Nonsurgical Approaches to Glioblastoma

Moshe Baitelman

Moshe Baitelman graduated January 2019 with a B.S in Biology and a minor in Information Technology. He is currently pursuing medical school.

Abstract

Due to the sensitivity of location, brain cancer is one of the most difficult and deadly known cancers. There are various forms of cancer in the brain with many shared characteristics as well as unique manifestations in each. While cancers originating in the central nervous system present in several ways, the most common forms are high grade gliomas generally, and glioblastoma or anaplastic astrocytomas specifically. With the advent of technology, researchers have been able to propose and refine extensive profiles of these relentless tumors, enabling greater and more successful treatment profiles to be developed. Where treatments used to consist primarily of chemotherapy and surgery, research has enabled the development of immunotherapy and gene therapy techniques as well as alternative treatments to take on the caustic disease.

Introduction

The foremost distinction of brain tumors is between primary; those that originated within the brain itself, and secondary; those that developed beyond the blood brain barrier and migrated to the brain under metastatic conditions. Secondary tumors account for the majority of all brain cancers and approximately 50% of these tumors metastasize from lung cancers (Gallego, 2015). According to the 2014 World Cancer Report, patients diagnosed with glioblastomas were measured to have a median survival time of 12-15 months post diagnosis with fewer than 5% of patients living beyond 3 years (Patchell, 2003). Under the World Health Organization's grading protocol for central nervous system (CNS) cancers, glioblastomas (GBM) are a grade IV glioma (Bleeker, et al., 2011). This is the most common form of primary brain tumor and the most fatal found in humans, accounting for 15% of all intracranial neoplasms (Bleeker, et al., 2011; Young, et al., 2015). The average age at diagnosis is 64 years old and the approximate annual incidence of GBM per 100,000 population is 3.19 cases, making an understanding of the disease and its treatment of paramount importance (Ostrom et al., 2017). GBMs develop rapidly de novo in astrocytes, star-shaped neuroglial cells suspected to play a role in blood brain barrier maintenance and neurotransmitter management (Kolb & Whishaw, 2009). This swift development occurs without any previous primary or lower grade lesion development that may indicate or preclude the GBM onset, making early diagnosis and adequate treatment more difficult.

Since 2005, the first-line standard of care has been established as surgery followed by chemotherapy, most often with temozolomide (TMZ), and radiotherapy. Despite the established longevity of this standard, its efficacy is highly questionable and there is still recurrence in the majority of cases (Patchell, 2003). To complicate matters further, there is no second-line standard of care for glioblastomas and recurrence is consistently fatal (Roy, et al., 2015).

Discussion: Difficulties with Conventional Treatments

One of the significant factors contributing to the aggressive character of GBMs is their infiltrative nature. Astrocytes have extensive networks of processes forming from their cell bodies, creating an intricately branched structure extending throughout the nervous system. Where some cancers merely invade their host tissue, the cells of grade IV astrocytomas penetrate their host with protrusions. These processes weave a complex margin with host tissue that blurs the border between invasive and host cells. This makes complete surgical resection nearly impossible as practitioners are unable to differentiate between, and separate, the healthy and malignant tissue. As a result, recurrence is extremely high in GBMs and alternative treatments must often be considered post-surgery when complete resection is not achieved (Patchell, 2003).

Beyond the inability to properly remove gliomas, the benefit of surgery is questionable. In a study of recurrent brain tumors, researchers found no significant benefit to a second resection, though a first resection did confer an increased overall survival (Suchorska et al., 2015). Nonetheless, the nature of the neurosurgical procedure lends itself to various complications. Along with the wound and postsurgical medical complications, brain surgery runs the risk of systemic and cortical injuries, damaging physical and mental health with the most common risk of neurological impairment (Jackson, et al., 2016). Research also found that the acquisition of motor and language deficits post-surgery is linked to decreased survival rates compared to patients without surgical brain damage (McGirt et al., 2009). The results of these studies indicate that the benefit of surgery is questionable.

