How can targeting hTERT in glioblastomas enhance treatment efficacy and reduce tumor recurrence?

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Abstract

This paper looks at the potential of targeting hTERT to treat glioblastoma. hTERT is a protein that helps elongate DNA ends. Glioblastoma is an aggressive and malignant brain cancer. The hTERT promoter mutation is common among glioblastoma and other cancers and causes the overproduction of hTERT, which gives the tumor traits like rapid proliferation, resistance to treatment, and an immunosuppressive environment. hTERT is targeted by treatments like immunotherapy, DNA vaccines, inhibitors, and CRISPR. All of these are promising options for treating cancers like glioblastoma, but they also have limits and challenges. Targeting hTERT could also and already has helped treat other cancers and related diseases.

Introduction

Glioblastoma is a type of brain tumor that is the most malignant, common and refractory (Davis). Glioblastoma represents 50.1% of the malignant brain tumors in the world. Furthermore, they are the most deadly and treatment resistant cancers (National Brain Tumor Society).

Life of a patient with this cancer includes constantly worsening headaches, vomiting, nausea, defective senses, and seizures (AANS).Symptoms may vary based on the patient and stage of cancer. Only 6.9% of people that get glioblastoma survive 5 years or longer (National Brain Tumor Society).

There are thousands of different types of brain cells (Dana Foundation). Neurons are brain cells that send signals across your brain, and are responsible for allowing you to think, move, and react. Another common brain cell type is glia. Glia, meaning glue in Latin, do more than just glue your neurons together, they are known to be actively participating in facilitating signal sending and maintaining brain health.

There currently is no cure for glioblastoma (Mayo Clinic). There is treatment which includes surgery, radiation, and chemotherapy (AANS). Surgery is limited in the brain. Neurosurgeons have to be careful, since the brain is delicate and important. Furthermore, the tumor spreads into brain tissue and tumors that enter brain tissue cannot be removed by surgery. Surgery can help get rid of the bulk of the tumor cells, but not the entire tumor. After surgery a radiation phase begins which can be in some cases radiosurgery, targeting the tumor and attacking it with radiation, or conventional radiation cycles which includes killing cells with radiation at a frequency that also gives time for normal cells to recover. This radiation can safely kill tumor cells in tissue. Then chemotherapy begins with temozolomide which helps decrease tumor replication.

Telomeres are generally linked with life expectancy. In cellular biology, they are known to be the ends of chromosomes. Telomerases are enzymes that help maintain telomere length, by lengthening chromosomes. Telomeres in healthier people maintain chromosome integrity and stability, and the telomeres decay rate drops in healthy people that have habits such as good nutrition, low stress, and exercise. A specific notable enzyme's name is hTERT. Telomeres shorten every cell replication, shortening of telomeres results in a decrease in chromosome integrity and stability. This allows for mutations, and mutations in DNA can cause cancers and tumors. Shorter telomeres, if shortened enough to have an advantage in cancer cells, due to the decreased stability, the lifespan of the cell also drops. Mutations in hTERT gene overproduce telomerases which lengthen telomeres and skip replication checkpoints, which results in

uncontrolled replication (Hackett and Greider). This hTERT mutation has lower survival rates than wild-type cancers and has an 80% occurrence rate in glioblastoma (Yan et al.).

Current treatment for glioblastoma includes slowing down or trying to mitigate the effects of glioblastoma (Hackett and Greider). Mutations resulting in overproducing hTERT, creates an immunosuppressive environment, by circumventing mitosis checkpoints and stabilizing chromosomes. Attempting to inhibit hTERT, can help get rid of the immunosuppressive environment which increases the efficiency of the current treatment process significantly (Yan et al.).

Section 1: strategies for targeting hTERT in glioblastomas

There are two types of telomere strands: single stranded and double-stranded (Di Nunno et al.). The shelterin complex acts to protect telomeres and as a cofactor for hTERT. The RNA molecule (hTR) which comes from hTERT and it guides hTERT towards DNA ends, and provides a template for hTERT. hTERT directly opposes the “end replication problem” which is the natural erosion of telomeres. The “end replication problem” is responsible for cellular senescence and eventually apoptosis (cell death). Not only does hTERT delay apoptosis, it also suppresses growth inhibitory factors, and DNA damage responses.

