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ARTICLE

Brain Cancer Treatment with Gene Editing

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ABSTRACT

The first method is used gene editing to knock out the PD-L1 receptor located on the T cell surface so that PD-1 on the cancer cell surface cannot combine with the PD-L1, in that case, T cell can identify the abnormal cell and kill it. At the beginning, researchers use protein-guided editing technology, but it is not easy to control and not specific enough, so they choose to use CRISPR-Cas9 to edit the target gene. Comparing with the traditional protein-guided nucleases, CRISPR-Cas9 system is more easy-handle, highly specific, and it is an more efficient tool for engineering eukaryotic genomes; because CRISPR-Cas9 system aims to edit the targeting genes by tiny RNAs guiding the Cas9 nuclease to the target site by base pairing.The second treatment is mainly used "fighting cancer with cancer".Because living tumor cells have the ability to home and target tumors, thus, if those living tumor cells can be engineered to secrete therapeutic agents, the tumor cells can be effectively cured. Shah's team picked the agent interferon-β (IFN-β). However, this idea of treatment is limited by the premature cell death due to autocrine toxicity.The researchers solved this problem by first using CRISPR Cas9 to knock out the IFN-β–specific receptor (IFNAR1) in inherently IFN-β–sensitive syngeneic tumor cells, and subsequently engineered them to constitutively produce IFN-β for tumor cell targeting and simultaneous immunomodulation. These therapeutic cells are further designed to coexpress granulocyte-macrophage colony-stimulating factor (GM-CSF) that facilitates the differentiation, proliferation, and recruitment of dendritic cells (DCs). The last approach can stop cancer cell repairing their DNA when it gets damaged.

1. Introduction

Brain tumors are short for intracranial tumors, which often cause neurological dysfunction and can be life-threatening in severe cases. Brain tumors are classified into benign and malignant tumors just like other parts of the body. Meningiomas and pituitary tumors are benign tumors of the brain with high incidence rates. Meningiomas and pituitary tumors are benign brain tumors with a high incidence rate. The term "brain cancer" usually refers to malignant brain tumors, and glioma is the most common type of brain cancer. Gliomas are the most common type of brain cancer. Most brain malignant tumors recur and have a high rate of disability

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and mortality, and are one of the key challenges for neurosurgery to overcome. Like most cancers, the cause of gliomas is still unclear, and the prevailing view is that genetic variations in individual cells in the body are the source factors leading to the development of gliomas. Factors such as the environment, food, emotions, and infections may all lead to cell mutations(Wang et al., 2023).

Genome Editing, also known as genome engineering, is a type of genetic engineering that involves the insertion, deletion, modification or replacement of DNA in the genome of a living organism. The difference between this and earlier genetic engineering techniques is that earlier genetic engineering techniques randomly inserted genetic material into the host's genes and genome, whereas gene editing inserts gene fragments at specific locations. Several approaches to genome editing have been developed. A well-known one is called CRISPR-Cas9, which is short for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9. CRISPR-Cas9 technology, in essence, is more like a pair of scissors that cuts the DNA and then uses the cell's own repair to control the failure of a particular gene. This technology, after continuous testing to ensure the accuracy of gene editing, can be used to treat diseases caused by genetic mutations, as the trait cannot be expressed when the disease-causing gene is disabled, thus achieving therapeutic effects.

2. Treatment 1

In the immunotherapy field, T cells have one negative regulator is PD-L1, which can combine with dentritic cells(DCs) or tumor cells and recognize the PD-1 receptors on these cells, then, PD-L1 will act on the PD-1 to kill the DCs or tumor cells. They have proved a new method breaking the checkpoint of T cells is useful and feasible(Su et al., 2019). This result gives a new way for targeting checkpoint inhibitors, improving the curative effect of T cell based adoptive therapies as well. On the other hand, scientists have already discovered that the immunization caused by tumor vaccines and cancer vaccines does not always bring the clinical advantage. It must be noted is that a large percentage of tissues are relayed on PD-L1 expression, since PD-L1 influences the limitation of T cell reaction, thus, using medicines to break the tolerance of PD-L1 and PD-1 blocking antibodies still has risks. In that case, recently, RNA-guided endonucleases has been invented, called CRISPR(clustered regularly interspaced short palindromic repeats) and CRISPR-associated (Cas) 9. Comparing with the traditional protein-guided nucleases, CRISPR-Cas9 system is more easy-handle, highly specific, and it is an more efficient tool for engineering eukaryotic genomes; because CRISPR-Cas9 system aims to edit the targeting genes by tiny RNAs guiding the Cas9 nuclease to the target site by base pairing. In their previous work, they have used mice and rats to achieve the efficient gene targeting by so-injection of single cell embryos with Cas9 mRNA and sgRNA. After that, they succeeded finishing similar experiments in Cynomolgus monkeys.

