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Stem-Cell Based Therapy in the Treatment of Glioblastoma Multiforme

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ABSTRACT

Glioblastoma Multiforme is a grade IV detrimental tumor occurring in nearly 13,000 Americans annually, presenting a serious and often fatal toll on the individual suffering from the tumor. However, the resistant properties of the cells which together form the tumor pose a variety of issues in terms of treatment, including violently spreading throughout the central nervous system, disrupting the blood-brain barrier, as well as having a survival rate of forty percent. There has yet to be a guaranteed effective treatment for Glioblastoma, however, a form of treatment known as stem-cell therapy utilizing neural stem cells has recently gained interest as a common form of treatment. The various forms of treatment for Glioblastoma Multiforme other than primarily stem-cell based therapy as well as the composition of these stem cells has been presented and analyzed in this paper, in order to present and suggest which form of treatment for this tumor is the most effective.

Introduction

An agglomeration of tumors located in the central nervous system consisting of the brain and spinal cord as well as aggressively spreading throughout these two regions of the human body, gliomas constitute for approximately twenty-five percent of all neurological tumors, deeming them the most common variation of brain tumors. Gliomas originate from three types of glial cells, or cells which support the function of neurons. These three categories of glial cells are composed of ependymal cells, which are cells that regulate cerebrospinal fluid, oligodendrocytes, or cells which produce myelin that surrounds and protects axons, as well as astrocytes.

Astrocytes account for nearly seventy percent of gliomas with Glioblastoma Multiforme being a subdivision of astrocytes. In addition, gliomas are categorized based on their variation of glial cells as well as their mutation. In general, tumors are rated on a scale from one to four, with a tumor rated level four being the most invasive and fatal possible. On the other hand, tumors with a low rating often do not require intense therapy. The components which form these neurological tumors also depend on neoplasms. Neoplasms are a tightly clustered collection of tissues that take place when cell growth and division occur at an unusually rapid rate or when apoptosis, or programmed cell death does not occur at the correct moment. Neoplasms are resistant in nature, meaning they can often resist various forms of treatment for Glioblastoma Multiforme, posing a challenge to the treatment of this tumor.

Moreover, Glioblastoma Multiforme tends to undergo a process known as metastatic seeding, when cells that are cancerous disperse to other locations of the body. As a result, individuals who suffer from Glioblastoma Multiforme tend to lose their life within the span of approximately one year, due to current forms of treatment for this tumor being relatively new and not very effective, which poses the question as to which method of treatment is the most effective and reliable.

What is Stem-Cell Therapy and How is it Executed?

Stem-cell based therapy is a form of treatment for a broad spectrum of diseases and conditions and is not limited to treatment only neurological conditions. Georges Mathé, a French oncologist, discovered the potential stem cells possessed in 1958, and saved the lives of six individuals who were exposed to exceedingly high levels of radiation. Additionally, stem cells are precursor cells, meaning they continually develop and experience growth until they possess the capability to form a new type of blood cell through differentiation, a cellular process which creates a distinction in the function of the cell. To retrieve and manipulate stem cells until they reach this form, they must be extracted from the individual in need of stem-cell therapy.

However, these stem cells can also be retrieved from another individual, as long as the stem cells have a similar genetic structure, causing siblings to be the most likely donor to possess stem cells with a similar genetic makeup due to siblings receiving their genome from the same source, their parents. Stem-cells can be derived from a variety of locations, including but not limited to the blood, bone marrow, and dental pulp, and separating the stem cells from these materials and then returning these substances back into the body. Although, the most simplistic extraction location is to retrieve stem cells directly from neural tissues, as well as using adult stem cells, since these cells are directly applicable to treatment of neurological conditions.