A promoter is a part of DNA, which attracts proteins to read the gene section of the DNA, produce an RNA, and eventually produce a different protein. hTERT promoter region mutations are dangerous since they help glioblastoma proliferate (Hackett and Greider). In one study, 80.2% of 276 IDH wild-type glioblastoma patients had a hTERT promoter mutation (Giunco et al.) In the most common type of glioblastoma, a mutation in hTERT promoter results in over production of hTERT, which increases the expression of its functions in cancers (Hackett and Greider). Suppressing growth inhibiting factors, and delaying apoptosis, results in uncontrollable growth, which gives glioblastoma and other malignant cancers their aggressiveness.

hTERT needs to be transcribed then translated to form as a protein (Krause). Transcription is when RNA is produced from your DNA, and translation is when the RNA is translated into amino acids to form a protein such as hTERT. In transcription there are factors that are known as transcription factors which determine if RNA is produced and how much is produced. Inhibitors are transcription factors that oppose RNA production, so the protein won’t be produced. If hTERT or hTERT production is inhibited, glioblastoma replication will be more controlled due to the decrease in hTERT (Hackett and Greider). Two such telomerase inhibitors for hTERT, imetelstat and KML-001, are active agents in clinical trials and do have medical use (Tao et al.). Imetelstat is used to treat children with refractory, recurrent, central nervous system malignancies or solid tumors. KML-001 and cisplatin, a chemotherapy drug, did have an intended effect in human trials (Edelman et al.). Unfortunately, KML-001 and cisplatin also had side effects including a suppression of blood marrow (Myelosuppression) and delay in recharge of heart ventricles (QTc prolongation). GRN163, which inhibits a mechanism of hTERT hTR RNA, has led to incredible success suppressing growth via injection *in vivo* (D’Alessandris et al.). The reviewed treatments treat various cancers with the same mutation. The reviewed treatments are not targeting glioblastoma with the hTERT promoter region mutation. Success in treatment development on telomere targeting telomerase inhibitors for cancers could look like, reducing tumor growth, improving survival rates, and passing clinical trials.

Clustered regularly interspaced short palindromic repeats (CRISPR) is a method to treat different diseases and medical problems. CRISPR is a very precise tool that can modify and cut genes (Chehelgerdi et al.). CRISPR finds a specific target portion of DNA using a programmed RNA guide, which locates based on palindromes in DNA (Max-Planck-Gesellschaft). The DNA segment gets cut using an enzyme such as cas9 (Chehelgerdi et al.).CRISPR's importance in cancer treatments lies in its abilities to find and edit mutations that allow the cancer to grow uncontrollably. Such as a mutation of the promoter for hTERT, which is also present in most glioblastoma and mutations can be targeted by treatments with higher precision when using CRISPR (Zhao et al.).In fact, hTERT has been successfully targeted using a CRISPR technique with an sgRNA guided CjABE which is a Cas9 from the bacterium *Campylobacter jejuni* fused with an adenine base editor guided by sgRNA. This technique has been efficient in correcting the promoter hTERT mutation, which is a possible treatment course for glioblastoma. The only FDA approved CRISPR treatment is for sickle cell disease and its treatment costs $2,200,000 USD which isn't affordable for most people (Hassanein). This price comes from the amount of money put into developing and testing and creating the treatment.

Immunotherapy is an emerging technology which modifies the immune system to help cure disease or cancers. Immunotherapy works by enhancing the immune system's ability to recognize and/or attack diseases. Antigens stimulate your immune system. For cancers specifically, tumor associated antigens (TAAs) are targeted in cancer immunotherapy (Mizukoshi and Kaneko). When a cancer cell dies TAAs break into peptides that are recognizable by major histocompatibility complexes (MHC) which activate T cells which find and kill mutated cells. Making broken down hTERT peptides immunogenic since hTERT is a TAA, and due to this property, hTERT can be a target for immunotherapy. Inoculation of peptides to increase the activity of MHC class II is a form of immunotherapy called peptide vaccines (Wang et al.). Improving the immune system, to recognize and attack high concentrations of hTERT can help fight glioblastoma internally within the patient.

Another form of immunotherapy known as DNA vaccines work by overexpressing TAA proteins (Wang et al.). Introducing TAAs *in vitro* to dendritic cells and reintroducing the dendritic cells is also another form of immunotherapy for cancer. Acceptance and commitment therapy (ACT) therapy modifies and enhances a patient's immune cells (TILs) before reintroducing them to the patient's body. Many chemicals are produced and tested attempting to successfully execute one of these strategies, and many are proceeding in clinical trials and are succeeding. These chemicals can only target specific locations like (BPH) for pancreatic cancer. Also in the process many of these procedures and chemicals result in killing immune cells along with cancer cells. A chemical that succeeded in gene therapy is RZ-001 along with atezolizumab, but only targets hepatocellular carcinoma (Sava). Research shows the possibility of using multiple immunotherapy strategies at once will be most effective and most likely what future studies are moving towards (Mizukoshi and Kaneko). With advancing technology and newer studies coming out quickly on telomerase, it is quite possible to find a remedy for glioblastoma via immunotherapy.

