Discovering New Targets and Chemotherapy Drugs to Overcome Glioblastoma:

Review of the Literature

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Abstract

Glioblastoma multiforme is among the most aggressive brain tumors with minimal prognosis and high recurrence. There is need to identify new targets for chemotherapy drug targets and treatment options to combat the virulence of the tumor. To better identify future directions of research, this review has compared the two processes of identifying new chemotherapy drug targets, including S1P, *smpd*, *Sphk*, ugcg, pannexins, and glutamate and testing potential treatments. Findings from this review, suggest that a combinatorial therapy that involves targeting those six genetic and cellular components will be optimal for treating glioblastoma. More research needs to be conducted to identify more chemotherapy drug targets and novel treatments options.

Introduction

The glioblastoma multiforme (GBMs) are the most common brain tumors. They originate in glial cells, the protective cells that support brain neurons. GBMs account for more than 50% of all central nervous system (CNS) tumors of glial origin, and GBMs grade IV, the most advanced GBMs, account for 4% of all cancer deaths in the USA annually.

Current treatment strategy for GBM includes a surgery followed by radiation therapy and post-radiation administration of temozolomide (TMZ). TMZ works as an alkylating agent that binds to the DNA and slows down cell growth. Despite all these treatments, the average survival of patients with GBM is still no more than 15 months, due to the high level of recurrence (Connell-Albert, 2012, p. 22). Because of that, there is a need to discover novel drug targets, for example, specific molecules essential for cancer growth, to develop new chemotherapeutic drugs to block those targets and slow disease progress.

Before drug researchers test potential chemotherapy drugs, they have to identify the pathway of the drug target. This review investigates original research, which involved identifying chemotherapy targets for GBM and then testing chemotherapy drugs. Six different targets were the subject of the original research studies reviewed, and they all affect the S1P signaling, which is an important process in intercellular interaction, and, preventing tumor growth. The six targets are (1) *S1P1* receptor, (2) sphingosine kinase (*Sphk*), (3) pennexins and glutamate, which control the S1P synthesis and maintenance. Also, (5) the ugcg gene and (6) sphingomyelin phosphodiesterase (*smpd*) monitor the synthesis of S1P derivatives, namely, glycosphingolipid and sphingomyelin, respectively. The *S1P1* acts as a receptor for the S1P molecule, directing the release of T cells from the thymus and the spleen, and, essential for the immune response counteracting GBM. Sphingosine kinase (*Sphk*) directs the synthesis of S1P by phosphorylating of sphingosine. Pannexins are gap junctions that increase cell-to-cell signaling via ATP release, stimulate cell growth via glutamate release, and activate the inflammasome for fighting viruses at the last stage. The ugcg gene controls the first committed step in glycosphingolipid synthesis, and the *smpd* catabolizes the SM, a lipid found in subcellular membranes, which directs release of hypothalamic hormones.

The six targets identified in the studies reviewed proved to be able to influence the S1P signaling and act as potential targets for controlling GBM growth. We need to take them to the next step, where we test them clinically. Some chemotherapy drugs have been tested clinically successfully. We need to conduct more research for identifying new chemotherapy drug targets and chemotherapy drugs.

Methods

We selected five of the six articles used in discussing the chemotherapy targets from a published review by J. R.Van Brocklyn (2007), retrieved from the ProQuest database, Mini Reviews In Medicinal Chemistry journal. Two of the studies were from The Journal of Biological Chemistry, Allende et al. and Ishii et al.

The remaining three were from The Journal of Molecular and Cellular Biology, Mizugishi et al., Genesis, Yamashita et al., and Proceedings of the National Academy of Sciences, Stoffel et al.

Selecting publication titles that contain the names, neuro, brain, cancer, or gene led to choosing Brocklyn's review article. Words from the subject list included: male, female, rats, middle-aged, gene expression, etc. Names that do not pertain to brain cancer, like oncology and nature, were excluded. The sixth chemotherapy target study along with the three studies testing chemotherapy drugs came from the GMU MARC system based on the recommendation of Dr. Ancha Baranova, the co-director and lead scientist of the GMU-INOVA research lab and who oversaw the dissertation by Connel-Albert at GMU. The testing of chemotherapy drugs is nearly standard, so Connel-Albert's research was selected as an example.