The idea of using stem cells as a form of therapy for Glioblastoma Multiforme was likely considered due to their ability to alter into multiple types of tissues. Since Glioblastoma Multiforme rapidly spreads to several locations in the body and especially the Central Nervous System, the use of stem-cells would be an effective form of treatment. Furthermore, stem-cell therapy is when exogenous stem cells are dispersed into the body or endogenous stem cells are stimulated. The mobilization of endogenous cells is utilized when attempting to treat or manage degenerative disorders, whereas exogenous stem cells are made use of when gliomas are on the severe side of the spectrum. Essentially, stem cell based therapy has introduced a new perspective on the treatment of mild, moderate, and severe neurological and other disorders.

Classification of Stem Cells

In general, stem cells are classified by their location of origination in the human body. For Glioblastoma Multiforme, the two main variations of adult stem cells derived from the brain are Mesenchymal Stem Cells (MSC's). MSC's are derived from bone marrow, adipose tissue, the umbilical cord, and dental pulp. The other primary variation of stem cells derived from the brain are neural Stem Cells, typically abbreviated as "NSC's." Neural stem cells tend to be found in the dentate gyrus, or the region in the brain responsible for memory as well as being found in the subependymal zone, the layer of cells beneath the ependyma, or the membrane the surrounds regions of the brain and spinal cord that contain fluids. Neural stem cells are effective due to their ability to regenerate neurons, astrocytes, and oligodendrocytes.

A Closer Look at Neural Stem Cells (NSC's)

Neural stem cells are primarily located in the hippocampus and subventricular locations of the brain and possess the ability to transfer substances throughout the entirety of the brain. For instance, neural stem cells also can migrate towards hostile masses of gliomas and other types of neurological tumors and cancers, but the reason for this migration, however, is unknown. For the NSC's to migrate towards the tumor, it can take up to fifty minutes in length after they are administered as well as taking up to five days for these stem cells to fully expand and cover the vicinity of the tumor they are being used to treat. Factors that affect the rate of migration for neural stem cells include the number of tumors present in the brain as well as the distance the stem cells are injected from the tumor. More than one tumor being present in a specific region affects the process of tumor growth reduction because the concentration of neural stem cells per tumor is significantly decreased due to

there being multiple tumors to treat rather than only one. As a result, the recovery time of the patient suffering from Glioblastoma Multiforme can be further delayed. Essentially, neural stem cells play a major role in the treatment of Glioblastoma Multiforme and must be administered in a particular manner in order to ensure brief but effective treatment.

