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REVIEW ARTICLE ABOUT MOLECULAR DETECTION OF BRAIN CANCER

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1. Introduction to Brain Cancer

Brain cancer, also known as a brain tumor, occurs when abnormal cells grow rapidly in the brain or its surrounding tissues. Brain cancer can be primary, beginning in the brain, or metastatic, originating in other body parts like the lung, breast, skin, kidney, or colon. Brain cancer poses serious challenges due to the intricacies of the human brain itself, as well as the presence of the blood-brain barrier, which provides an effective shield against various substances in the bloodstream. An accurate diagnosis of a brain tumor is vital for treatment, which often requires invasive surgery. Therefore, a newer and more effective diagnostic approach is critical to improving clinical practice.

Brain tumors are the most prevalent type of solid tumors after hematological malignancies in children and young adults under the age of 20. Brain and central nervous system tumors are the second leading cause of cancer death in this age group. In adults aged 20 to 64, brain tumors rank the seventh most common cancer. Cancers of the brain and other parts of the nervous system rank ninth for cancer deaths in these age groups. However, brain tumors account for only a small portion of all cancers in adults aged 35 to 84 (only about 3%).

Traditionally, brain tumors are categorized based on their location (supratentorial vs. infratentorial), behavior (benign vs. malignant), and tissue origin (neuroectodermal, mesodermal, or ectodermal). According to the central nervous system tumor pathology classification established by the World Health Organization, there are neuroectodermal tumors identified in patients of all ages (e.g., astrocytoma, oligodendroglioma, ependymoma) and benign mesodermal tumors (e.g., meningioma). Tumors of ectodermal origin also occur in the brain, such as pituitary adenoma, craniopharyngioma, and pineocytoma. However, the classification is complex and difficult for non-specialized healthcare professionals.

The brain, with a wet weight of approximately 1,500 g, possesses unique structural, functional, and biological characteristics that differentiate it from other organs. These variables pose formidable challenges to the early detection and subsequent management of primary brain tumors and brain metastases. Moreover, an array of metabolic and physiological hurdles limits optimal drug delivery for the treatment of brain pathologies, particularly those targeting primary or metastatic tumors. [1][2]

1.1. Types and Prevalence of Brain Cancer

Brain cancer is a malignant or abnormal growth of brain cells that can occur in the brain, spinal cord, optic nerves, and cranial nerves. Tumors that originate in the brain are called primary brain tumors, while brain cancer can occur at any age, certain types occur primarily in children. Metastatic brain tumors are a more common type of neoplasm and refer to cancerous cells that have spread to the brain from other organs. After cancers of the lung, breast, colon, and melanomas, brain tumors are the fifth most common site of metastatic cancer. Tumors affecting the brain generally fall into two major groups: tumors that have a neuroectodermal

origin (accounting for 40% of cases), which includes gliomas and medulloblastomas, and those of non-neuroectodermal origin (accounting for 60% of cases), which include meningiomas, pituitary tumors, peripheral nerve tumors, etc. Beyond their often fatal consequences, brain tumors present unique and fascinating problems of treatment, biology, and genetics.

Meningiomas are the most common tumor in adults at 39%, followed by glioblastomas at 12.5%. Gliomas are the most common type of primary malignant brain tumors. They arise from glial cells which provide support for neuronal cells. Treatments for tumors of the CNS are aggressive and often unsuccessful. The blood brain barrier (BBB) prevents common chemotherapeutic drugs from reaching the tumor site, and surgical resection may leave satellite tumor cells in normal tissue. In children, brain tumors are most often located in the posterior fossa, while in adults these tumors are more commonly found supratentorial (above the tentorium cerebelli).

While brain cancer remains rare compared to cancers in other organs, the incidence of cancer of the brain and other related sites has risen in the last decade, most notably in individuals 80 years and older, from 0.7% to 63.3%. Brain tumors are more commonly reported in men than in women. Overall, the lifetime risk of developing a brain or central nervous system (CNS) tumor has been estimated to be 1 in 188 for men and 1 in 227 for women.

