Review

The Molecular, Neurological, and Clinical Features of Diffuse Intrinsic Pontine Glioma

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Abstract: Diffuse intrinsic pontine glioma (DIPG) is recognized as a pediatric brainstem cancer with a 0% survival rate. On a molecular basis, DIPG commonly results from mutations in histone H3, specifically a mutation in the *H3K27M* gene, that promotes tumorigenesis and results in presentation of this fatal brainstem tumor. DIPG is challenging to treat, as surgical intervention is inefficacious due to the location where the glioma resides. To date, traditional treatments such as radiation, chemotherapy, and immunotherapy have not increased survival rates and have only been successful at relieving symptoms. Future therapeutic approaches such as proton beam radiation, Chimeric Antigen Receptor T Cell (CAR-T) immunotherapy, and alternative epigenetic pharmaceuticals are under investigation for potential benefits. Various clinical trials have also explored these treatment procedures to discover potential increases in survival rates in both animal and human studies. In this review, we will evaluate the pathology and molecular characteristics of DIPG, the current and future approaches to DIPG treatment; and, lastly, we will discuss clinical trials that have been completed to develop successful treatment options.

Abbreviations:

BBTB: Blood Brain Tumor Barrier;
CAR-T: Chimeric Antigen Receptor T Cell;
CED: Convection Enhanced Delivery;
CN: Cranial Nerve;
DIPG: Diffuse Intrinsic Pontine Glioma;
PBT: Photon Beam Radiation Therapy;
pHGG: Pediatric high-grade glioma;
RT: Radiation Therapy.

Keywords: Diffuse Intrinsic Pontine Glioma; DIPG; pediatric tumors; cancer; CAR-T; pons; histone mutation

Introduction

Diffuse Intrinsic Pontine Glioma (DIPG) is a rare and fatal pediatric brainstem cancer with 200-300 new cases introduced in the United States (U.S.) each year (1,2). From the onset of symptoms and diagnosis, children are typically given eight to fourteen months to live (2). With the anatomical region of DIPG being the brainstem, specifically the pons, there are currently no successful treatment options, resulting in a 0% survival rate (3). The aggressive symptoms of DIPG, occurring in those aged five to ten years old, rapidly diminish the quality of life and result in most children receiving palliative care just four months after their initial diagnosis (4).

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Methods

The primary source for article selection was the PubMed electronic database. The team reviewed appropriate free articles between the years 2012 and 2022 using keywords or search terms such as "DIPG," "pediatric," "glioma," "brain tumor," "clinical trial," "CAR-T," "pons," "radiation therapy," "histone mutation," and "treatment." Articles and authors were individually evaluated for quality by the team, and reference lists were used to find further relevant studies. Only studies evaluating children 0-18 years of age with Diffuse Intrinsic Pontine Glioma were used. Studies including patients with DIPG over the age of 18 were excluded.

Discussion

Clinical Presentation

In DIPG, the tumor within the pons causes patients to present with brainstem dysfunction or cerebrospinal fluid obstruction including cranial nerve (CN) palsies, long tract signs, and ataxia (5-7). Typically, the first symptom that presents is abducens nerve palsy that results in esotropia or diplopia. Following CN palsy, other common symptoms include difficulty walking, loss of balance, weakness, clumsiness, limited eye movements, and an asymmetric smile (5,7). Some of the long tract signs that occur are increased tone, hyperreflexia, clonus, and the presence of a Babinski reflex. Symptoms of increased intracranial pressure can present in some patients due to the hydrocephalus caused by the expansion of the pons. Other symptoms such as behavioral changes, night terrors, and school difficulties have also been reported (5).

Pathology and Molecular Characteristics

Based on the guidelines from the World Health Organization, DIPG can be classified as a grade II, III or IV tumor either by biopsy or autopsy (8). The tumor invades white and gray matter structures of the brain, and the majority have necrosis, mitotic figures, and microvascular proliferation (9).