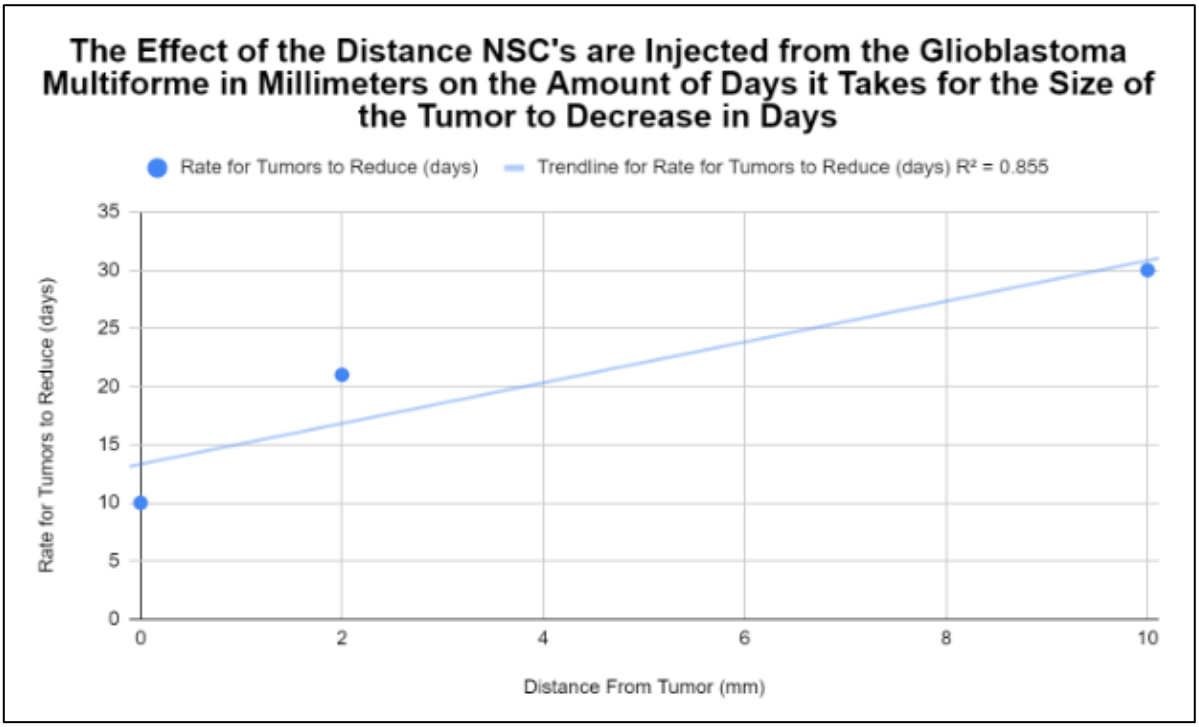

Figure 1. The scatter plot pictured above depicts the relation between the distance neural Stem Cells are injected from the tumor in millimeters and the number of days it takes for the tumor to decrease in size until it reaches a sub-detection level according to a recent study conducted by the Department of Pharmaceutical Sciences. The correlation coefficient of the graph (R-value) of 0.92466 portrays a strong and positive relationship between these two variables, meaning as the distance the NSC's are injected from the tumor increases, the number of days it takes for the tumor to decrease to a minimal size increase as well. Therefore, it can be derived from this data that injecting the neural stem cells as close to the tumor as possible leads to the most effective and short-lasting treatment time as the time it takes for the reduction of tumor growth decreases significantly. Created and copyrighted by Shreya Ambekar.

Causes of Glioblastoma Multiforme

As of right now, the exact underlying cause for the formation of Glioblastoma Multiforme is still unknown. However, those who are of older age groups and those with other genetic conditions tend to have a higher likelihood of suffering from this condition. This unknown reason poses an obstacle to finding a dependable and nearly always effective form of treatment as well.

Structural Composition of Glioblastoma Multiforme

Glioblastoma Multiforme, as all other neurological tumors, primarily consists of invasive tumor cells, including both Glioblastoma stem cells as well as tumor initiating cells. These components form the entire tumor when combined. The invasive tumor cells are a major reason as to why Glioblastoma Multiforme is fatal in nature for most individuals who suffer from this condition. Glioblastoma Multiforme consists of invasive tumor cells, a

tumor core, neuronal and glial progenitors, neurons, oligodendrocytes, microglia, astrocytes, and capillaries. The neuronal progenitors can initiate tumor growth and the neurons are also capable of producing specific molecules and growth conditions that allow the tumor to further grow. Similarly, oligodendrocytes present in Glioblastoma Multiforme are more often than not cancerous and are capable of spreading to other locations in the Central Nervous System other than just the brain. Microglia act as a form of communication between tumor cells through the use of chemokines and growth factors, also stimulating the invasion of tumors, just like the astrocytes present in the tumor. Additionally, capillaries consist of Glioblastoma Multiforme (GBM) cells which invade areas encompassing blood vessels. Essentially, these various components of the tumor are all responsible for the severely invasive and lethal nature of Glioblastoma Multiforme.

Structural Composition of Neural Stem Cells

Neural stem cells are utilized for the treatment of Glioblastoma Multiforme due to their ability to effectively differentiate into both glial and neuron cells as well as effectively responding to certain stimuli, which are both used in the treatment process. Furthermore, neural stem cells consist of a nucleus responsible for control over the cell's activities. The nucleus of these stem cells are surrounded by dendrites, a type of cell that receives signals from other neurons, a cell body known as the soma which contains the nucleus, a myelin sheath that surrounds nerves as a form of protection, a node of Ranvier which are sections of a neuron that are not enclosed by the myelin sheath, axons, axon terminals branching off synapses, as well as a nerve impulse traveling from the location of the dendrites towards the direction of the axon terminals.