Results

Six of the papers reviewed discussed possible chemotherapy targets, while the remaining four focused on testing chemotherapy drugs.

Preliminary research investigating novel targets for tumor therapy

To identify a molecule as a target for chemotherapy, the gene controlling that molecule is knocked down then the physical and neurological effects are analyzed (Fig 1). Inhibiting cell growth was an indicative that the target might be successful in slowing GBM growth. Yamashita et al. (2005) followed a standard set of the procedures that most other gene targeting experiments also pursue. They began by preparing the gene containing the S1P allele from a mouse library, and then cross it over with a null allele. The resulting gene was the knocked down gene for the S1P. Polymerase chain reaction (PCR) was used to magnify the genome and was followed by the Southern analysis to confirm the gene sequence. They injected the recombinant gene into mice, which were mated, and the resulting offspring, the chimeric mice, were also mated (Ugcglaxp x Ugcglaxp). They used the offspring in the analysis. Sections from these offsprings' brain, kidney, spleen, and liver were then taken and tested through histology, Western blot, flow cytometry, Alamar blue test, and compared to the normal.

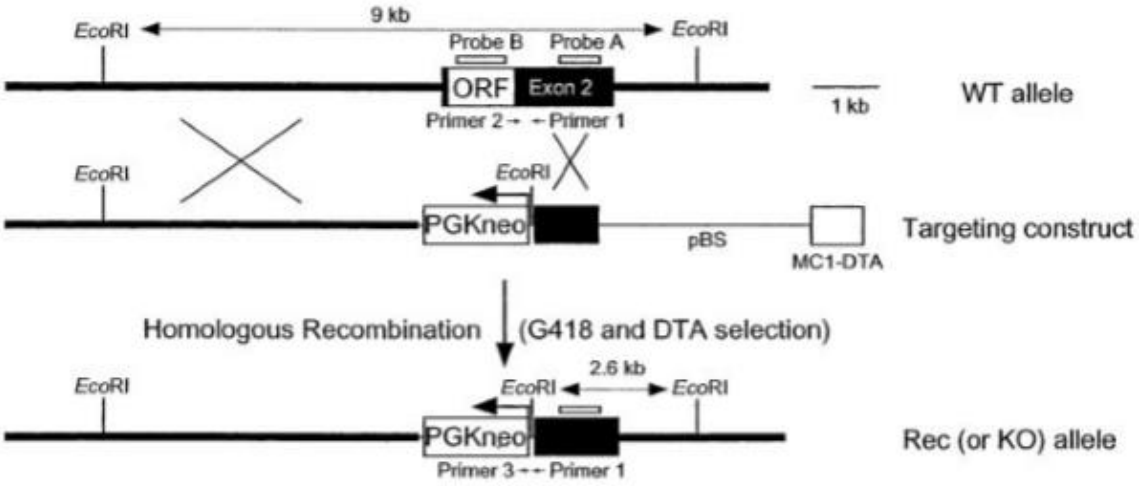

Figure 1. Targeted disruption of SphK2 gene schematic representation.

Using the S1P1, sphingosine kinase, and pannexins to synthesize and maintain the S1P. Ishii et al. (2002) attempted at investigating the G protein-coupled receptor *S1P2*, one of five receptors, to determine if it serves redundant roles with *S1P3*, which they targeted in a previous study. They found that *S1P2*-null mice had normal life expectancy, whereas *S1P2S1P3* double-null mice died during the perinatal period. This outcome suggested that *S1P2* and *S1P3* play a conjunctional role in controlling cell signaling and therefore, cellular growth and development. Since GBM causes uncontrolled cell growth, using chemotherapy to enhance the *S1P2* and *S1P3* genes and, cell signaling can reduce GBM growth.