Chemotherapy treatments generally follow the attempted resection procedures but have limited success due to the impermeability of the blood brain barrier to foreign chemicals (Deeken & Loscher, 2007). TMZ is a common chemotherapy drug for gliomas and has demonstrated mild success against GBM and anaplastic astrocytoma but its overall effectiveness is slim and is accompanied by a cocktail of undesirable side effects (Friedman, et al, 2000). The established first-line standard of care is precarious and insufficient. As a result, research has turned to alternative methods by which to treat and manage this deadly.

Genetic Analysis

In order to purposefully theorize and discuss possible solutions and treatments, GBM manifestation must first be understood. Occurring primarily in older patients, GBM progresses quickly and with low survival rates. A minority of cases have been seen in younger patients with a history of epilepsy connected to progressive low-grade gliomas (Ostrom et al., 2015). To address the lack of understanding, research continues to look for commonalities in tumor onset. As genomic instability is an enabling characteristic

of cancer, efforts have been made in the field to source a primary effector with genetic basis. As of yet, there is only one confirmed molecular predictive factor for GBM - methylation of the O6-methylguanine-DNA methyltransferase (MGMT) promoter (Bleeker, et al, 2011). The relative dearth of information on the molecular basis for GBM requires additional research.

Whole-genome single nucleotide polymorphism (SNP)-based array analysis of gene expression in GBM patients has indicated several genetic changes that shed some light on the nature of the illness. Notably, loss of heterozygosity frequently occurs on the 17p gene, which contains the tumor protein 53 (p53) (The Cancer Genome Atlas (TCGA), Research Network, 2008). Loss of heterozygosity refers to the deletion of a chromosome portion with the corresponding homologous segment being duplicated to compensate for the loss of gene neutrality (Bleeker, et al, 2011). The significance of this mutation is reflected in the loss of genetic code for p53. p53 encodes a nuclear phosphoprotein involved in regulation of cell proliferation. In other words, wild type p53 is a tumor suppressor gene. Mutations in p53 not only cause loss of tumor suppression, they can activate p53 to an oncogene in a negatively dominant fashion implicating these mutations in a number of cancers (Finlay, et al., 1989). Further, loss of heterozygosity of chromosome 10q is associated with poor rates of survival and is the most common genetic alteration found in primary and secondary GBM's. This chromosome contains various tumor suppression codes, most notably the region containing ANXA7, an epidermal growth factor receptor (EGFR) inhibitor, and deletions contribute to a proliferation of cell growth; a distinguishing behavior of cancerous tumors (Yadav et al., 2009).

Additional research focuses on genetic amplifications associated with GBM. Specifically, amplification of the gene for EGFR on chromosome 7 has been shown to be a consistent characteristic in glioblastomas (Finlay, et al, 1989; Bleeker, et al, 2011). Focal amplifications (amplifications containing a small concentration of genes) correlate with overexpression or mutation of EGFR, leading to subsequent activation of the PI3K/AKT pathway, another indicator of poor prognosis (Beroukhim et al., 2007; Phillips et al., 2006). General amplification of entire chromosomes, specifically chromosome 7, has shown correlation with the activation of the Met axis, a codependent cycle with Hepatocyte Growth Factor (HGF) which furthers the occurrence of cell proliferation (The Cancer Genome Atlas (TCGA), Research Network, 2008).

Another genetic component considers IDH1 mutations. The IDH1 gene codes for isocitrate dehydrogenase 1, a critical component of the citric acid cycle, catalyzing the conversion of isocitrate to α-ketoglutarate. Mutations of this gene have been discovered in 12% of glioblastomas (Watanabe, et al., 2008). Mutations of this enzyme do not appear to cause loss of function, rather, cancer-associated IDH1 mutations alter the reaction of the enzyme, enabling it to catalyze the NADPH-dependent reduction of α-ketoglutarate to R(-)-2-hydroxyglutarate (2HG). As many humans have a reduced capacity to dispose of 2HG in an efficient manner, overaccumulation of the metabolite has been implicated in the formation of malignant brain tumors (Dang et al., 2009). 2HG also has a penchant to activate NF-κB, a prominent protein complex that controls cytokine production, cell survival, and DNA transcription, implicating it in many cancers, including various leukemias (Chen et al., 2016).