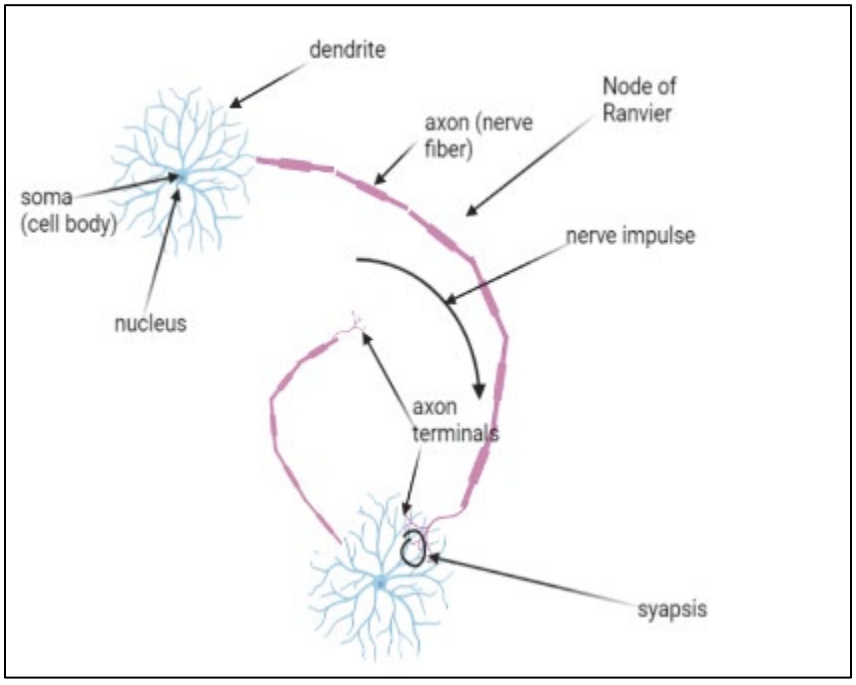

Figure 2. Pictured above is a diagram of the structural composition of neural stem cells with the components described above. Furthermore, a diagram of a Schwann cell is pictured in the diagram as well, depicting that around the node of Ranvier is nerve fiber as well as containing myelin as a form of insulation for the cell. Created and copyrighted by Shreya Ambekar.

The Transfer of Molecular and Chemical Signals in the Brain

Understanding the role and function of neurotransmitters in the brain is an essential component of the engineering of stem cells to treat Glioblastoma Multiforme in order to synthesize effective forms of treatment which nearly identically replicate the normal function of stem cells in the body. Moreover, neural stem cells are required to transfer molecular and chemical signals in the brain and play a significant role in the treatment of Glioblastoma Multiforme. Axons, the region of neurons where electrical nerve impulses travel to be transferred to other neurons, vary in length depending on the type of neuron they are located in. The axons which act as a connection between the brain and the spinal cord are typically the lengthiest. Within axons themselves are axon collaterals, branches that span along the edge of neurons.

Furthermore, the purpose of axon collaterals is to transfer information in the form of signals from neuron to neuron throughout the brain. Synapses, or contact points between axon terminals on one side of a neuron and dendrites on the other side of another neuron are the location where neurons communicate, and chemical signals and impulses are transferred as well. Therefore, axons that are non-functional due to damage contain neurons with neurotransmitters which are unable to effectively communicate and transfer signals between neurons. Axons which are not functional account for various neurological conditions, such as Alzheimer's Disease. Depending on the particular function of the axon, some axons are encompassed in a fatty substance known as myelin, which acts as insulation for the neurons. Additionally, myelin allows axons to transfer signals over long distances.

