with the blood-brain barrier (BBB). The study investigated common leakage points, particularly in the thoracic spine region and at the cranial base near the cardiothoracic interface, through a systematic mapping approach. The presence of bacteria in the CSF of brain cancer patients was also investigated, aiming to identify widely used strategies for targeting cancerous cells and detecting enzymes within CSF. Further insights pinpoint specific regions where leakage occurs and highlight particular proteins and enzymes implicated in this pathological process, showing how the discharge of tumor by-products contributes to CSF damage. Consequently, this research enabled the identification of enzymes and tumor cells in CSF and introduced a novel component that characterizes tumor-associated cerebrospinal fluid.

Keywords: Brain cancer, Cerebrospinal fluid, Blood-brain barrier, Enzymes

Introduction

While cancers of neural origin are frequently associated with high mortality, the precise causes and the intricate cellular and molecular mechanisms driving these diseases remain only partially understood. Despite advances in understanding different brain tumor types, there is limited clarity on how these tumors expand and inflict damage beyond their initial sites. This investigation employs a longitudinal prospective approach aimed at evaluating cerebrospinal fluid (CSF) leakage, with a specific focus on enzymes derived from brain malignancies and the array of secreted proteins they contain. It is hypothesized that these biological agents contribute to compromising the integrity of the blood-brain barrier. This qualitative research utilizes deductive reasoning and draws from cohort study models to gather relevant clinical samples. The researcher collects CSF discharge originating from cancer patients, particularly from areas along the spinal column—most notably the thoracic spine—and from the cranial base near the cardiothoracic junction.

The primary objective includes identifying the anatomical sites where these secretions breach the blood-brain barrier, utilizing magnetic resonance imaging (MRI) techniques, and subsequently cataloging the protein and enzyme profiles found in the CSF. According to Cleeland [1], cancer is a collection of diseases characterized by uncontrolled cellular proliferation and potential infiltration into surrounding tissues. This unregulated division arises when the natural cycle of cellular death and regeneration is disrupted, allowing damaged or unnecessary cells to persist and multiply abnormally [1].

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Tumors are classified as malignant when they possess the ability to invade neighboring tissue or metastasize to distant areas through vascular or lymphatic systems. In contrast, benign tumors generally remain localized and, although they may grow substantially in size, they typically do not reoccur post-removal. However, benign brain tumors can still pose serious risks due to their location. Cancer's origin is rooted in genetic alterations that regulate cell behavior, particularly in terms of growth and division [1].

Cancer can develop virtually anywhere in the body, which is composed of billions of cells. Normally, these cells proliferate and replace old or damaged ones as needed. Enzymes—acting as biological or organic catalysts—play a crucial role in accelerating chemical processes in living organisms. Once extracted, these enzymes can also serve vital functions in industrial and scientific applications [2].

Research questions

RQ1: What are the common methods of detection of cancer cell enzymes in the CSF?

RQ2: Which research targets compare missing enzymes in the selected studies?

RQ3: What are the common bacteria sampling of brain cancer in CSF discussed in the research?

Literature review

Detection of cancer cell enzymes in the CSF

The brain and spinal cord, forming the central nervous system (CNS), are often affected by complex malignancies that require advanced diagnostic and therapeutic strategies. Among these, establishing effective biomarkers has emerged as a critical priority in improving diagnostic precision, monitoring disease progression, and evaluating therapeutic efficacy. Cerebrospinal fluid (CSF) has become a major focus in the search for molecular signatures indicative of CNS cancer. While many molecular candidates have shown potential, only a limited number have progressed to consistent use in clinical diagnostics. This section explores the biochemical elements in CSF reported in scientific studies as potential brain tumor indicators and analyzes why many of these proposed markers have not yet transitioned into standardized CNS cancer management protocols [2].

RQ1: What are the common methods of detection of cancer cell enzymes in the CSF?