Using sphingosine kinase (Sphk)-1, Sphk2, and Sphk3 to control cellular signaling and growth, lymphocyte trafficking and distribution, and neurological as well as vascular disorders. Allende et al. (2004) investigated the effect of deleting the *Sphk*1 gene on S1P signaling and lymphocyte trafficking and distribution. They determined that the *S1P1* levels decreased but did not diminish as a result of deleting the *Sphk*1 gene because they saw that signaling, as well as trafficking and distribution, were maintained at lower levels. They suggested that other enzymes besides *Sphk*1, like *Sphk*2, lysophospholipase D, S1P lyase/phosphatase, or ceramidase, contribute *S1P1* production. These other proteins, in addition to *Sphk*1, should be targeted when synthesizing chemotherapy drugs to upregulate *S1P1* expression and counteract the lack of intercellular communication and lymphopenia caused by the GBM.

Carrying on Allende et al. (2004)'s work, Mizugishi et al. (2005) investigated the effect of *Sphk*2-/- and *Sphk*1-/- *Sphk*2-/--double null mice on neural and vascular development. Mice that lacked *Sphk*1 and *Sphk*2 had related complications to the *S1P1*-null mice and mice with multiple receptor mutations, like a higher incidence of vascular developmental defects, cranial hemorrhage, and neuronal tube deficiencies (NTD). Since carcinogenic mycotoxins are, potentially, responsible for disrupting the normal *Sphk*ases' activities, causing abnormal growths as well as NTDs, this study could direct future research to use the *Sphk*ases as potential targets for chemotherapy to increase S1P levels. Allende et al. and Mizugishi et al. confirm that *Sphk*1 and *Sphk*2 work in conjunction to bring about S1P signaling and increase lymphocyte trafficking and distribution, with Allende et al. adding that lysophospholipase D, S1P lyase/phosphatase, or ceramidase could, potentially, be other enzymes contributing to that process.

Using Pannexins to increase inflammasome activity, intercellular interaction, and inhibit glutamate release. Connel-Albert (2012) attempted at using the biotinylation and phosphatase assays to determine the location and post-translation modification, respectively, of pannexin (panx) gap junctions. Showing that phosphorylation is not necessary for pannexins activation through the phosphatase assay, she concluded it is glycosylation that is more important. Likewise, the biotinylation assay revealed that the panx channels do not insert in the plasma membrane without glycosylation. The phosphatase and the biotinylation assays, therefore, confirmed that glycosylation is a major event for pannexins to enter in the plasma membrane and maintain their function.

Furthermore, Western blot analysis showed that GBM patients had decreased amount of panx2. Since panx2 functions in activation of the inflammasome, and panx1 increases the release of ATP into the extracellular membrane where it goes through a cascade of events to increase intercellular communication, both panx2 and panx1 play a role in inhibiting tumor invasiveness and growth. Would could direct future research for chemotherapy drugs to enhance panx2 and panx1 glycosylation, and, panx2 sumoylation and palmitoylation to increase the inflammasome activation as well as intercellular communication. Increasing panx glycosylation, *Sphk*1 and *Sphk*2, and *S1P1* receptors can increase S1P synthesis and maintenance, with Connell-Albert adding that panx will increase glutamate release and cause cancer growth. Therefore, if we increase panx function we also have to inhibit glutamate release.

Increasing S1P signaling and inhibiting tumor growth by increasing glycosphingolipid and sphingomyelin synthesis, and increasing the cAMP levels.