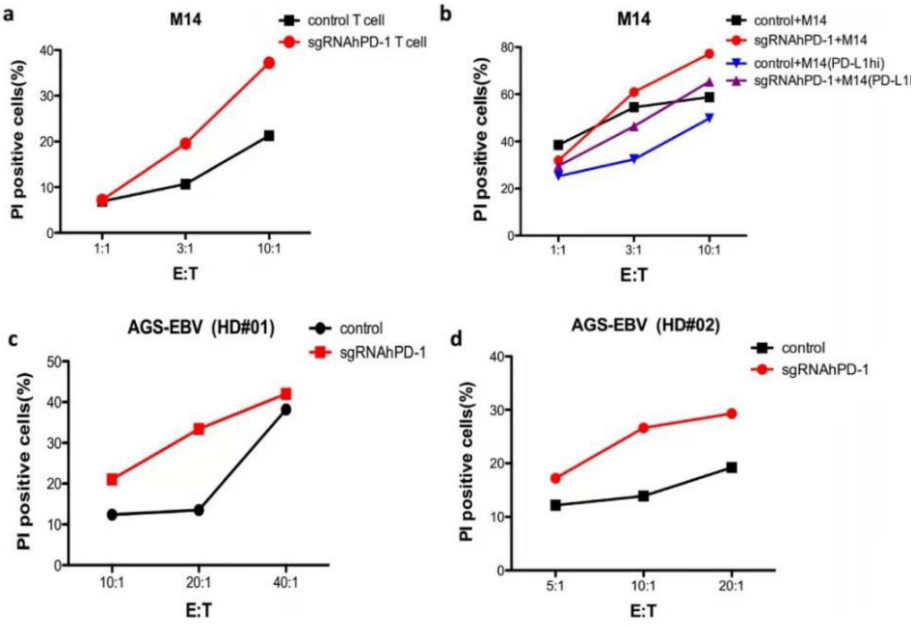

Figure 1. Enhanced cytotoxicity of the hPD-1 KO primary T-cells(Su et al., 2019)

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Patient or healthy volunteers contributes to the T-cell reprogrammed by sgRNA:Cas9 or control were cultured in vitro with IL-2 to co-culture with PD-L1 expressing tumor in different effectors to target cell ratio(E:T). The cytotoxic reactivity of the effector T-cells was measured using CFSE/PI cytotoxicity assay. Fig.1 illustrates the relative percentage of double-positive cells out of CFSE-labeled tumor cells. In graph (a), the hPD-1 KO T cells oor control T cells from melanoma patient were co-cultured with CFSE labeled M14 cells at E:T of 1:1,3:1, 10:1 respectively. After 6 hours, PI was added and the cells were analyzed by flow cytometry. In graph(b), the hPD-1 KO T cell or control T cells from a melanoma patient were co-cultured with CFSE labeled PD-L1-lo-M14 or PD-L1-hi-M14 cells at E:T of 1:1, 3:1, 10:1, respectively. After 6 hours, PI was added and the cells were analyzed by flow cytometry. In graph (c) and (d), the hPD-1 KO T cells or control T cells from healthy donor #01 and healthy donor #02 were co-cultured with CFSE labeled AGS-EBV cells at ratio (E:T) of 5:1, 10:1, 20:1, or 10:1, 20:1, 40:1, respectively. After 16 hours, PI was added and the cells were analyzed by flow cytometry. The above experiments have been repeated 3 times with similar results(Su et al., 2019).