Leptomeningeal disease, also termed neoplastic meningitis (NM), represents an uncommon but severe progression of cancer that involves the entire central nervous axis by infiltrating the meninges that envelop the brain [2] and spinal cord. Clinical manifestations vary and may impact cranial nerves, cerebral structures, or spinal pathways. Given that the disease pervades the full extent of the subarachnoid and CSF spaces, therapeutic strategies and staging must consider this extensive distribution. However, in cases of primary brain tumors, neoplastic meningitis is rarely examined as an isolated phenomenon [3]. Recent evaluations have analyzed this condition in the context of primary brain neoplasms, where its detection is often reliant on CSF cytology and neurological symptomatology, which can vary along a rostrocaudal gradient. Despite these methods, average survival rates for individuals with NM remain dismally low, spanning only a few months post-diagnosis. The condition develops when malignant cells penetrate the leptomeninges and circulate within the subarachnoid space, an event seen in approximately 4–15% of patients with solid tumor malignancies [3], making it a poor prognostic marker.

In both solid and hematologic cancers, NM is a lethal outcome resulting from neoplastic seeding of the subarachnoid compartments. Identifying cancer cell involvement in CSF early on is pivotal for prognosis and treatment, though it remains a diagnostic challenge. Disintegrin and metalloprotease (ADAM) and matrix metalloproteases (MMPs) have been identified as potential markers for extracellular matrix degradation, a process closely linked to blood-brain barrier compromise and metastatic infiltration. Research utilizing PrAMA technology employed FRET-based metalloprotease substrates with high specificity for detecting proteolytic activity in real-time from CSF samples of NM patients and healthy controls. By observing fluorescence shifts over time, researchers can monitor protease behavior dynamically. Analyzing the cleavage of five distinct FRET substrates simultaneously allows for the profiling of protease activity, facilitating the identification of unique enzymatic patterns across different disease states. Even minimal volumes of CSF were sufficient to generate reproducible and sensitive cleavage profiles. The rate of cleavage per substrate was computationally correlated through PrAMA analysis with specific proteases, including heightened activity of MMP-9, ADAM8, and ADAM17 in CSF collected from patients. These enzymatic behaviors were also responsive to

batimastat (BB-94), a known metalloprotease inhibitor [4]. Such enzymes contribute significantly to the deterioration of the blood-brain barrier. However, because these proteases act indirectly, CSF cytology may still present as negative, underscoring the limitations of traditional cell-based diagnostics. Ultimately, PrAMA profiling of CSF offers a powerful tool for the sensitive and early detection of disease, potentially transforming the diagnostic landscape in everyday clinical contexts.

One of the defining characteristics that set cancer cells apart from their normal counterparts is their enhanced ability to migrate. This heightened mobility enables them to infiltrate surrounding tissues, making direct cell movement a plausible mechanism for local cancer invasion. Researchers have identified a particular compound that appears to enhance the motility of cancer cells. Although its precise function is still under investigation, this compound is suspected to significantly contribute to the local spread of cancer. The discovery holds promise, as targeting this molecule may inhibit its role in promoting cancer cell movement or block its initial production within tumor cells [4].

In the context of diagnosing brain malignancies, especially those with leptomeningeal involvement or secondary metastatic infiltration of the central nervous system, the examination of cancer cells in the CSF through microscopic evaluation remains the diagnostic gold standard [5]. Specimens for myeloid cytology are typically acquired either intraoperatively during tumor resections or by collecting fluid through lumbar puncture or intraventricular (PCV) reservoir access [6]. Despite multiple collection methods, lumbar puncture remains the most widely endorsed approach for retrieving CSF samples to detect malignant cells originating from primary CNS cancers [7, 8]. To minimize erroneous interpretations caused by tumor cell shedding during or shortly after surgery, a waiting period of one to two weeks is generally advised before performing diagnostic cytology on postoperative CSF samples [8–10].

Accurate assessment of microscopic imaging relies on refined cytoplasmic analysis methods. Standard protocols call for the immediate processing of about 7.5 mL of CSF, as cellular degradation can lead to a 50% loss within two hours post-collection [7, 11]. These samples are subjected to centrifugation using Cytospin® at 800 g for approximately 3 to 5 minutes, followed by air drying for 10 to 15 minutes, then stained using the May-Grünwald Giemsa (MGG) technique for another 10 to 15 minutes [12].

A newer innovation in this field is ThinPrep, a liquid-based cytology method that enhances the identification of malignant cells in CSF, particularly when evaluating solid tumors. This technique preserves cellular morphology and involves isolating cells via high-efficiency filtration and light electrostatic adherence to glass slides. Samples are mixed with 10 mL of a preservation buffer and left to settle for 15 minutes. Following this, the prepared slides are fixed in 95% ethanol for another 15 minutes and stained using the conventional Pap smear protocol [12].