Collectively, malignant brain tumors are relatively uncommon, accounting for less than 2% of all malignancies. However, they are the most common solid tumor in children and the leading cause of cancer-related deaths in this group. Seizures are the most common presenting symptom of brain tumors, affecting 43% of patients. Other common symptoms include headache (39%), focal weakness (25%), nausea and vomiting (22%), ataxia (15%), and personality changes (11%). [3][4]

2. Molecular Biomarkers in Brain Cancer Detection

Early and accurate detection of brain cancer is crucial for improving patient prognosis and treatment outcomes. Conventional imaging techniques often fall short in providing definitive diagnoses, leading to increased reliance on molecular biomarkers. Advances in molecular biology and bioinformatics have paved the way for the identification of new molecules that significantly enhance the detection capabilities of this devastating disease.

In recent years, numerous molecular biomarkers, including genetic mutations and epigenetic alterations, have emerged as potential candidates for brain cancer detection. Genetic mutations, including single-nucleotide variations, amplifications, deletions, and chromosomal rearrangements, have been identified as important events in the initiation and progression of brain tumors. For example, isocitrate dehydrogenase 1 (IDH1) mutations, which are more common in low-grade gliomas and secondary glioblastomas, have been associated with improved overall survival. An improved multiplex droplet digital PCR (ddPCR) assay has been developed to detect seven IDH1/2 mutations and provide a breakpoint analysis.

With improved biomarker detection performance and analytic workflows, the assay showed high sensitivity and specificity in detecting brain tumors compared to non-cancer controls. This ddPCR assay could be used to screen for low-grade or intermediate-grade brain tumors and assist in the differential diagnosis of glioblastomas.

Similar to IDH1 mutations, several chromosomal alterations, including 1p/19q co-deletion, 10q loss, and 7 gain, have been observed in delicate gliomas. Because most of these alterations in non-neoplastic tissues are closely associated with genomic abnormalities in tumor cells, the analysis of ddPCR-derived CNV could serve as a possible screening assay for brain tumors. [5][6]

Epigenetic alterations, especially the hypermethylation of key genes, have been found in various types of tumors, leading to the inactivation of several tumor-suppressor genes. Methylation changes in genes such as

p15INK4B, MGMT, ATP5C1, and CDH13 have been demonstrated to serve as effective biomarkers for brain cancer, although their performance in detecting brain tumors in biofluids requires further research.

2.1. Genetic Mutations

Brain cancer, also known as primary brain tumors, is a term used to describe a variety of abnormal growths within the brain or its surrounding structures. The genesis of these tumors is often due to genetic alterations, which can be heritable or acquired during a person's lifetime. Genetic alteration, in this context, refers to the mutation of the structure and/or function of a gene and/or a genome. Mutations or alterations in genes involved in a cell's growth regulation can lead to aberrant cell cycle and uncontrolled cellular proliferation. The genes involved in the regulation of growth are classified as proto-oncogenes and tumor suppressor genes. Uncontrolled proliferation due to aberrations in proto-oncogenes leads to tumor formation. On the other hand, loss or inactivation of growth-regulatory tumor suppressor genes causes aberrant proliferation of a cell. Alteration of tumor suppressor genes, such as deletions, point mutations, or epigenetic silencing, could lead to tumor formation. Conversely, mutations or amplifications in proto-oncogenes may also play a pivotal role in leading to gain-of-function events resulting in aberrant cell proliferation. Structural aberrations such as translocation, amplification, or inversion involving genes encoding growth-regulatory proteins would be involved in the development of various tumors.

2.1.1. Focal Genetic Alterations

Focal genetic alterations leading to the aberration of a single gene or a few genes have been highlighted as a systematic approach in identifying specific oncogenes and tumor suppressor genes in a tumor. Broadly, there are three categories of focal genetic alterations: (i) amplification (or overexpression) or insensitivity of proto-oncogenes, (ii) loss of heterozygosity (LOH) or inactivation of tumor suppressor genes, and (iii) translocation involving either a proto-oncogene or a tumor suppressor gene. [7][8]

2.1.2. Amplifications

The development of comparative genome hybridization (CGH) and fluorescence in situ hybridization (FISH) techniques has revived interest in the genesis of tumors and underlying genetic alterations. By detecting chromosomal copy number change, a systematic study of chromosomal alterations associated with various neoplasias could be performed. Correlations between specific oncogenes and tumor type or histological grade could be established, elucidating the role of specific oncogenes in tumor formation. [9][10]

2.1.3. Loss of Heterozygosity

The detection of uniparental disomy (UPD) has led to the refinement in the physical localization of tumor suppressor genes. Formal proof of the involvement of specific genes with a given tumor-type (i.e., gene is a true tumor suppressor gene) has been revealed by the studies of germ line mutations and characterization of knockouts. Association of tumor suppressors with development of syndromes or familial cancer predisposition is another indication that the disruption of these genes would lead to tumor formation.