The key problem of immunotherapy is the effective activation of tumor reactive T cells and the inhibition of checkpoint inhibitor. The latest result of the checkpoint blockade targeting the PD-1 or PD-L1 pathway has shown significant antitumor responses in patients with advanced melanoma, lung cancer, and other cancers with a durable clinical response. T cells activated in the absence of PD-L1 or PD-1 co-stimulation are functionally activated, exhibiting increased proliferation by stimulating dc or tumors, producing higher levels of Th-1 cytokines, particularly IFN-γ, IL-2, and TNF-α, and enhancing lytic activity. Previous studies have demonstrated that blocking PD-1 or PDL1 with monoclonal antibodies can improve IFN-γ production and cytotoxicity *in vitro* and *in vivo*. Here, they also demonstrated that these sgRNA hPD-1:Cas9-modified primary T cells from healthy donors or advanced cancer patients exhibit enhanced IFN-γ production by stimulation of relevant peptide antigens, and they found that disruption of PD-1 improved tumor cell lysis, possibly due to PD-1 or PDL1 interaction-mediated reversal of immune resistance. IFN-γ is one of the Th1 cytokines, which mediates cellular immune responses, activates cytotoxic T cells, and indirectly regulates tumor lysis through multiple mechanisms. Therefore, they believe that IFN-γ indirectly activates cytotoxicity in their case. Furthermore, in their system, gene editing using Cas9:sgRNA-mediated T cells from patients and healthy donors elucidated cytotoxic improvements in tumor cell lines on two PD-L1-positive target cell lines, and further confirmed this by inducing PD-L1 expression on target cells. In order to obtain good results using PD-1 or PD-L1 inhibition strategies, the expression of its receptor PD-L1 should be considered. They have used a lot of approaches, such as ethics statement, plasmid expression vectors, T cell activation and electroporation, *in vitro* generation of autologous DC, *in vitro* expansion of PD-1 KOT cells, and flow cytometry. If this method is used in the brain cancer treatment, the PD-L1 on the surface of the T cell are broken *in vitro*, and then inject the modified T cell into the brain, then the modified T cell will attack the cancer cells in the brain, since there are no PD-L1 combined with PD-1, so the cancer cells cannot hide or escape from the assault from T cell.

3. Treatment 2

Researchers found a way to eliminate brain tumor cells efficiently via CRISPR Cas9. The main idea is to repurpose cancer cells to develop a therapeutic that kills tumor cells and stimulates the immune system to both destroy primary tumors and prevent cancer. They transformed living tumor cells into potent agent that drives both tumor killing ability and antitumor immunity.

Once researchers tried using inactivated therapeutic tumor cells (ThTCs) in order to trigger robust immune cell trafficking to the tumor site, resulting in the induction of an antitumor immune response in different cancer types. Yet this approach showed no clinical benefit, due to the lack of direct cytotoxic effect on tumor cells and the inability to trigger a strong antitumor immune response.

In contrast, living tumor cells have the ability to home and target tumors. Thus, if those living tumor cells can be engineered to secrete therapeutic agents, the tumor cells can be effectively cured. They picked the agent interferon-β (IFN-β), owing to its direct effects, such as inhibition of tumor cell proliferation and angiogenesis, and indirect effects, such as activation of antitumor immune responses. However, this idea of treatment is limited by the premature cell death due to autocrine toxicity.

The researchers solved this problem by first using CRISPR Cas9 to knock out the IFN-β–specific receptor (IFNAR1) in inherently IFN-β–sensitive syngeneic tumor cells to avoid autocrine toxicity, and subsequently engineered them to constitutively produce IFN-β for tumor cell targeting and simultaneous immunomodulation. These therapeutic cells are further designed to coexpress granulocyte-macrophage colony-stimulating factor (GM-CSF) that facilitates the differentiation, proliferation, and

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recruitment of dendritic cells (DCs). GM-CSF expression promotes DCs' capacity for antigen cross-presentation, costimulatory molecule expression, and proinflammatory cytokine production, thereby priming the immune system for long-term antitumor responses.

To eliminate the possibility of unwanted secondary tumor initiation, we implemented a dual safety kill-switch comprising herpes simplex virus–1 thymidine kinase (HSV-TK) and rapamycin-activated caspase 9 (RapaCasp9) in these ThTCs(Chen et al., 2023). The switch can be activated if needed to eradicate the ThTCs, making this dual-action cell therapy safe, applicable, and efficacious. These ThTCs were tested in mice with advanced glioblastoma; different mice strains were used, including one that contained bone marrow, liver, and thymus cells derived from humans, mimicking the human immune microenvironment. It was found that the therapeutic tumor cells could eliminate the tumors efficiently, significantly increasing survival rates and providing long-term immunity against recurrent and metastatic cancer(Chen et al., 2023).

Through contrast groups in experiment, researchers found that stimulating type I IFN signaling activities within the tumor microenvironment is likely to improve therapeutic efficacy for patients with cancer.