The current theory about DIPG is that it results from mutations in histone H3 (9-11). The H3 family of histones are made up of H3.1, H3.2, and H3.3, which are all structurally and functionally related. The histones, along with their numerous chaperones, are important for allowing dynamic availability to specific genomic loci (12). H3.3 is encoded by both the *H3F3A* and *H3F3B* genes, while H3.1 is encoded by multiple genes. The mutation that appears in 70-84% of DIPG is the *H3K27M* mutation, which results in lysine being changed to methionine at K27 (9,10). There has been some evidence showing an association between the histologic grade of the tumor and the presence of the H3.3 mutation. However, the H3K27M mutation can still be found in DIPG with grades II, III, or IV tumors (13). The position of this missense mutation disrupts the highly conserved N-terminal of the protein, which negatively affects the epigenetic regulation of gene expression (14). In addition, it can affect the nucleosome structure and its interactions with transcription factors. Research has found that this specific mutation, K27M, is a gain of function mutation that blocks the activity of the polycomb repressive complex 2, which plays a role in chromatin remodeling and epigenetic silencing of genes (PRC 2) (10,15). While the mutant H3 variants are not high in number in DIPG cells, they do employ a dominant-negative effect that initiates histone trimethylation or dimethylation of all H3K27 in all H3 variants and the wildtypes (1,16). The dominant-negative effect results in upregulation of gene expression at loci with the loss of H3K27me3. Conversely, genomic loci that gain the H3K27me3 have decreased expression of the accompanying genes, which some researchers believe contributes to tumorigenesis (16,17). Also, H2K27me3 is found increasingly more within brain regions that also have H3K4 trimethylated, which usually promotes active gene transcription. The genes with both marks are said to be "bivalent" because they have both active and silent marks, which is also found to be involved in oncogenic pathways (1,16).

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While the H3K27M has been found to increase cell proliferation, research has shown that the mutation alone is not able to produce DIPG (18). Instead, the mutation must be accompanied by *PDGFRA* overexpression or *TP53* loss to result in tumorigenesis. Combinations of the three mutations were studied to determine which had the greatest effect on tumorigenesis. Having all three mutations lead to the transformation of mouse neural stem cells into tumors, which had similar gene expression to that of human DIPG cells (18,19). Additionally, not only does H3K27M initiate the formation of DIPG, but it is also required for maintenance of the formed tumors. Studies found that deletion of the mutation in DIPG tumor cells had negligible effect on cell growth *in vitro*, but inhibited tumorigenesis when implanted in mice (18,20,21). Other mutations, like overexpression of the c-Met pathway and MYCN amplifications, found in autopsy samples of DIPG patients are topics of ongoing research to determine their part in tumorigenesis (8).

Current and Future Approaches to DIPG Treatment

Radiation

Current treatments for DIPG enhance one's quality of life by temporarily relieving symptoms, but future advancements are necessary to prolong the life expectancy of patients diagnosed with DIPG (28). Radiation therapy (RT) is a standard treatment used for DIPG (28,29) and utilizes a six-week regimen (28) of X-rays to deliver photons, or energy, to the glioma and damage cancerous cells and prevent tumor growth (30). While RT has been the only treatment known to alter the natural progression of DIPG, (31) improving 70-80% of patient's symptoms (2), this treatment does not prolong survival rate appreciably (28,29) and can leave patients with debilitating side effects from radiation toxicity (2,29). Photon Beam Radiation Therapy (PBT) may be a safer approach to treating DIPG (32). PBT is a promising treatment for primary gliomas, as it substitutes photons with protons to deliver less radiation to deep tissues surrounding the cancerous tumor and yields improved survival rates versus traditional RT practices (32). While the use of PBT is encouraging, more research and clinical trials are needed to thoroughly assess the risks and benefits (33).

Chemotherapy

Another common approach to treating DIPG is chemotherapy. Chemotherapy inhibits cell proliferation and tumor metastasis (34) by administering (usually intravenously or orally) drugs that kill cancer cells (34). Much like RT, chemotherapy does not extend a patient's survival rate, which could be due to this treatment's lack of penetration through the blood-brain barrier (BBB) (34,35). As a treatment for DIPG, chemotherapy presents many complications. Tumor cells carry ABC transporters, also known as ATP Binding cassette efflux transporters (36). Specifically, tumor cells carry ABC1. ABC1 is a P-glycoprotein responsible for multidrug resistance that is expressed in both tumor cells and at the BBB (36). With the ABC1 transporters at the blood brain barrier, 60% of drugs, including those used in chemotherapy treatments, are recognized and are unable to get past the BBB (36). Tumor cells that carry ABC1 transporters proliferate in the Blood Brain Tumor Barrier (BBTB), which makes most gliomas difficult to target with chemotherapeutic drugs (37). The BBTB is the BBB that has been altered by pathological conditions, like DIPG. The BBTB disrupts the BBB and allows for minimal chemotherapeutic drugs to pass, however, not enough to significantly decrease tumor growth and proliferation (37). Due to DIPG being an extremely invasive tumor, areas that are unable to be reached by chemotherapeutic agents are constantly proliferating (36). This results in areas outside of the glioma becoming chemoresistant, due to the increased number of tumor cells, and subsequently, an increased number of ABC1 transporters (36,37). Because of the chemoresistance of the BBB, as well as the increasing resistance as tumor cells proliferate, chemotherapy has not been as effective as many have hoped.