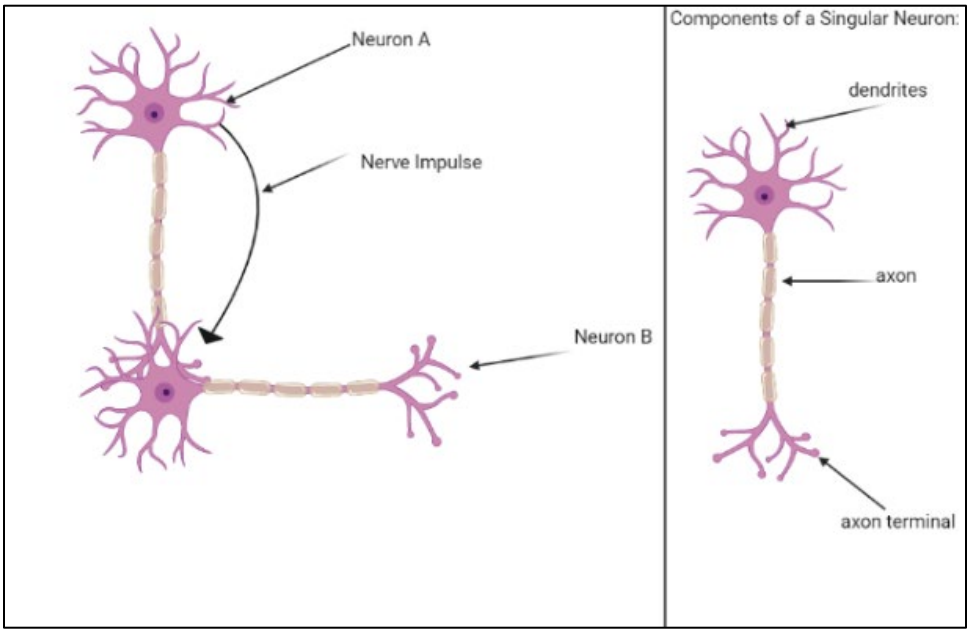

Figure 3. Pictured above is a diagram on the transfer of signals between two neurons: Neurons A and B. The nerve impulse, or electrical signal is transferred through the axon of Neuron A towards the axon terminal on Neuron B. This signal passes through the neuron in the form of neurotransmitters and is processed in order to produce a product, an example of which could be the formation and production of a protein. Created and copyrighted by Shreya Ambekar.