2.2. Epigenetic Alterations

Intrigued by the intricate mechanisms underlying tumorigenesis, researchers have turned their attention to the realm of epigenetics. This fresh perspective emerged in the 1940s and 1950s as scientists explored non-genetic, inheritable traits that could influence phenotypes. Such traits could include epigenetic alterations like promoter methylation and chromatin remodeling. Recent studies have revealed that these epigenetic alterations are crucial in the development of glioblastoma. Epigenetic modifications, such as DNA methylation and histone acetylation, play a significant role in the inhibition of tumor suppressor gene expression and the induction of oncogenes, resulting in aberrant epigenetic states in cancer. It is

hypothesized that these epigenetic changes, which are partially reversible, can aid early diagnosis of tumors if they show a specific profile. As a step further, the research aims to detect novel epigenetic alterations in gliomas that can serve as biomarkers for early detection, identification of tumor types, and separation of patients from healthy individuals.

This work will address these alterations and determine the existence of specific epialleles in tumor samples. It will begin with an introduction to DNA methylation and the basic methylation processes and modifications on the SNVs. Then it will describe various methods for detecting DNA methylation, focusing on the most up-to-date and relevant methods such as whole genome sequencing methylation kit. In gliomas, large-scale studies on methylation and its alterations have been performed, and this chapter summarizes how epigenetic alterations have been discovered and detected in glioma. It will continue with research on epigenetic alterations in gliomas, focusing on the most relevant studies. Additionally, this work will explain the methods adopted for brain cancer biomarker discovery, consisting of methylome screening, tumor identification based on genomic sequencing data, and the identification of probes that show differences in methylation. Furthermore, this work will highlight the results and significance of findings of aberrant DNA methylation of specific promoters in gliomas and the first evidence that SILAC-acetylated lysine peptides can be enriched from complex protein mixtures by sensing the lysine acetylation state. [11][12]

3. Techniques for Molecular Detection

Molecular techniques for the detection of brain tumors are used to analyze mutations, rearrangements, methylations, gene expressions, copy numbers, and surrogate markers associated with molecular subtypes. Current research focuses on using easily available biopsies, such as frozen, paraffin-, and cytology-based samples. Biomarkers for diagnosis, classification, monitoring of the therapeutic response, and precision medicine (targeted therapy and personalized medicine) are studied. The most common and relevant techniques are presented. For each technique, the principle, possible applications related to brain tumors, advantages, and disadvantages are described.

Polymerase chain reaction (PCR) was invented in the 1980s and is still widely used today. Despite all the new developments, it is still in the guidelines for glioma stratification as a simple, cheap, and easily applicable procedure. The mutation of isocitrate dehydrogenase 1 (IDH1) at amino acid 132 (R132H) is the most common mutation in adult gliomas (in about 80% of cases), making it a good candidate for biomarker development. The sequenced nucleotide changed the codon from ATG (coding for methionine) to ACG (coding for threonine). Mutations of IDH1 or its homologue IDH2 lead to the accumulation of 2-hydroxyglutarate, affecting DNA methylation and decreasing the activity of alpha-ketoglutarate-dependent enzymes, including the ten-eleven translocation (TET) family of DNA hydroxylases. Factors that usually promote demethylation can no longer act, enhancing promoter methylation and inhibiting the transcription of several genes involved in cell proliferation and differentiation. This change is called the CpG island methylator phenotype (G-CIMP) and can be detected using restriction enzyme digestion or sequencing of bcDNA. There are two major drawbacks: a small amount of non-wild type bcDNA (about 1%) in IDH1 mutant patients might limit sensitivity, and an enrichment step would be required for many cytological samples, but it could be performed automatically using a liquid-handling robot.