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A traditional form of chemotherapy, intra-arterial (IA) chemotherapy, requires the use of a catheter to be inserted into the femoral artery (38). A microcatheter is then inserted to explore the blood vessels of interest (34) and chemotherapeutic agents are directly administered in concentrated doses to the cancerous region (31). By directly delivering chemotherapeutic agents to the glioma by the arterial system, side effects from chemotherapy toxicity can be bypassed (31). An experimental chemotherapeutic strategy for DIPG treatment that also has selective targeting actions is Convection Enhanced Delivery (CED) (38). CED can bypass the BBB and increase tumor uptake of chemotherapeutic agents (38) and can do so efficiently by utilizing hydrostatic pressure to drive fluid flow instead of relying on passive diffusion (39). A 2019 study conducted with an *in vitro* experiment that explored the potential benefits of CED by administering tumorigenesis pathway inhibitors to patient and mouse derived cell lines (40). Results from this investigation indicate that CED with these chemotherapeutic agents successfully inhibits growth of DIPG cells in vitro to ultimately prolongs survival (40). More pre-clinical trials are necessary to be completed before thoroughly assessing potential benefits of this procedure, but CED seems to be a promising treatment.

A meta-analysis (41) conducted from 1987-2005 consisted of an accumulation of DIPG investigations regarding its characteristic of being inoperable and potential alternative chemotherapy therapeutics. The first experiment analyzed the use of chemo-radiotherapy in which the progression of disease was observed by MRI and the average survival of twelve months was documented (41). The second study was an intensive high-dose course of chemotherapy (which involved chemotherapeutics such as cisplatin/etoposide, cyclophosphamide, vincristine, and methotrexate) and a subsequent course of myelosuppressive chemotherapeutics, radiation, and maintenance chemotherapy (41). The first four patients followed the chemotherapy schedule outlined within this report, but the use of immunosuppressants severely intensified present or novel neurological deficits in each case (41). Most patients in this study died due to tumor progression with the average survival age being thirteen months (41). La Madrid et al, examined the combinatorial immunotherapy treatment of cisplatin and etoposide followed by isotretinoin before, during, and after focal irradiation (41). This treatment was well-tolerated by the patients with no significant adverse events disclosed and the average survival of each patient was twelve months (41). La Madrid et al explored the effects of intravenous vinorelbine before, during, and after irradiation (41). Vinorelbine was administered in a saline solution with anti-nausea medications and given before infusion and disease progression was observed by MRI (41). Multiple patients developed multiple transient episodes of monolateral peripheral facial nerve palsy during treatment, but these symptoms subsided after infusion (41). While the average survival rate was nine months, this specific investigation did involve two patients who survived without disease progression for a maximum of 48 months after treatment (41).

CAR-T Immunotherapy

While novel chemotherapy and radiation procedures may prove to be effective DIPG treatments, a specific form of immunotherapy called chimeric antigen receptor therapy (CAR-T) may offer promise in the future. Immunotherapy has been a long-established method to treat cancerous tumors (42), DIPG has special challenges that a clinician administering treatment must consider (42). Since the brainstem controls important functions for daily life, any therapies given to DIPG patients must limit the destruction and inflammation of healthy tissue around the glioma (42). In CAR-T, T-cells are collected from the patient's bloodstream and are genetically engineered to express an artificial receptor for monoclonal antibodies that bind to cancer cell antigens before being infused back into the patient (43). By undergoing CAR-T cell infusion, the patient's T cells have antitumor characteristics that help fight glioma progression with little to no effect on non-cancerous cells (44). Even though most reports utilizing CAR-T cells for pediatric tumors are xenograft studies or phase one clinical trials (45), this form of immunotherapy gives

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clinicians hope for finding treatments that allow DIPG patients to have longer survival rates (45).

Future Epigenetic Alternatives

Since 80% of DIPG cases possess the H3K27M mutation as their underlying pathology (46), scientists are looking to the current advancements of epigenetics as an option to prolong patient lifespans (46). Histone methylase and demethylase inhibitors are being utilized to hinder H3K27M-expressing DIPG cells and brainstem gliomas (46). Even though these approaches may seem promising, epigenetic modifications have been found to be unsuccessful at treating other central nervous system gliomas (47). The lack of function from these treatments could be due to off target activity and the lack of effective drug concentrations in the central nervous system (47).