Despite its diagnostic utility, CSF cytology presents several significant limitations **(Table 1)**. Primarily, this technique depends on the visual identification of atypical cells in CSF using gamma staining, and clinicians are responsible for determining malignancy based solely on morphological features. Consequently, CSF cytological analysis is inherently qualitative and lacks objective quantification or standardized confirmatory measures [13]. Moreover, since malignant cells may be intermittently or minimally present in the CSF, initial samples may not capture these cells, leading to false-negative results. Thus, if the first cytological assessment returns negative, follow-up sampling is strongly recommended to improve diagnostic accuracy [14]. Additionally, although CSF cell analysis is highly selective in detecting cancer cells, it tends to be hypoallergenic, presenting another limitation in its clinical reliability.

Existing work

Table 1 outlines different detection techniques and strategies used for identifying brain cancer biomarkers in cerebrospinal fluid, along with their advantages, disadvantages, and corresponding references.

Table 1. Targeting cerebrospinal fluid for the discovery of brain cancer biomarkers

Approach	Method	Pros	Cons
	Cytanalysis of CSF: This involves the microscopic examination of cerebrospinal	Demonstrates high specificity [5-7].	Due to limited sensitivity, false-negative outcomes are frequently encountered [3, 15-17].

	fluid to identify cancerous cells.		
Detection of cancer cells in the CSF	Cytometry analysis: this technique assesses the expression of surface proteins on cells, providing valuable insights into their characteristics.	The automated process facilitates quick analysis [5, 18]. Requires only a small volume of cerebrospinal fluid [9, 17].	When cell counts drop below 25 cells/μL, both false positives and false negatives may occur; additionally, distinguishing between mitotic and neoplastic cells remains challenging [16].
	Other tools: techniques like DNA cell measurement or fluorescent hybridization can be employed on-site to analyze the chromosome content of cancer cells in cerebrospinal fluid.	Genetic abnormalities detected by these methods can help identify malignant locations [19].	These approaches often suffer from inadequate sensitivity [20].
Detection of biochemical molecules secreted by cancer for CSF	CSF protein analysis: this method focuses on identifying and quantifying proteins in the CSF, and assessing their completeness.	Protein patterns in cerebrospinal fluid from certain brain tumors can help differentiate between tumor subtypes and stages [21-24].	Diagnostic performance may be limited by only moderate levels of sensitivity and specificity [25].
	MicroRNA CSF analysis: this involves measuring the levels of microRNA in cerebrospinal fluid to gather molecular insights.	MicroRNA detection in cerebrospinal fluid offers excellent specificity and chemical stability [26, 27], needing minimal sample amounts, which enables repeated monitoring of molecular changes throughout cancer treatment [28].	The reliability of biomarker specificity can be compromised by variations in expression levels and the unidentified origin of certain markers [28-33].

Table 1 presents approaches utilizing cerebrospinal fluid to identify brain cancer biomarkers.

Breakdown enzymes

Certain normal cells produce breakdown enzymes that play a role in immune defense and tissue repair. These enzymes help the body eliminate infections by targeting pathogens and cleaning up damaged tissue during the healing process. In numerous cancer types, elevated levels of these enzymes have been identified. Some tumors also attract a significant number of enzyme-secreting white blood cells as part of the body's immune defense against cancer. Although the origin of these enzymes is still under investigation, their presence is believed to contribute to the invasion of surrounding healthy tissue by cancer. As malignant cells degrade nearby normal tissues, nearby blood vessels can become compromised, resulting in hemorrhaging [4].

RQ2: Which research targets compare missing enzymes in the selected studies?

As tumors grow, their nuclei often expand away from local blood vessels, limiting the supply of oxygen and nutrients to the tumor core. Just like normal cells, cancer cells need these elements to survive. Endothelial cells, which line blood vessels, respond by releasing angiogenic signals that promote endothelial cell migration, proliferation, and specialization. These chemical signals initiate angiogenesis—the development of new blood vessels—which is essential for tumor progression. Without adequate blood supply, a tumor's growth remains restricted to a minuscule size. However, once vascular development is stimulated, tumor growth accelerates as it becomes nourished by a dense network of newly formed vessels [34].