Also, it has been confirmed that IFN-β is the ideal therapeutic agent since IFNAR1/2 are expressed at the mRNA level with a relatively low range of variations across different types of cancer samples. Similarly, IFNAR1/2 was expressed universally across different IFNreg clusters. Being one of the most aggressive and immunosuppressive tumor types, primary and recurrent glioblastoma (GBM) in TCGA were specifically verified to have a comparable expression of IFNAR1/2. Hence, it is proved that making IFN- β the agent is widely applicable for tumor targeting(Chen et al., 2023).

4. Treatment 3

In an effort to get more people out of their predicament, there are now a variety of methods for treatment. This treatment stop cancer cells repairing their DNA when it gets damaged. They do that by blocking the PARP (poly adenosine diphosphate- ribose polymerase) protein. The class of PARP inhibitors is the most established of the DNA damage response modifiers. They are understood to prevent the DNA damage repair through several mechanisms. PARP also have essential roles in homologous recombination, non-homologous end joining and alternative end joining. In order to maintain normal physiological functions, cells must have multiple DNA damage detection and repair mechanisms to enable timely and accurate repair of damaged DNA.When single-strand DNA is damage, it can repair by mismatch repair, nucleotide excision repair or base excision repair. Whereas the double-strand DNA can only be repaired by homologous recombination (HR) or non-homologous end joining (NHEJ) . PARP inhibitors sometimes interferes with the base excision repair pathway.

The class of PARP inhibitor is the most established of the DNA damage response modifiers and canonically interferes with the base excision repair pathway. Through figure 2, there are about three stages of PARP inhibitors action involved in the process called RARylation.

When the single-strand DNA breaks, it drives double-strand DNA breaks. In the case of double-strand breaks, it is rare, but the situation is much more serious, and if it is not repaired in time, the cell's DNA becomes unstable and the cell eventually dies. So there are two main ways to repair double-stranded DNA breaks. One is non-homologous end-joining (NHEJ) repair, which is more like an emergency fire-fighting captain, whether the repair is correct or not, to connect the broken DNA. The other is the homologous recombination (HR) repair pathway, which involves a large number of proteins such as BRCA, ATM, RAD51, etc.. , of which the most well-known is the BRCA protein. BRCA1 and BRCA2, they are both genes that produce tumor suppressor proteins, which help the body repair damaged DNA, thus ensuring the stability of the cellular genetic material. When these genes are mutated, tumor suppressor proteins are not formed properly, which leads to DNA damage repair method, homologous recombination, being affected as well. BRCAI/2 is a component of the HR pathway. For BRCA-associated malignancies, patients carry a germline BRCAI/2 mutation at one allele in all cells, followed by the complete loss of the second allele in cancer cells, which is a mandatory step in carcinogenesis. HRD is due to the deletion of the BRCAI/2 allele in the cancer cells, and therefore when used in conjunction with a PARP inhibitor, the synthetic lethality would be tumor-specific and will not affect normal cells(Sim et al., 2022).

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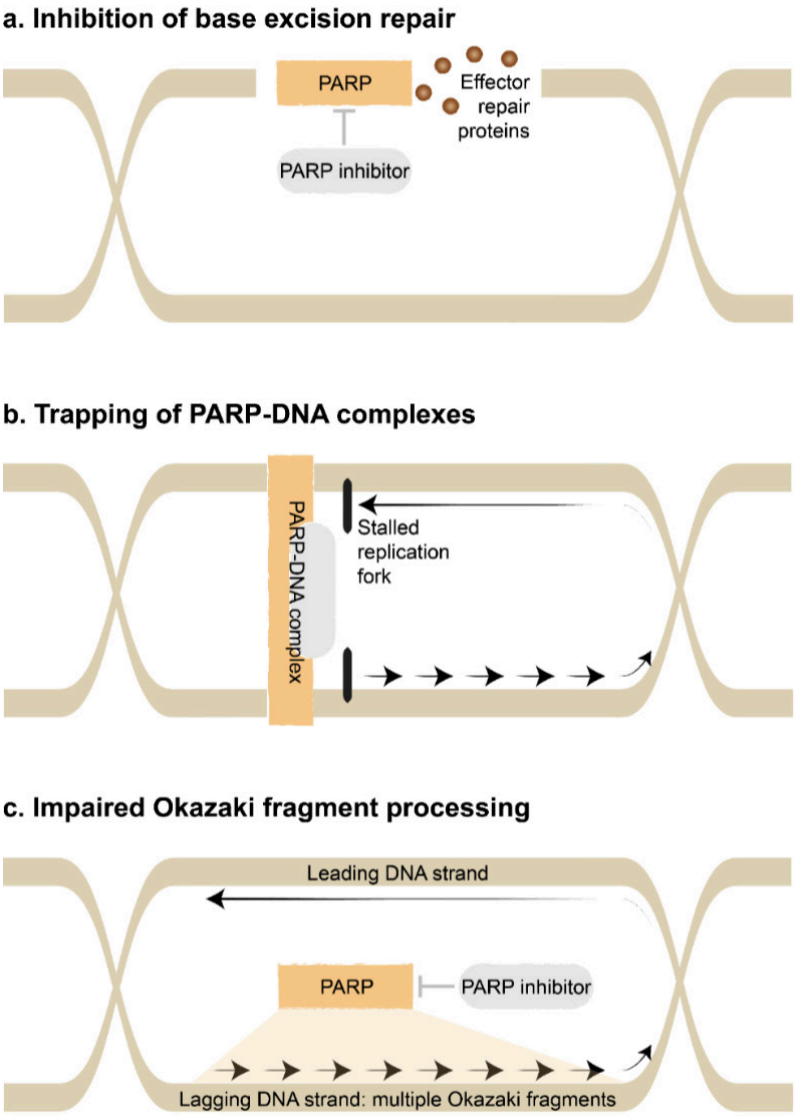