Section 2: benefits and potential impact on treatment outcomes by targeting hTERT

Targeting hTERT promoter mutations provides a means to predict and measure cancers, due to its high concentrations in tumors (Yang et al.). 85%-90% of tumors express high concentration of hTERT, and studying hTERT has strong potential to teach a lot about tumors. Targeting hTERT has created viable treatments for breast cancer. Treatment strategies targeting

hTERT lead to successful trials for glioblastoma in mice *in vivo* and will most likely be in future treatment strategies (Aquilanti et al.). Treatment success can be based on a patient's tumor stage, since it takes 30–80 days *in vitro* to kill cancer cells and this strategy would not be applicable in advanced cases. Multiple different strategies and chemicals to combat cancers and tumors emerged from targeting hTERT, and many are promising and in trials. Even so, immunotherapy, CRISPR, and DNA vaccines are very promising. These treatments seem to be only a possible addition to current treatment plans and not a complete remedy. Targeting hTERT has led to an improvement in diagnostics for tumors. Unfortunately, in trials, hTERT inhibitors aren't performing well and have many limitations (D'Alessandris et al.).
A DNA vaccine named phTERT (INO-1400/1401/9012/5401) has been in phase 1 and 2 of clinical trials targeting glioblastoma but failed to show clinical results (Wang et al.). Many clinical trials for immunotherapy treatments such as dendritic cells (DCs), DNA vaccines, ACT, and TAP cells have taken place since 2004 which passed clinical trials 1 or 2 for the following tumors: metastatic prostate cancer, acute myeloid leukemia, metastatic melanoma, pancreatic cancer, breast cancer, melanoma and prostate cancer. Many pharmacological molecules succeeded in inhibiting hTERT transcription in vitro and vivo like butylidenephthalide, MZ-5-156 (GH-RH antagonist), CRISPRi approach, and suramin (Pennisi et al.). In vitro and vivo costunolide and eribulin have succeeded in indirectly inhibiting hTERT as imetelstat, and GRN163 have succeeded in inhibiting hTR, a component of hTERT. CRISPR treatment targeting hTERT is also a possible option as sgRNA guided CjABE successfully converted a mutated hTERT promoter to treat HCC (Zhao et al.). Research on treatment options targeting hTERT can lead to an increase in treatment efficacy for various cancers such as glioblastoma for which no cure exists.

Glioblastoma recurrence is a clinically important problem due to its aggressiveness and the sensitivity of its location. The recurrence rate is 90% in 2 years which is high compared to other cancers (myTomorrowsTeam). Recurrent cancer is when cancer returns after treatment (National Cancer Institute).This happens when a small, undetectable amount of cancer cells don't die, and replicate until there are enough to notice the cancer cells again. Reducing tumor recurrence requires killing as many cancer cells as possible. Recurrence can occur anywhere, but is most likely to reappear near or at the site of the original tumor. Glioblastoma can appear anywhere in the brain or spinal cord, but mostly reappears where the tumor originally was located (Moffitt). Surgery may not be repeated due to patient health or loss of blood in the brain. Chemotherapy can require a change in dose or different set of chemicals to treat recurrence. Radiation isn't typically done multiple times due to increasing chance of radiation-induced cancer and is generally avoided the second time around. Given the limitations of conventional treatments, some of the alternatives mentioned in this paper may prove to be particularly useful. For example, effective inhibition of CRISPR can eliminate unlimited proliferation and immunosuppressive environments due to hTERT providing an immunosuppressive environment by circumventing mitosis checkpoints and decreasing DNA damage response (Yan et al.; Hackett and Greider). Additionally, efficient immunotherapy targeting hTERT can boost the efficacy of the immune system, due to hTERT being a TAA which activates the immune system (Wang et al.). These properties of hTERT, allow it to be a possible method to reduce tumor recurrence, since these methods fight tumors at the molecular level. These properties also provide a possible target to treat glioblastoma, and decrease recurrence rates.

Conclusions & Future Directions

Overall, glioblastoma is a tumor of cancerous glial cells which are located in the brain (Dana Foundation). hTERT promoter mutation is a common mutation among glioblastoma and other cancers. Likewise, hTERT promoter mutations result in an increase of hTERT production which is beneficial for the tumor since overproduction of hTERT can lead to delayed apoptosis, allowing for damaged cells to continue to grow and multiply (Di Nunno et al.). Therefore, hTERT promoter mutations create an ideal environment for cancer cells to proliferate. Directly targeting hTERT is a promising method to treat cancers like glioblastoma. While there is no cure for glioblastomas at the moment, emerging treatments are showing immense promise to treat this disease in the future (Mayo Clinic). Possible treatments targeting glioblastoma include immunotherapy, inhibition, and gene editing. Common limitations include cost, sensitivity of the brain, a blood-brain barrier that prevents certain chemicals from entering the brain, and the refractory nature of glioblastoma.

Targeting hTERT to treat cancer can create an entirely new branch of cancer treatment. Immunotherapy, CRISPR, DNA vaccines, and direct inhibition of hTERT, its mechanisms, or even transcription factors are all possible treatments that have limitations of which some can be overcome and some cannot. This provides hope for treating dangerous refractory cancer which currently have no remedy like glioblastoma.

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