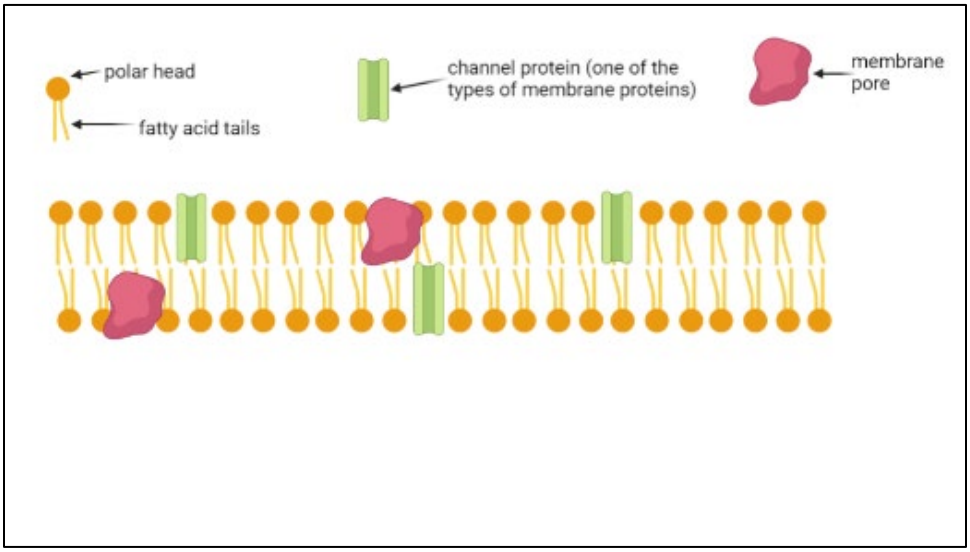

Figure 4. Pictured above is one of the structural components of myelin, a fatty substance present in cells, and the component portrayed is the phospholipid bilayer. Myelin is composed of a phospholipid bilayer membrane, with polar "heads" which are hydrophilic and fatty acid nonpolar "tails" which are hydrophobic. Due to the differences in polarity, the phospholipids arrange themselves in a manner so that the fatty acid tails face each other, and the heads of the phospholipids face the outer atmosphere. The purpose of this arrangement is to form a semi-permeable membrane around the neurons, and only allow a select-few substances to enter and exit the cell. There are also several membrane proteins in the phospholipid bilayer that forms myelin, and the purpose of these proteins is to aid in the transport of abnormally sized materials and materials whose properties do not allow them to enter the membrane span across the membrane such as channel and carrier proteins. Another purpose of these proteins is to serve as enzymes or receptor proteins to receive particular signals. The transport of materials across the membrane without the aid of the proteins is done without the use of Adenosine Triphosphate (ATP) and the proteins utilize ATP to transport materials which are unable to cross the semipermeable membrane on their own. Created and copyrighted by Shreya Ambekar.

Stem Cells in the Treatment of Glioblastoma Multiforme

Stem cells are effective in targeting and the treatment of various neurological pathologies, including one of the most common ones, Glioblastoma Multiforme. As mentioned earlier, stem cells must expand and cover the tumor in order to successfully reduce the size of the tumor. The primary reason found for why neural stem cells and Mesenchymal Stem Cells were able to expand was due to cell surface markers, organic compounds attached to the cell membrane, experiencing abnormally high rates of gene expression, causing these genes to produce an excessive number of substances, especially proteins. These newly produced substances were being transferred to the area of the tumor and eventually encompassed the tumor.

Moreover, stem cells traveled in the direction of neoplasms due to cytokines, substances secreted by other cells in the brain. Cytokines which were excreted during the treatment of Glioblastoma Multiforme with stem-cell therapy include hypoxia-inducible factor-1a, the vascular endothelial growth factor, as well as the hepatocyte growth factor and extracellular matrices, or a network interconnecting various cells and tissues. Due to this property of stem cells, they have sparked interest because they can work their way around the barrier present between the blood and the brain. Fortunately, neural stem cells do not produce toxins, nor do they disrupt the regular functions and processes of the brain. Essentially, the use of neural stem cells in the treatment of Glioblastoma Multiforme has become widespread due to the properties of these stem cells as well as the substances they are able to secrete.

Trials and Results from Experiments with Stem-Cell Based Treatment

As of right now, there have been several trials conducted in order to experiment with stem-cell based therapy and determine how effective this form of treatment is. In addition, studies have been performed on animals, the data derived from these clinical trials portrayed that stem cells (primarily neural stem cells), were effective in confronting neoplasms. Furthermore, it was also concluded that stem cells were also effective in combating tumors as well as significantly reducing their intensity. The stem cells used in these trials were artificially engineered in order to produce the most efficacious results. Another piece of information acquired from the experimental trials was that these engineered stem cells were able to induce apoptosis, or programmed cell death in the cells which formed the tumor. This process prevented the Glioblastoma Multiforme tumors from both further growth and spread.