Combining the ugcg gene with Cre recombinase in the presence of nestin promoter to reverse mutation in glycosphingolipid synthesis. The studies identifying the potential chemotherapy drug targets differ in the type of gene they synthesize and study. For example, Yamashita et al. (2005) structured their study to investigate the effect of the ugcg gene on survival and neurologic behavior. They attempted at knocking the gene down by two different ways, flanking it with LoxP sites or presenting it with the Cre recombinase under control of the nestin promoter. They found that the ugcg gene flanked by the LoxP sites still retained their level of expression, whereas those recombined with the Cre recombinase had died during gastrulation or lived with impaired neurological behavior. These results were similar to previous studies investigating ganglioside-deficiency. Given that glioblastoma in humans can cause ganglioside and glycosphingolipid deficiencies, future studies could use Cre recombinase under control of the nestin promoter to reverse the possible mutation in the ugcg gene in GBM patients and, slow GBM growth.

Ishii, et al. (2002) have also discovered that S1P can control the cAMP levels in two ways--the PTX-sensitive cAMP inhibition and the PTX insensitive-cAMP activation. Since high cAMP levels inhibit GBM (Hill, Moreno, Lam, Haqqani, and Kelly 2009), we could direct future studies to chemotherapy drugs that increase cAMP levels, and, slower the GBM growth. Yamashita et al. and Ishii et al. confirm it is important to work on S1P, with Ishii et al. also maintaining that it is important to regulate the cAMP levels.

Using sphingomyelinase phosphodiesterase (smpd)-3 to control lipids and amino acids' trafficking in the membrane of the Golgi in hypothalamic neurosecretory organs, and, regulating the secretion of hormones, like growth hormone, and indirectly, controlling tumor growth. Stoffel, Jenke, Blöck, Zumbansen, and Koebke (2005) investigated *smpd* 2-/- and *smpd*3-/--deficient mice to see their physiological response. Immunohistochemistry of sex organs and the pituitary gland revealed growth retardation and delayed puberty for mice that had *smpd*3-/- mutations in their Golgi. Since *smpd*3 plays a critical role in trafficking of lipid and protein products in Golgi membranes of hypothalamic neurosecretory neurons, they suggested its elimination caused the dwarfism because of some hormone misregulation, like the FSH, TSH, IGF-1, GH. GBM could also result from excess GH levels in the tumor masses. Therefore future GBM chemotherapy studies could target *smpd*3 to decrease the GBM growth (Mezey, Treszl, Block, Vizkeleti, Juhasz, Klekner, et al. 2014; Ogilvy-Stuart and Gleeson 2004). A weakness in this study could be that they have stayed at the surface and did not investigate the mechanism with which the ceramide, a product of SM, plays a critical role in the cell trafficking, beyond suggesting their topology in the Golgi membrane. Future research in this area could lead to developing new drugs to target that process to regulate GH levels. Researchers agree that increasing glycosphingolipid and *smpd*'s synthesis will increase S1P signaling with Ishii et al. adding that it is also important to increase cAMP levels to decrease GBM growth.

The current state of targeting research. It is important to synthesize and maintain the S1P molecules by enhancing the *S1P1*, *Sphk*, and pannexins. It is also important to increase the S1P signaling once we synthesize the S1P molecules by increasing the glycosphingolipid and *smpd*. If we increase inflammasome activation and intercellular communication without inhibiting cancer growth by increasing cAMP levels and inhibiting glutamate release from panx, GBM will remain to grow. Therefore, it is important as we increase the S1P levels and signaling also to inhibit glutamate release and increase cAMP levels.

Chemotherapy: testing different drugs having specific cellular targets

After identifying different chemotherapy drug targets, including sphingosine kinases, sphingosine-1 phosphates, and pannexins, researchers identify different drugs to act on the targets to control tumor growth. Connel-Albert (2012) investigated the effect of natural therapeutics, like Schweinfurthins, and their synthetic counterparts, the Schweinfurthin analogs, which could be useful in case of scarcity of natural products, in treating GBMs. Her methodology is typical for testing chemotherapy drugs: She prepared the different sets of murine astrocytomas, ran Alamar tests, and calculated the GI50 values, the concentration of the drug at which it can inhibit 50% of cells' growth, which became her basis for determining the drug efficacy.

She also tested the FDA approved Chloroquine (CLQ), Nelfinavir (NFV) and perifosine (PER), and the non-FDA approved Phosphatidylinositol ether lipid analog (PIA)-6 and OSU-03012. Finally, she tested PI-103, Tricirbine, and Rapamycin.