When the fluid influx into a tumor surpasses the capacity of surrounding tissues to absorb it, the result is edema, accompanied by a rise in interstitial pressure and possible cyst formation. In central nervous system tumors, such as those in the brain or spinal cord, this often leads to peritumoral cysts—fluid-filled sacs adjacent to the tumor. These cysts can provoke clinical symptoms and are commonly observed in association with central nervous system malignancies. With advancements in imaging and tissue analysis, the mechanisms behind cyst formation and spread have come under closer scrutiny. Research indicates that peritumoral cysts and associated

edema result from increased vascular permeability, mediated by local tumor factors or abnormal hemodynamic pressures within malformed blood vessels. Edema and cysts form when this fluid leakage exceeds the tissue's absorptive capacity. Therapeutic interventions that limit vascular permeability or surgically remove the tumor have been shown to alleviate these symptoms, suggesting the tumor's direct role in initiating the edema that precedes cyst development [35]. Modern oncology is grounded in the understanding that the same molecular systems governing normal cellular processes—such as growth, differentiation, and programmed death—are also involved in cancer progression when disrupted. The current scientific consensus suggests that cancer arises due to alterations in these regulatory networks, causing normal cells to adopt malignant behaviors. This study examines both the enzymatic profile of tumor cells and the frequency of peritumoral cyst formation, which involves the accumulation of protein-rich fluid. It also investigates the role of local mediators that increase vascular permeability and the impact of abnormal tumor vasculature on fluid dynamics. These insights aim to quantify tumor fluid content—whether cerebrospinal fluid or another type—and correlate it with the severity of edema. In addition, the study assesses cerebrospinal fluid leakage symptoms through spinal tap evaluations in brain tumor patients, as well as the tumor types involved [36].

RQ3: What are the common bacteria sampling of brain cancer in CSF discussed in the research?

Bacteria sampling of brain cancer in CSF

Analyzing cerebrospinal fluid (CSF) for microbial presence plays a critical role in identifying infections like meningitis, a condition characterized by bacterial invasion of the brain and spinal cord's protective layers. Various pathogens, including viruses, fungi, and bacteria, can contribute to central nervous system infections, including both meningitis and encephalitis. During CSF examination, tests focus on detecting signs of infection such as the presence of bacteria, increased white blood cell levels, and specific chemical markers. These tests also aid in diagnosing autoimmune disorders that affect the nervous system, such as multiple sclerosis (MS) and Guillain-Barré syndrome.

Lymphoma can spread into the cerebrospinal fluid (CSF) when cancer cells move from the breast, lung, or other parts of the body. Once cancer cells enter the CSF, they may settle in the brain or spinal cord and begin to proliferate. To investigate the disease, a bacterial test is conducted on the CSF. This test is primarily used to identify cancer cells within the CSF, the fluid that surrounds the brain and spinal cord. It is most commonly performed when the cancer type is known to spread through CSF, such as in cases involving endometriosis. The test measures the pressure within the CSF and collects samples for further analysis. CSF testing is also useful for diagnosing neurological disorders, including infections like meningitis and injuries to the brain or spinal cord.

Additionally, the author uses bacterial assays to assess the presence of vascular endothelial growth factor (VEGF), also called vascular permeability factor (VPF), a signaling protein that promotes blood vessel formation. VEGF is a member of the platelet-derived growth factor family of cystine knot growth factors. VEGF levels were quantified using ELISA on matched CSF and serum samples obtained from patients. These groups included individuals with solid tumors and meningeal carcinomatosis (MC, n = 11), those with brain metastases but no MC (n = 12), patients with nerve syndromes associated with tumors (n = 4), viral and bacterial meningitis cases (n = 15), and a group of individuals with non-neoplastic, non-infectious neurological conditions (n = 100). The VEGF index was calculated using CSF/serum albumin ratios to estimate the intrathecal production of VEGF. Immunofluorescence labeling of VEGF was performed on breast cancer-associated brain tumor samples.