Ultimately, the strongest evidence of a genetic aspect to GBM is that of the MGMT promoter. While TMZ treatment in general has shown some benefit, much of its effectiveness is diminished by this molecular occurrence. TMZ acts by modifying the O6-position in guanines. This translates to DNA lesions leading to DNA cross-links preventing cell replication. MGMT can remove the alkyl groups and contribute to TMZ resistance but not when methylated. Because about 50% of patients exhibit MGMT methylation, the effectiveness of TMZ is considerably lessened in approximately half of all cases (Bleeker, et al., 2011). Research is being done particularly in this area to better understand the environment surrounding methylation and prospectively harnessing the power of methylation to increase TMZ effectiveness (Lee et al., 2018).

Additional aberrations in genetic behavior exist in GBM with varying degrees of frequency. With more tools becoming available, it is apparent that the picture of GBM is not clear and further genetic research is required. Nonetheless, these tools enable researchers to identify the most prevalent deviations from typical behavior. For example, many alterations occur within three primary pathways; the p53, RB, and PI3K/AKT pathways, and appear to occur in a mutually exclusive fashion (The Cancer Genome Atlas (TCGA), Research Network, 2008). This defines a narrower scope of application, hopefully resulting in the development of rational therapeutic techniques and drug design. These genomic approaches may ultimately contribute to more individualized therapies for greater patient longevity.

Immune Evasion and Immunotherapy

In addition to the complications involved with gene aberrations in malignant gliomas, the impressive rate at which GBMs evade natural immune response opens another point of study for prospective treatments. Avoidance of the immune system was added as a defining characteristic of cancer in 2011 and an examination of the natural immune response has helped develop vaccinations and treatments for various diseases (Hanahan & Weinberg, 2011). Nonetheless, the ability of tumors to dodge these receptors requires a better understanding, specifically in the brain. In line with original theories, the CNS is immunologically privileged. Unfortunately, it is not quite as privileged as once suspected. While early theories assumed this privilege was a result of isolation of the brain by the blood brain barrier,

newer research refutes this. Peripheral immune cells do in fact cross the barrier, but CNS neurons and glia regulate immune responses in a different fashion than other cells (Carson, et al., 2006). Researchers have demonstrated this deviance from the standard immune system using experimental foreign grafts to rabbit brain. These grafts were rejected at a much slower rate than grafts to less unique locations (Medawar, 1948). An additional indication of CNS immune system inadequacy is the immune response of the brain in multiple sclerosis patients. The immune system of affected individuals appears to contribute directly to the proliferation of the disease rather than fighting it (Hemmer, et al., 2015). Despite these deficiencies, analysis suggests proper manipulation of the immune system can assist in the fight against brain cancers, achieved through understanding the mechanism by which tumors evade it.

ICT-107 is a multiple-antigen-pulsed dendritic cell vaccine containing multiple tumor-associated antigens (HER2, TRP-2, gp100, MAGE-1, IL13Rα2, and AIM-2) known to interact with glioma stem cells. These antigens may be ideal for vaccination as their introduction to the dendritic cell tumor environment stimulates T-cells to generate glioblastoma specific cytotoxicity (Huang et al., 2017). This immunotherapy was the first such trial to reflect significant results in terms of overall, progression-free survival in newly diagnosed GBM (McGranahan, et al., 2017). A phase III trial is awaiting additional funds to further investigate the effectiveness of this vaccine (Phuphanich et al., 2012). Another vaccine in phase II trials is Gliovac. This vaccine utilizes autologous antigens from the patient subjects combined with allogeneic antigens (antigens with a dissimilar genetic makeup from that of the patient) derived from other GBM patients. Beyond using tumor lysate, the Gliovac vaccine is designed similarly to ICT-107 to encourage the development of cytotoxic T-cells to enhance the struggling immunological response (Schijns, Virgil E J C et al., 2015). Further studies examine the link between the CTLA-4 receptor, a constitutively active receptor on the Treg gene, and its downregulation of the immunologic response in active T cells (Leach, et al., 1996). By blocking CTLA-4, researchers have seen considerable success with enhancing antitumor immunity, rejecting both artificially introduced and pre-established tumors. The rapid growth of immunotherapeutic treatments stresses the importance of a more comprehensive understanding of the immune system and its reactions to foreign in vivo developments. More specific information on marker antibodies will go a long way in furthering treatments, not only for GBM but also for other immuno-evasive diseases.