Various platforms established IDH1 R132H immunohistochemistry, from fully automated (e.g., Ventana) to manual. The biggest problem is heterogeneity; it can be lost throughout recurrence or not detected in a significant portion of tumors. Nevertheless, a lack of this mutation can rule out low-grade gliomas. In a much smaller proportion of cases, the IDH2 mutation (R172) can occur, requiring a different antibody (Dako) for detection. The IDH1 R132H antibody is widely used, but for research purposes, it is advisable to look at other possible mutations. 1p19q co-deletion testing can be performed using FISH; however, it is not the primary detection method for oligodendrogliomas, as it may give false negatives on small tissue cores. Additionally, FISH requires trained personnel and is usually labor-intensive. As an alternative to FISH,

melting temperature analysis of co-deleted probes can be performed using real-time multiplex PCR with the same lengths of amplified regions (usually primers designed for ~200-bp fragments). The method is applicable to frozen samples and fixed-paraffin samples. Excellent sensitivity/specificity data have been published. Methylation profiling using the Illumina Infinium MethylationEPIC platform can be performed using DNA isolated from cryoprotection agent-fixed tissue, formalin-fixed paraffin-embedded tissue, and cytology specimens. [13][14]

3.1. Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is an important technique of molecular biology which is used to amplify a specific segment of DNA and produce thousands of identical copies of that DNA. PCR is a three-step process: denaturation, annealing, and extension. Denaturation is the first step in which the DNA is heated for about 5 minutes to separate its two strands. Next is the annealing step in which the DNA is cooled to about 50-70 degrees Celsius so that the primers can bind to its complementary sequence of the DNA. The final step of PCR is extension in which the temperature is increased again and a heat-stable DNA polymerase, Taq polymerase, adds nucleotides to the DNA strand starting from the primers. These three steps of PCR are repeated about 25 to 35 times so that the DNA would be amplified exponentially.

To analyze the PCR products, gel electrophoresis is commonly used. The PCR products are applied in agarose gel wells and an electric current is passed through the gel which causes the negatively charged DNA to run towards the positive electrode. The smaller the DNA fragments, the faster they move, thus they are separated by size. The DNA in the gel can then be stained and visualized by UV light. Usually, primers are designed to amplify specific DNA of an organism, and so it is used to identify the presence of the DNA by analyzing the size of the bands seen on the gel.

PCR can be used to detect mutations in a DNA sequence. One of the primers in the PCR is designed to amplify the wild-type sequence of DNA, and the other primer is designed to amplify the mutant sequence. The DNA sample is isolated from the organism, and PCR is performed. If the organism has the wild-type DNA, it would amplify this DNA and the band on the gel would be at the expected length. However, if the organism has a mutant DNA, it would amplify this mutant DNA, and thus the band on the gel would be at a different size compared to the wild-type. [15][16]

3.2. Next-Generation Sequencing (NGS)

In recent years, next-generation sequencing (NGS) techniques have been developed, standardized, and utilized to assess a variety of types of nucleic acids. NGS is defined as high-throughput sequencing methods that allow the sequencing of the base pairs within an organism's genome. These methods assess millions of sequences simultaneously, allowing for greater coverage and scalability while incurring lower per-base cost than other sequencing methods, such as Sanger. In a single NGS run, hundreds of megabases to terabases of sequencing data can be attained. Fourth-generation technologies are currently being created to try to analyze single DNA molecules in real time. Current instruments can sequence >1 billion bases per run. This signifies the coming of age of next-generation sequencing technology and the prospect for a larger variety of uses in clinical laboratories. Protocols and targeted tests are rigorously standardized prior to implementation in routine use. The complexities of bioinformatics analysis must also be embraced prior to consideration of both small and large-scale sequencing.

Two recent large-scale whole-exome sequencing studies have been reported where primary and metastatic human glioblastomas underwent sequencing and matched normal DNA profiles were able to reveal somatic alterations not previously recognized. These approaches identified previously unrecognized somatic alterations in the mouse models of glioblastoma and metabolic dysregulation due to sequence alterations of isocitrate dehydrogenase 1 and 2. These comparative studies reveal the need for cautious interpretation of any variant identification due to differences in the populace and genome sequencing technologies employed.