Schweinfurthin. Only one human glioma and two mice astrocytoma cell lines out of the remaining six cell lines were sensitive to schweinfurthin and its analogs. This outcome contradicted with previous findings, which showed more cell lines being susceptible to the drug. Connel-Albert attributed this discrepancy to the difference in confluency in her culture compared to the other previous experiments. The cancer cells could also have undergone molecular changes, which gave them more resistance. Also, scheinweinfurthin's high molecular weight (M.W.) (~500 Da) yields it ineffective in penetrating the blood-brain barrier (BBB), which only has permeability for M.W.s that are less than 400 Daltons. The new molecular changes that Albert witnessed and which give resistance to the tumor cells could be manipulated to keep only those which are advantageous to the drug's reactivity. However, scheinweinfurthin analogs could be beneficial to determine toxicity, the mechanism of drug action and novel targets, and also could be of benefit in case of scarcity of natural products.

Nelfinavir, Chloroquine, Perifosine, PIA-6, and OSU-03012. She found that OSU-03012 inhibited the PDK1/Akt in the PI3K/Akt/mTOR pathway, yielding 80% maximum inhibition of the cell viability by acting as a cytostatic, inhibiting the cell cycle at the G1 phase. PER produced 90% maximum inhibition by apoptosis. CLQ and NFV each yielded more than 80% inhibition (Conell-Albert, 2012). At low levels, NFV has cytostatic effects, while at high concentrations it has cytotoxic effects. PIA-6 did not have a response, and she suggested that P13K might be a better alternative. While testing NFV as a chemotherapeutic drug, Connell-Albert also attempted at gathering data in support of the published report, that Nelfinavir can treat estrogen negative receptors (ER-) because the cytotoxicity of Nelfinavir is irrespective of ER manifestation. She was able to gather data to support that hypothesis. One limitation of this study is that inhibiting dysregulated pathways, like the Akt pathway could result in negative feedback loops, which enhance the tumor growth.

PI-103, Tricirbine, and Rapamycin. Connel-Albert (2012) concluded that the PI-103 is the most efficient in dephosphorylating Akt. It is very delicate by acting as a cytostatic compared with the control, meaning it causes the most dephosphorylation. Following, that is the Tricirbine, which caused same effect but less pronounced. Following is the Rapamycin, which produced the least effect on the Akt (almost no change); but at subtle doses (1 pm), it inhibited the downstream effector molecule completely.

The current state of research for chemotherapy drugs. Cytostatic drugs which showed high efficacy in vitro, like Rapamycin, OSU-03012, NFV at low levels, and PI-103, can be used in combination to decrease

cellular growth and division of GBM without damaging the body cells. Using the combination therapy can help overcome the feedback loop, which enhances the tumor growth. Since in vitro analysis does not predict the toxicity in vivo, it may be a limitation of this study, and we need to retest the drugs clinically. Additionally, the Alamar blue assay may introduce unwanted variables. The utilization of compounds with low cytotoxicity, which causes less harm to the body compared to its chemotherapy drugs and radiation counterparts, gives this study an advantage.

Conclusion

The highlights from the papers reviewed are targeting the genes that synthesize and maintain the S1P. To identify more chemotherapy drug targets, new signaling molecules, including different types of sphingosine phosphates, pannexins, and sphingosine kinases, need to be investigated. Scientists could use knowledge of neurological drugs that are already in use to guide which pathways they could target. Then, they would evaluate the effect of knocking down the molecules responsible for maintaining these trails on tumor growth. To facilitate intercellular interaction, it is important to increase the S1P function through increasing glycosphingolipid, regulating the *smpd* syntheses, and increasing cAMP levels while inhibiting glutamate release. Use of combinatorial therapy composed of cytostatic drugs that, simultaneously, targets different molecules and genes could prove successful in slowing cancer growth without increasing negative feedback.

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