Treatment with Infection

A seemingly counterproductive method of treatment has been considered to address incomplete resection of glioblastoma tumors. While the actual number of surgical site infections after malignant brain tumor resection is not well established, data indicates the rate to be about 3.4% (Uzuka et al., 2017). A speculative theory supported with anecdotal evidence presented the idea that patients who developed postoperative bacterial infections and survived had a radically increased overall survival. A study reported four cases of patients with surgical site infections living well beyond the expected range of survival for glioblastoma (Bowles, et al., 1999). This theory has been subjected to several trials and though not statistically significant, results showed that many infected patients live longer on average though reverse logic may be cloud results. Infection may correlate with increased life span because patients that live longer are more likely to develop an infection, not because infection lengthens survival (De Bonis et al., 2011).

Antiangiogenic Treatments

Antiangiogenic treatments have shown a lot of promise in cancers management and particularly in that of GBM as second-line treatments. The rationale for antiangiogenics is strong; as cancers are highly vascular, preventing the growth of blood vessels to supply the tumor should help deplete tumor resources and severely retard malignancy. Tumors are known to exploit the body's nutrient and oxygen supplies and require enhanced networks of blood and lymphatic vessels to facilitate this resource abuse (Nishida, et al., 2006). Vascular endothelial growth factor (VEGF) is a signaling protein that stimulates the creation of blood vessels and has received increased attention as an indicator of oncogenesis (Pavlova & Thompson, 2016). To address this, researchers developed Bevacizumab, a monoclonal antibody that counters the effects of VEGF and has been approved for trials in the United States for some time. Dosed intravenously, Bevacizumab has been used independently as a monotherapy and in pairs or groups of antiangiogenics for a multi therapeutic approach in various cancer types. In early trials, a statistically significant number of patients demonstrated an improvement in overall survival, but randomized phase III trials have been unable to demonstrate an appreciable improvement in overall survival required to move this form of therapy forward (Bleeker, et al., 2011).

Retroviral Delivery

A promising treatment technique addresses issues with alternative treatments. Retroviral replicating viruses (RRVs), are a novel way of treatment delivery and contain a wide variety of unique characteristics that allow for highly specific gene transfer in an efficient manner. Pharmaceutical companies are combining many of the above therapies into a single, comprehensive treatment method for recurrent high-grade gliomas with the goal of extending these concepts to further treatments.

Retroviruses are positive-sense RNA viruses. These obligate parasites cannot reproduce or complete their life cycle without a host cell. Once within a host, these viruses use the enzyme reverse transcriptase to produce DNA from the viral RNA

