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Suppress the proliferation process of glioblastoma by breaking off the tumor - microenvironment interaction by CRIPSR -mediated technology

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Abstract:

Glioblastoma is a notorious malignant tumor and traits in cancer provide strong drug resistance. Current Traditional clinical treatments include chemotherapy and surgery, had been proved that showed insufficient effect towards glioblastoma, leading to the requirement of new therapy ------ CRISPR mediated technology therapy application. CRISPR is prior options for gene therapy drug design and using gene-editing principle from the molecular level. Scientists research evaluated the CRIPSR gene therapy had ability in editing related functions of glioblastomas and inducing an immune response by blocking the metabolic activity of cancer cells to achieve anti-cancer effects. We discussed the recent identified potential CRISPR gene therapy targeting sites in glioblastoma gene therapy, including clinical traits and in vivo experiments verified the value in future clinical treatment applications. We also discussed the relationship between the target sites of the glioblastoma genome and the traits they related with. Targeting the angiogenesis regulator factors leads to suppression of glioblastoma proliferation and inhibit the tumor growth. Mediated the tumor micro environment by enhancing antigen present process and reduce glioblastoma interaction with immune cells, could up regulated the anti-tumor immune response. The is also a possibility of combining immune therapy and CRIPSR gene editing therapy together by Car-T therapy. We also discuss the potential delivery method for CRISPR gene- editing therapy , compare the AAV virus method and nanocapsule for assist the edited gene reach the target sties and avoid off-target rate. The CRISPR therapy could be the mainstay in glioblastoma treatment by directly target the glioblastoma metabolism to inhibit tumor proliferation, and could increase survival rate in child glioblastoma treatment as the heterogeneity of glioblastoma is high.

Keywords: CRIPSR -mediated technology; glioblastoma; cancer; gene therapy;

Introduction:

Back in the day the existence of glioblastoma was a source of despair , the disease was famous for its high recurrence(1) , extremely aggressive(2) ,tumor trait and poor length of survival (3) . As a malignant brain tumor , the microenvironment around the brain tumor contributes a lot in dismal prognosis (4), and the glioblastoma stem cell enhance glioblastoma drug resistance towards chemotherapy(5) . The traditional treatment have proven inefficient , include chemotherapy, Radiation, even the combination drug therapies, as the plasticity and heterogeneous propertie from the glioblastoma stem cell lineage (6) leads to drug resistance and immune escape mechanism. In this situation, application of new immune therapy into the clinical treatment being important and in haste, and the gene-editing technology , or gene therapy as an emerging and rising technology , may provide unexpected effect.

1. Reasons for choosing gene therapy as new therapy:

More researches proven that gene therapy is a promising approach and strategy for glioblastoma (7) . In this article, we will discuss the up to date research of the driven factors of glioblastoma and the newest gene therapy study process , include new target sites , engineered cell , delivery vectors . We believe that there is a relationship between the new discover of pathogenic mechanism that leads to glioblastoma and apply gene therapy of that for mediate the immune response towards cancer cell (8) .Although under present clinical therapy, glioblastoma still maintain an incurable situation which the survival length is less than 2 years , but (2) increasing reports of success experiments of gene therapy experiments in vitro and in vivo indicated that the combination of gene therapy and immunotherapy may be the future (9). There are different aspects for targeting : directly eliminate the mutated cells' survival and replication ability by overturn the inter cellular molecular metabolisms of tumor cells and inhibit the elements that leads to glioblastoma; suppress glioblastoma proliferation like cut out oncogene or disturb the protein synthesis process that involve in cancer pathogenic mechanism by using edited gene sequence target special RNA 's translation activity. Another option is enhance patient immune system response towards the tumors by relieve glioblastoma 's ability of hijack immune cells in brain microenvironment (10) .There is a factor that made immune therapy complex and more hard to harness positive results from clinical trials, is that tumor microenvironment could down - regulate immune cells' anti-tumor ability , it was a hypothesis , but increasing researches verify the hypothesis to a established fact , same in glioblastoma (11), the cancer cells inhibit antigen present process , reduce regulation and even induce immune cell like NK cell and T cell apoptosis. At present, the size and number of genetic experiments involving the immune escape ability to suppress glioblastoma are not large , but these experiments have demonstrated that when the interference of cancer cells on immune cells is blocked, for example, the gene expression of cancer cell paracrine proteins used to evade immune checkpoint detection is suppressed , the immune system can automatically resume an effective anti-tumor response (12)the majority of patients do not respond to this form of immunotherapy. New approaches are required to overcome resistance to immunotherapies.","container-title":"Cancer Gene Therapy","DOI":"10.1038/s41417-021-00369-7","ISSN":"1476-5500","issue":"6","journalAbbreviation":"Cancer Gene Ther","language":"en","license":"2021 The Author(s discover the available targeting sites of molecular mediator from induce mutation factors become necessary for the gene therapy .