In terms of the studies performed on human individuals, it was derived that administering exogenous neural stem cells into the dentate gyrus was responsible for the production of endogenous neural stem cells. After conducting a trial with a species of mice, data obtained from these trials proved that injecting neural stem cells underneath the epidermis and into the basal layer of the skin led to a longer period of survival for the mice. Injecting the stem cells underneath the skin also led to an increase in the production of the caspase-3 molecule, an effector and apoptotic cell. This variety of cell undergoes a series of molecular steps until its programmed death, meaning that neural stem cells could directly impact degenerative glioma cells which pose a hazard to the body. Furthermore, the artificially engineered neural stem cells were synthesized so that they would express the genes IL-4 as well as IL-12, which are both effective in reducing the intensity and size of the tumor. Therefore, the process of only using stem-cell based therapy to treat Glioblastoma Multiforme is not possible due to there still being discoveries and experiments conducted.

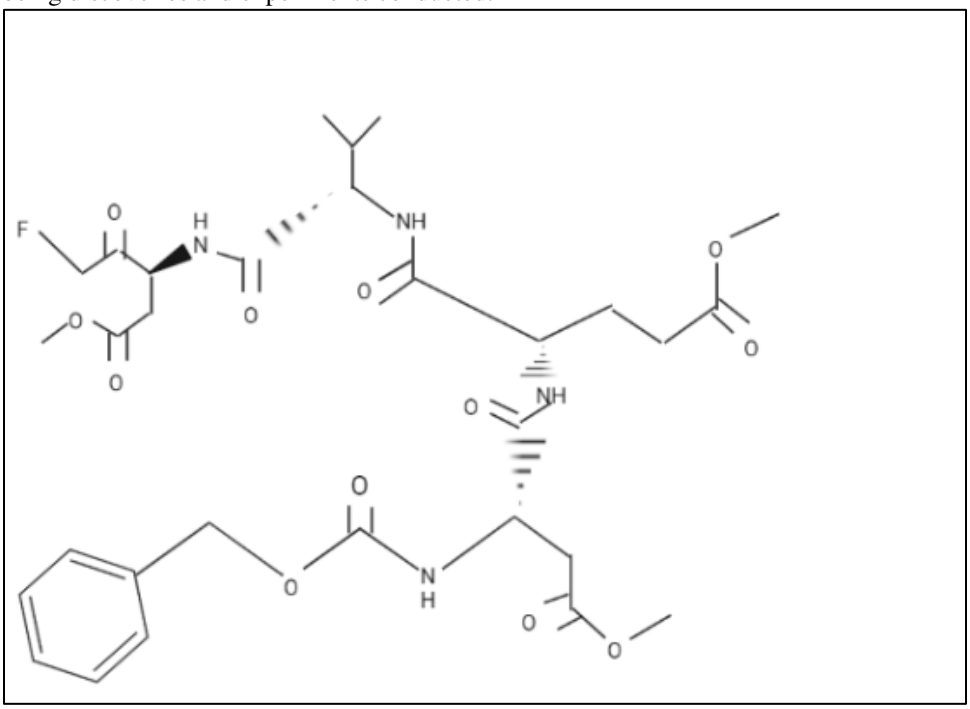

Figure 5. Pictured above is a structural diagram of the Caspase-3 molecule, also known as $C_{30}H_{41}FN_4O_{12}$ and is also an inhibitor protein, or an enzyme. The Caspase-3 molecule interacts with both the Caspase-8 and Caspase-9 molecules. Created and copyrighted by Shreya Ambekar.

Disadvantages of Stem-Cell Based Therapy

Although there are many positive and beneficial aspects to utilizing stem-cell based treatment for Glioblastoma Multiforme, there are still several disadvantages that appear with them. For instance, there have been multiple discrepancies and inconsistencies between conducted experimental trials as well as in the effects of the treatment. This variability has primarily been detected when experimenting with MSC's and not as often with NSC's. One possible reason for these inconsistencies could be the diversity of the MSC's experimented on, specifically the differences in the properties of the fetal (embryonic) and adult stem cells used, with adult stem cells being more effective during the treatment process since they are multipotent, compared to pluripotent fetal stem cells.