sequences. This DNA strand is then incorporated into the host cell genome with an integrase enzyme. By transferring it's genetic data into the host, the virus ensures it will be reproduced when the host reproduces. The applications of these viruses can allow scientists to produce desired proteins and genetic products for treatment purposes. These viruses confer many advantages to the treatment of cancers and in particular, of glioblastoma. One such benefit of RRVs is the selective acceptance within cells. It has previously been established that tumors exhibit a distinct loss of immunity and that a central aspect of cancer development is the evasion of the immune system. Cell populations naturally assess tissue to prevent proliferative, uncontrolled growth, and cancers evolve to avoid the anti-tumor response. This evolution evades immune checkpoints and regulators but also enables immunoediting, the process by which cancers read, react, and adapt to immune processes. By disabling the anti-tumor response, cancer cells can replicate continuously unchecked (Butt, et al., 2004). The lack of immunity that permits cancers to exist also creates an opportunity for medical intervention. RRVs are generally recognized by healthy cells and dealt with accordingly by the normal immune system, but the reduced defense mechanism of cancer cells allows the RRV to incorporate genetic components into the cancer gene pool. This selective acceptance safeguards healthy tissue while priming cancer cells. Another key feature of RRVs is the ability to spread non-lytically and without an exceptional inflammatory response. Gene products are not produced until the virus is activated and this avoids an inflammatory response from the innate immune system (which is not heavily compromised). Coupled with a nonlytic retroviral infection, this delivery system enables researchers to target specifically cancerous or immunocompromised cells (Logg, et al., 2012).

The Tocagen therapy relies on an engineered retrovirus known as Toca 511 (vocimagene amiretrorepvec) for delivery. Toca 511 is a murine leukemia virus that infects dividing cells, preferentially integrating into cells that are immune-deficient — in this case GBM cells. In addition, the retrovirus has no trouble crossing the blood brain barrier, an issue that arises with other treatments. The virus contains the genetic code for an altered cytosine deaminase (CD), a protein enzyme that catalyzes the reaction converting cytosine residues to uracil (Ostertag et al., 2011). In trials, after several cycles of RRV treatment, researchers found a high concentration of CD present in tumor cells, but none or insignificant levels expressed in quiescent cells. The retrovirus delivery method efficiently targets tumor cells and integrates its genetic contents into the DNA of the host cell without affecting the genetic integrity of healthy cells in the body.

While the success of the genetic transfer is significant, the expression of optimized CD within tumor cells is of little consequence. Further action is required to have an impact on cancerous cells. 5-fluorocytosine (5-FC) is subsequently administered as a prodrug, a drug in an inactive form. Despite widespread uptake of 5-FC by the majority of cell types, this inactivity prevents any notable response from quiescent or typical cells. However, in the Toca-511 pretreated tumor cells, 5-FC is converted by CD to 5-fluorouracil (5-FU), a potent antineoplastic cancer drug used as the drug of choice for colorectal, esophageal, gastric, and many other cancers. While the precise mechanism of 5-FU is unknown, it is suspected to interfere with DNA synthesis, protein synthesis, and RNA processing (Pinedo & Peters, 1988). Collectively, these contribute to an incompatible state for the tumor cell leading to apoptosis and subsequent removal by the immune system. Furthermore, 5-FU has also been shown to destroy myeloid-derived suppressor cells. These cells help evade the immune system and elimination of these suppressor cells allows the patient's natural immunity to assist in tumor destruction (Cloughesy et al., 2016). An additional advantage of the engineered 5-FU produced in Toca-511 allows the active drug to diffuse through the cell membrane, giving the potential to have therapeutic effects reaching beyond the cytosol of the parent cell (Hemmer, et al., 2015). There is strong evidence to suggest this phenomenon, called the bystander effect, occurred in preliminary trials and it resulted in an improved overall survival rate as well as an improved recurrence free outcome. The Tocagen drugs are undergoing clinical trials but the progress they signify in science is considerable and opens the door to a proliferation of improved treatment techniques.

Multi-Therapy

The multi hit model of cancer, also known as the Knudson hypothesis, is the theory originally proposed in 1953 hypothesizing a stepwise component to oncogenesis. The theory suggests that cancer onset is not the result of a single mutation, rather the outcome of many mutations, creating conditions for the perfect storm that is cancer (Nordling, 1953). Knudson's hypothesis has greater validity after "Hallmarks of Cancer" was published by Weinberg and Hanahan (Hanahan & Weinberg, 2000). The hallmarks reiterate the idea that cancer is multi-faceted and more complex than a single aberration in physiology and development. The theory gives rise to an alternate approach to treatments; if the disease is multifaceted, then so must be the treatment plan. Various treatment proposals utilize this theory for multi-hit therapy. Some antiangiogenic treatments are being applied in conjunction with others for a comprehensive approach. Several attempts have been made, or are now in trials, that couple effective treatments like that of TMZ with newer medications to enhance their effectiveness. In concurrence with TMZ, patients have been tested with a wide variety of pharmaceutical cocktails, including gefitinib and erlotinib, a pair of EGFR inhibitors (Bleeker, et al., 2011). Patients have also been treated with radiotherapy and Gliadel wafers. These wafers are intracranial implants containing carmustine, a nitrosourea