Figure 2. In (a) As soon as PARP discovers a gap in the cytotoxic DNA where a single strand break exists, it binds to it, and this binding activates the catalytic activity of PARP inhibitors. Later in (b), as the PARP inhibitors-PARP-DNA complex accumulate, it cause the replication fork to stall and collapse. (c), PARP acts as a chaperone for the multiple Okazaki fragments in the lagging strand DNA strand during replication, and this is also blocked by PARP inhibitors. Current research suggests that DNA damage repair-dependent PARPs mainly include PARP-1 and PARP-2, both of which accurately recognize DNA wounds and bind intimately to DNA. In the process of repairing DNA damage, PARP-1 plays more than 90% of the function. It works by binding to DNA damage sites (mostly single-stranded DNA breaks) and catalyzes the synthesis of poly ADP ribose chains on protein substrates and recruits other DNA repair proteins to the damage site to repair the DNA damage. PARP inhibitors result in the inability of PARP proteins to shed from DNA damage sites by binding to the PARP1 or PARP2 catalytic site(Sim et al., 2022).

5. Conclusion

In conclusion, the first is to use CRISPR Cas9 technology to edit T cells, removing the PD-L1 receptor on the surface of T cells *in vitro*, so that PD-L1 cannot bind to PD-1 on the surface of cancer cells, thereby attacking cancer cells. The second method is to use CRISPR Cas9 technology to edit the IFN-β receptor of cancer cells, allowing the edited cancer cells to attack other cancer cells and tumor cells to achieve the purpose of treatment. The third approach is to use radiation therapy to destroy the DNA of cancer cells, and then implant PARP inhibitors, so that DNA repair is blocked, so that DNA inactivation can be used to remove cancer cells. From our perspective, the first method is successfully used mice and rats, even in Cynomolgus monkeys to achieve

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the efficient gene targeting by so-injection of single cell embryos with Cas9 mRNA and sgRNA. In addition, it is an more comprehensive, because it is not easy to ignore any cancer cells, but this system attack other normal cells by accident. The second one is lack of clinical experiment, although it is relatively perfect in theory, since it will not attack normal cells or omit cancer cells. And the last one, is limited to the time period, which is only suitable in the gene repairing, but it is the only one, in these three methods, widely used in the clinical treatment, especially in the ovarian cancer treatment.Although PARP inhibitors have limitations in the treatment of brain cancer, PARP inhibitors have benefited patients with breast, pancreatic, and ovarian cancers that carry BRCA gene mutations and are widely used in medicine.However, by targeting the DNA damage repair pathways, PARP inhibitors cause an accumulation of DNA damage and genomic instability. Additionally, the first two approaches are used in CRISPR Cas9, this gene editing technology, comparing with the traditional protein-guided nucleases, CRISPR-Cas9 system is more easy-handle, highly specific, and it is an more efficient tool for engineering eukaryotic genomes; because CRISPR-Cas9 system aims to edit the targeting genes by tiny RNAs guiding the Cas9 nuclease to the target site by base pairing.

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