Additionally, another potential reason for the discrepancy could be because animals were primarily being experimented on in trials, rather than humans, since stem cell based therapy is mainly used to treat humans. Furthermore, the trials and collection of data for stem-cell therapy with neural stem cells is relatively scarce because the artificially engineer stem cells have yet to be designed to combat and prevent reoccurrence of the neoplasms which could possibly lead to malignant cells in patients who have already recovered from the tumor. Ultimately, since the exact underlying cause of Glioblastoma Multiforme is still unknown, finding a specific treatment that could prevent the tumor from occurring or further intensifying in individuals is a difficult dilemma to solve. As a result, there will still be disadvantages to the current and what appears to be the most effective treatment, stem-cell based therapy to replace degenerative, damaged cells.

Other Forms of Treatment

Currently, forms of treatment for Glioblastoma Multiforme other than stem-cell based therapy exist. One of these forms of treatment includes chemotherapy, which is utilized for tumors which are not highly invasive nor intense. Since there is a barrier between the blood and the brain known as the blood-brain barrier, drugs given for the treatment of cancerous cells known as chemotherapeutic drugs, prevent penetration in the blood-brain barrier. However, this preventative measure reduces the effectiveness of chemotherapy itself. Additionally, unlike stem-cell therapy, chemotherapy has been seen to produce a negative impact on functional neurological stem-cells that are naturally present in the brain. Another form of treatment would be to administer high doses of radiation so that strong radiation waves kill any cancerous cells present in the body. These treatments have been effective due to both of these forms of treatment increasing the rate of the production of chemokines by abnormal cells because of hypoxia, or HIF-1α being secreted. Moreover, hypoxia-induced chemoattractants are responsible for guiding neural stem cells towards the gliomas for treatment. However, a disadvantage of both radiation and chemotherapy is that they are both not effective in the elimination of cancerous cells which grow at a slow rate.

In addition, there has been another approach for utilizing neural stem cells to be programmed in an alternative way where they would express enzymes that were an important part of the activation of particular prodrugs. These prodrugs would be responsible for reaching the Glioblastoma Multiforme tumor(s) and only release the prodrugs when the stem cells are near the tumors. Examples of these prodrugs that would be utilized include flucytosine and ganciclovir along with cytosine deaminase, which prevent DNA polymerase, an enzyme which forms Deoxyribonucleic Acid (DNA) molecules due to gathering nucleotides, from functioning. Thus far, using neural stem cells as a form of treatment has been the most effective form of treatment for Glioblastoma Multiforme because of oncolytic adenoviruses in gliomas. A final possible form of treatment includes the usage of stem cells as transporters to transport viruses which can replicate in tumor and cancerous cells, and eventually kill these cells. Unfortunately, their ability to approach the tumors is not sufficient, which leads to the potential of stem cells being combined, which could eventually lead to the further formation of tumors. However, there

is no specific or set form of treatment that is nearly always an accurate cure for Glioblastoma Multiforme, with forms of treatment still being investigated.

Conclusion

Essentially, a possible form of treatment for Glioblastoma Multiforme which has recently gained attention due to its effectiveness being higher than other forms of treatment is the use of stem-cell therapy. Neural stem cells extracted from the individual suffering from the tumor would be utilized to regenerate degenerative neural stem cells due to the tumor as well as locate and break down the tumor(s) present in the body. This type of stem cells was found to be most effective due to its induction of the molecule hypoxia-inducible factor-1a, allowing these stem cells to find a way around the blood-brain barrier, a task that has proven difficult for other forms of treatment that were tested. Although there has been variation in the results from trials conducted to test the use of stem cells as a form of treatment, the experiments conducted experimented on multiple variations of stem cells, including both fetal and adult cells. The data derived from the experiment led to the conclusion that adult stem cells were most effective in treating the Glioblastoma agglomeration. Overall, the most effective form of treatment for Glioblastoma Multiforme is the use of adult neural stem cells to locate and eventually decrease the intensity of the tumor.

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