alkylating agent, and give a steady treatment of chemotherapy to their host after being inserted into the brain (van den Bent et al., 2008). Direct insertion prevents the need to circumvent the blood brain barrier and offers a continuous administration of the drug for the duration of the treatment. As a nitrosourea, carmustine alkylates DNA strands, preventing normal gene expression in cancer cells and dampening tumor development. Further, contrary to previous research, resistance to a single alkylating agent does not predict resistance to alternate alkylating agents, allowing for a multi-agent approach to prevent cancer gene expression (Schabel, 1976).

The development of chimeric antigen receptor T-cell therapy (CAR-T) builds on the success of above-mentioned treatments such as retroviral genetic manipulation and immune system enhancement. This technique utilizes engineered T-cells to recognize specific markers in cancer cells and induce apoptosis. One lab at the Memorial Sloan Kettering Cancer Center focused on B cell acute lymphoblastic leukemia and is examining options to extend this therapy to gliomas. In the leukemia study, patients with relapsed B cell acute lymphoblastic leukemia had T-cells isolated from plasma and treated with a vector. The vector programmed T-cells to produce chimeric antigen receptor, a receptor that can be engineered to enable the T cells to recognize specific proteins found in cancers (CD19 in this case) (Brentjens et al., 2013). One particular advantage of CAR-T therapy is the use of the patient's own immune cells. Using autologous material decreases the incidence of rejection or other complications that frequently occur with the introduction of foreign cells (Almasbak, et al., 2016). CAR-T therapy has demonstrated high rates of success with above an 88% long term survival rate and is being evaluated for expansion to a greater variety of cancers, including high- and low-grade glioma (Wilkins, Keeler, & Flotte, 2017).

The range of ideas employed with multi-hit therapies and techniques takes advantage of the advances made in genetic and molecular research. Introducing therapies that make use of immunotherapy and genetic recombination, or that address multiple facets of angiogenesis are particularly useful in tackling the multidimensional aspects of cancer. Combination treatments allow scientists to develop a more comprehensive blend that can address the particular deficiencies associated with each individual medication and enable an extensive treatment plan for the greatest chances of survival.

Conclusion

Glioblastoma multiforme is a high-grade malignancy with a poor prognosis. While additional preventative research is required to improve diagnostic methods and screening, management methods are under rapid development to improve not only survival but also quality of life. A greater focus on intermolecular functioning within GBM helps provide a better understanding of how to effectively diagnose and recognize the cancer. Additionally, such information allows the introduction of periodic screening in higher risk individuals as determined by these advances. By analyzing intermolecular pathways such as kinase receptors and genetic modifications, researchers can develop steps or procedures to counter the cancer. This basic research extends itself heavily to the applied sector and allows for the creation of therapies and treatments that respond to the characteristics and actions unique to this disease and the complexities of its location.

Many treatments have already been proposed in an attempt to contain the rapid tumor proliferation associated with glioblastomas. With the knowledge gained from the success and failures of these treatments, adjusted multi-therapy approaches and novel replacement techniques have been developed. From genetics and immunological studies to bacterial and retroviral infections, these alternatives offer increased variability and possibility for patients. Despite the grim prognosis that typically accompanies the diagnosis of GBM, there is the potential for the cancer to no longer be as debilitating as it currently is or once was. Particularly through immunotherapy and gene therapy, as well as the use of antiangiogenics and nitrosourea alkylating agents, GBM can soon be combatted beyond the standard of surgical resection and chemotherapy.

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