The loss of telomeric sequence is a hallmark of gliomas, but little is known about how the sequence is lost. In a unique and creative study, fluorescence in situ hybridization can resolve losses of fractions of a single chromosome's telomere. Telomeric sequences are recognized and the potential uses of targeted enrichment of tumors characterized by detectable repetitive sequence alterations are elucidated. NGS technology is rapidly progressing into large-scale use for clinical human disease diagnostic applications on many systems. Work continues on refinement of bioinformatics and creation of standard reference material and protocols for use in large multi-laboratory studies. [17][18]

4. Challenges and Future Directions

The rapid development of new and improved molecular detection methods has greatly increased the sensitivity, efficiency, and ease in detecting different types of molecules, with applications in various fields. However, many of the new detection methods have not yet been evaluated for brain cancer detection. Thus, there remains a need for research conducting a comprehensive evaluation of current and emerging molecular detection methods for detecting brain cancer biomarkers.

Numerous current detection methods exist with strengths and weaknesses. While many methods are highly sensitive and easy to use, they also come with complexity in their use and design, time-consuming processes, the need for expensive equipment and experienced technicians, and the need for lab spaces. Evaluating and comparing the different molecular brain cancer detection methods provides insight into their strengths and weaknesses and interactions between the impact of a given biomarker and the performance of the detection method. Many barriers currently exist to research using new brain cancer detection methods and detecting biomarkers that can ensure the identification of all brain cancers. New brain cancer detection methods need to be able to successfully detect glioblastomas as they are the most common and aggressive type of primary cancerous brain tumor, and other methods need to be able to detect floating biomarkers as they are the most prevalent biomarkers in new brain cancer detection methods.

Recent advancements in molecular detection methods such as CRISPR, multimerization of different biosensing methods, nanotechnologies, and NIRF imaging combined with DNA nanoswitches hold promise for improving current molecular detection for brain cancer and overcoming obstacles associated with detecting brain cancer. New detection methods can disinfect a broader range of biomarkers and have sensitivity limits in interpreting attomolar concentrations and 1fg/mL. There is great promise showing that existing and newly emerging technologies could detect brain cancer. [19][20]

4.1. Limitations of Current Molecular Detection Methods

Currently available molecular detection methods for brain cancer, such as genomic profiling, transcriptomic analysis, proteomics, metabolomics, and bioinformatics, face multiple limitations. Some methods share intrinsic limitations, while others have technology-specific drawbacks. Many current methods rely on a comparative approach, contrasting biopsy samples from both healthy and cancerous brain tissues to tease out malignant indications. The primary challenge with this was described in a recent review. Despite the need for precise localization, cellular and genetic heterogeneity are substantial intra- and inter-tumor challenges, and invasive brain biopsy techniques for sampling have motivated this concern.

Some studies try to address glioma inter-tumor heterogeneity, focusing on targeting "subtypes" based on specific transcriptomic analysis; however, transcriptomic analyses can be misleading because they rely entirely on RNA extraction and assume uniformity of cellular components. A frequently overlooked confounding factor in transcriptomic analyses is "bystander" brain cells.

Another significant shortcoming shared by the comparative methods is exclusivity to tumor-bearing tissues generally focusing on identifying cancer-related alterations. Accordingly, these methods are incapable of recognition of brain cancer in non-tumor-bearing tissues in cases of initial presentation or further spreading

and seizure localization challenges. Such a need for early screening methods has been addressed by recent evaluations of free-circulating DNA analysis in plasma and cerebrospinal fluid that have shed some promising light, but have still landed on the outer edges of "public discussions" in the scientific research community, and their clinical assimilation is presently in its infancy.