All patients with MC exhibited higher VEGF levels in their CSF, with a mean concentration of 6,794.8 pg/mL, whereas the VEGF levels in their sera were comparable to other groups. After receiving anticancer treatment, VEGF concentrations in the CSF of CM patients decreased significantly. VEGF was undetectable in the CSF of patients with brain metastases who did not have MC. In patients with acute bacterial meningitis, the mean VEGF concentration in CSF was 38.6 g/mL, with only 9 of 17 patients showing detectable VEGF levels. The VEGF levels in bacterial meningitis patients were significantly lower than those observed in CM tumor cases (22.8 vs > 62.3), indicating a higher proportion of VEGF production within the CSF in CM patients with bacterial meningitis. In patients without persistent neoplastic or infectious diseases, CSF VEGF levels were below the detection limit of 25 ppm/mL [35].

Results and Discussion

The results will be evaluated in depth and compared to the proposed research questions to gain insights into the abilities of the readers.

RQ1: What are the common methods of detection of cancer cell enzymes in the CSF?

A retrospective meta-analysis revealed that the sensitivity for detecting cancer cells in cerebrospinal fluid (CSF) can be as low as 45%, a figure that varies based on the frequency of lumbar punctures conducted [20]. False positive results, due to a lack of sufficient CSF cells and the morphological similarity between benign and malignant cells, are found in about 10-20% of cases [17-19]. The inadequate techniques for obtaining and evaluating CSF cytology, combined with the lack of genetic analysis of tumor cells, undoubtedly contribute to the variability in sensitivity [3]. Therefore, although CSF cytology is currently employed in clinical settings, it is not a reliable method for assessing disease progression in brain or metastatic cancer cases [9].

RQ2: Which research targets compare missing enzymes in the selected studies?

Cancer cells cause significant changes in metabolic activity. Specifically, mutations in genes such as isocitrate dehydrogenase 1 and 2 (IDH1/IDH2) are pivotal for tumor growth in the central nervous system (CNS). This led to the assumption that the presence of malignant cells could indicate altered metabolite levels in the CSF. To explore this hypothesis, mass spectrometry was used to analyze samples from 129 patients, which included 8 healthy controls and 23 individuals with cancer (involving different CNS malignancies). Hierarchical cluster analysis revealed unique metabolite signatures in tumor samples, suggesting that these could help identify specific tumor types. The analysis found differences in 43 metabolites between healthy CSF and that of individuals with CNS or metastatic cancer. Pathway analyses showed distinct metabolic pathway variations, such as those related to glycine, choline, methionine, and glycolysis, between IDH-mutant and IDH-wildtype gliomas. Additionally, patients with IDH-mutant gliomas had higher CSF levels of D-2-hydroxyglutarate compared to other tumor groups or healthy controls. These findings support the idea that CSF metabolite profiling could be a useful clinical tool for diagnosing and monitoring CNS or metastatic cancers [36].

RQ3: What are the common bacteria sampling of brain cancer in CSF discussed in the research?

In cases of breast cancer metastasis to the meninges, immunohistochemical analysis revealed strong cytoplasmic staining for VEGF. Elevated levels of VEGF were found in the CSF of patients suffering from precancerous meningitis. The study offers preliminary evidence suggesting that VEGF in CSF could serve as an effective biomarker for both the identification and monitoring of therapeutic response in precancerous meningitis [36].

Conclusion

This research investigates the leakage of enzymes and cancer cells to understand the impact of cerebrospinal cancer on enzyme activity. The study posited that malignant cells in the central nervous system (CNS) could alter metabolic processes, leading to abnormal metabolite levels in cerebrospinal fluid (CSF) and that different CNS cancers might show distinct metabolite profiles. Mass spectrometry was employed to analyze the metabolites in CSF from both cancer-free individuals and those diagnosed with various CNS cancers. The findings revealed that CSF from healthy individuals exhibited normal metabolite profiles, whereas CSF from patients with CNS or metastatic nerve cancer displayed significant abnormalities. Additionally, patients with IDH-mutant gliomas had elevated levels of D-2-hydroxyglutarate in their CSF compared to those with other tumors or healthy controls, as confirmed through pathway analysis. These results underscore the potential of CSF metabolite profiling as a valuable clinical tool for diagnosing and monitoring CNS malignancies and metastatic cases. The research also highlights that enzyme analysis plays a crucial role in detecting tumor cells and offering therapeutic solutions. This work provides deeper insights into the diversity of cancer conditions and may aid in better understanding the underlying causes and treatment strategies within the cancer community.

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