Clinical Trials

Challenges of Treatment

While there are many ongoing clinical trials testing different treatments to be used for children with DIPG, there are many complications that limit safe treatment options. Because the glioma is located in the pons of the brainstem, surgical removal is not possible (22). The pons is responsible for many regulatory functions and removing a glioma from the pons could result in morbidity and mortality (23). In most brain tumors, normal brain tissue can be easily separated from the abnormal tissue; however, with DIPG, the tumor cells diffuse within the normal brain tissue, making it difficult to separate the normal from the abnormal tissue (24). Surgical removal of the glioma would risk the possibility of removing healthy, normal brain tissue (24). For similar reasons, biopsies are a controversial procedure in DIPG. There is, however, evidence that suggests stereotactic biopsy is the safest and now, most used method of retracting a sample from the glioma cells (25). Stereotactic biopsies are a more precise way of conducting a regular biopsy procedure. With the use of Magnetic Resonance Imaging (MRI) or X-Ray to detect a specific location, a stereotactic biopsy decreases the risk of affecting nearby areas of the brain (26). Some physicians, however, concluded that due to the risk of hemorrhage and other complications, biopsies are just as dangerous as surgical removal of a glioma (27).

ClinicalTrials.gov has been documenting trials since 2000 and is the world's largest registry provided by The United States National Library of Medicine. It is a database consisting of publicly and privately funded clinical trials on a wide range of diseases and conditions conducted worldwide. It provides the ability to track journal articles disclosing results in medical literature (48–50). A report published in 2019 reviewed the ClinicalTrials.gov database for all possible interventional clinical trials that included DIPG as a diagnosis of primary investigation. A notable finding from this article pertains to Burzynski Research Institute, which registered phase III trials assessing the effectiveness of antineoplaston therapy and RT versus RT alone. As of 2019, antineoplastons were not approved by the US Food and Drug Administration (FDA) to prevent or treat any disease. In addition, no randomized controlled trials showing effectiveness of antineoplaston therapy have been published in the literature at the time of publication in 2019. Even though the outcomes of the trials have been nonsignificant, they only make up approximately one-tenth of all clinical trials registered on ClinicalTrials.gov. This report shows that retrospection provides a unique view of the definition of failure. The definition of failure is dynamic. Although many clinical trials have not proven that an intervention can impact the DIPG prognosis, such findings inform clinicians of what not to investigate in the future (48).

In 2008 (51), it seemed that RT was the only treatment that provided any benefit to children with DIPG. However, even with potential radiation benefits, the average survival of these patients is usually never more than nine months (51). A prospective trial was performed using a BSG-98 protocol which utilized frontline chemotherapy alongside

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hematotoxic and nonhematotoxic cycles with the intent to delay the need for RT until the glioma progressed (51). Each cycle included three courses which were delivered monthly. The first course involved 1,3-bis(2-chloroethyl)-1-nitrosoureacisplatin while the second and third courses consisted of high-dose methotrexate (51). A historical cohort receiving local RT served as controls for this study. A significant increase in average survival spans with participants in the BSG-98 protocol (throughout the span of seventeen months) compared to those in the historical controls (throughout the span of nine months) was observed (51). The BSG-98 protocol led to significant findings, but issues arose due to costs from infection and hospitalization of patients (51).

An investigation (52) evaluated the antitumor effects of Delta-24-RGD in pediatric high-grade glioma (pHGG) and DIPG models. pHGG comprises approximately eight to twelve percent of all primary CNS tumors, is characterized by aggressive clinical behavior, and accounts for significant morbidity and mortality among children with brain tumors (52). Oncolytic virotherapy is an up-and-coming treatment using oncolytic viruses. This specific therapy is used because it selectively infects and damages cancerous tissues. Delta-24-RGD is a replication-competent adenovirus produced to replicate tumor cells with an abnormal RB (tumor suppressing) pathway and has been proven safe and effective in adult gliomas (53). This research showed that Delta-24-RGD had a significant antitumor effect in vitro in a panel of cell lines and *in vivo* in pHGG and DIPG orthotopic immunosuppressed and immunocompetent non-human animal models (52–55).

Conclusion

DIPG makes up approximately ten to fifteen percent of all pediatric brain tumors and more than 90% of children with DIPG die within two years of disease onset (47). Despite subtle advancements in DIPG molecular characterization, effective treatments have yet to be discovered (19) and current therapies have adverse side effects. With limited treatment options available, there is a critical need for continued retrospection to ensure that future efforts in clinical research are justified and poised for maximal impact and discovery (48). Although there have been many clinical trials involving DIPG treatments, there still has been no apparent improvement in the prognosis. Fortunately, future trials have triggered a recent burst of enthusiasm. Projects are emerging worldwide, as we can see that they may benefit from coordination and collaboration. The knowledge surrounding DIPG biology is increasing and translating this newfound understanding of the disease into clinical trials (along with utilizing the latest drug distribution methods) may eventually lead to more effective treatments in the future (38,56,57).

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