Each molecular detection method has its technology-specific limitations. Genomic profiling using massively parallel sequencing is still widely regarded as a gold-standard technology for identifying somatic point mutation, copy number, and structural alterations in cancer studies, illustrating variations in a broad spectrum of human cancers. However, the bi-component design of massively parallel sequencing necessitates conversion from tissue samples to qualified DNA libraries in a delicate and costly process that is susceptible to abundant reagent failing points, sample processing and handling, and bioinformatics workflow complications post-sampling. Moreover, high-coverage whole-genome sequencing is usually necessary for genome-wide biologically clear interpretations (e.g., a minimum of 85× for single-tumor whole-genome sequencing to diminish a vast number of false-positive "artifacts"). Even with such limitations, massively parallel genomic sequencing is now widely applied in clinical studies as a companion diagnostic method for targeted therapies. Nonetheless, this is still inapplicable to primary brain cancer in locations difficult to reach by neurosurgical resections or with initial deep-seated, multifocal, or disseminated presentation. [21][22]

4.2. Emerging Technologies

Emerging technologies are critically needed for the molecular detection of brain cancer for multiple reasons, including those explained in Section 4.1, namely the limitations in the specificity, sensitivity, speed, and affordability of currently available techniques. Promising emerging technologies fall into two categories: molecular detection technologies using different modes of analysis, such as chemiluminescence or microbubbles, and technologies that improve the individual steps of nanomaterial-based biosensors, such as the bioconjugation of nanomaterials. Other very innovative technologies can detect DNA methylation and RNA splicing.

In a paper published in the journal Biomedical Engineering Letters, authors from the University of Puerto Rico describe a chemiluminescence-based detection method for brain cancer in its early stages. In this methodology, an array of DNA probes is immobilized on a glass card that is repeatedly scanned by a fiber optics cable for quantification. A complementary target sequence, 19 nt long and bearing a 5' amino group, would hybridize to the probes in the presence of 6x SSPE buffer. The DNA hybrids were tagged with horseradish peroxidase that would catalyze the oxidation of luminol in the presence of hydrogen peroxide to generate an electrochemiluminescent signal. The intensity of the signal is directly proportional to the concentration of the final hybrids in solution. This proof-of-concept experiment could be further developed by integrating commercially available technologies.

In a paper published in the journal Scientific Reports, authors from the Institute of Engineering in Medicine, The University of California, San Diego provide insight into how microbubbles can be used to enhance the molecular detection of brain cancer using ultrafast ultrasound. Commercially available microbubbles could be conjugated to anti-hEGFRvIII and injected intravascularly, with brain-tumor xenografted mice being scanned 1 and 7 days later. This second scan revealed a 45% decrease in relative microbubble concentration in the tumor region, suggesting detection of molecular cancer progression after only 7 days of tumor growth. This proof-of-concept experiment could also be further developed for its optimization and commercialization. [23][24][25]

5. Conclusion

Molecular detection methods have a pivotal role in upgraded brain cancer diagnosis, prognosis, and treatment monitoring. The efficient and refined isolation of human brain tumor cells using LCM gave rise to

dozens of purposes, including comparative genomic hybridization (CGH), single nucleotide polymorphism (SNP) mapping, and/or expression profiling by microarrays. On the other hand, genetic/molecular alterations within the genomes of brain tumor cells, such as DNA loss/gain and methylation, were detected through Isothermal Amplification and high sensitivity detection methods, including Escherichia coli strand-displacing polymerase was used to amplify specific DNA targets in hyperthermophilic archaeon Thermococcus kodakarensis or multiple displacement amplification to amplify the whole genome of tumor cells.

More importantly, newest technologies, such as photothermal therapy (PTT) using nanomaterials (e.g., gold nanoparticles, graphene-based nanosystems, and ceramics) to combat brain tumor cells combined with localized molecular imaging, had been proposed and suggested the feasibility of early brain cancer diagnosis. Despite the fact that most studies were conducted in healthy rodent models or other animal species to avoid ethical problems, some developments were already performed in human cells/models. In the near aim, employment of combinations of different contrast and therapeutics agents simultaneously for early brain cancer detection/treatment shall be proposed. Each contrast agent has its own advantages in detection or treatment potency and supposed to be able to upscale the overall smartness and efficacy of the combined nanoplatform.

The proposed smart detection and therapeutic systems for the treatment of brain cancer could also be modified for monitoring and killing cancer cells in a noninvasive manner. Moreover, future developments could be proposed due to decreasing the diameter of the nanomaterials to the nanoscale (1–100 nm) resulting in higher penetration rates through the blood-brain barrier and hence improved access to tumor sites and a favorable interaction with tumor cells. Following thorough toxicological studies, most of the desirable properties of the proposed platforms could be transferred into commercial use to combat a wide range of tumors at early stages.

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