1.1 CRISPR background

Among the varies technology options, CRISPR, seems like a ray of hope , also is a ideal tool, to had the ability involve in glioblastoma gene therapy drug design of tumor cell itself and immunotherapy by modified T cell for gaining active immune response (13–17)CRISPR has become as much a verb as it is an acronym, transforming biomedical research and providing entirely new approaches for dissecting all facets of cell biology. In cancer research, CRISPR and related tools have offered a window into previously intractable problems in our understanding of cancer genetics, the noncoding genome and tumour heterogeneity, and provided new insights into therapeutic vulnerabilities. Here, we review the progress made in the development of CRISPR systems as a tool to study cancer, and the emerging adaptation of these technologies to improve diagnosis and treatment.","container-title":"Nature Reviews Cancer","DOI":"10.1038/s41568-022-00441-w","ISSN":"1474-1768","issue":"5","journalAbbreviation":"Nat Rev Cancer","language":"en","license":"2022 Springer Nature Limited","note":"publisher: Nature Publishing Group","page":"259-279","source":"www.nature.com","title":"CRISPR in cancer biology and therapy-","volume":"22","author":[{"family":"Katti","given":"Alyna"},{"family":"Diaz","given":"Bianca J."},{"family":"Caragine","given":"Christina M."},{"family":"Sanjana","given":"Neville E."},{"family":"Dow","given":"Lukas E."}],"issued":{"date-parts":[["2022",5]]}},"label":"page"},{"id":34,"uris":["http://zotero.org/users/16059431/items/BEGRH5P-

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W"],"itemData":{"id":34,"type":"article-journal","abstract":"Clustered regularly interspaced short palindromic repeats (CRISPR . Clustered regularly interspaced short palindromic repeats, or CRISPR nuclease-based genome editing , was a technology for eukaryotic DNA repair. CRISPR was first discovered from prokaryotes in research about RNA adaptive immunity on bacteria (18) where CRISPR system involve defense phage infection and plasmid transfer (19)non-specific RNA degradation. Here we analysed the defensive capabilities of LbuCas13a from Leptotrichia buccalis and found it to have robust antiviral activity unaffected by target phage gene essentiality, gene expression timing or target sequence location. Furthermore, we find LbuCas13a antiviral activity to be broadly effective against a wide range of phages by challenging LbuCas13a against nine E. coli phages from diverse phylogenetic groups. Leveraging the versatility and potency enabled by LbuCas13a targeting, we applied LbuCas13a towards broad-spectrum phage editing. Using a two-step phage-editing and enrichment method, we achieved seven markerless genome edits in three diverse phages with 100% efficiency, including edits as large as multi-gene deletions and as small as replacing a single codon. Cas13a can be applied as a generalizable tool for editing the most abundant and diverse biological entities on Earth.","container-title":"Nature Microbiology","DOI":"10.1038/s41564-022-01258-x","ISSN":"2058-5276","issue":"12","journalAbbreviation":"Nat Microbiol","language":"en","license":"2022 The Author(s ,the bacteria insert the sequence of exogenous DNA into the region of CRISPR and transcription start when the infection happen second time (20,21)we show the implementation of the CRISPR SWAPnDROP concept for the model organism Escherichia coli, the fast growing Vibrio natriegens and the plant pathogen Dickeya dadantii. We demonstrate the excision, transfer and integration of large chromosomal regions between E. coli, V. natriegens and D. dadantii without size-limiting intermediate DNA extraction. CRISPR SWAPnDROP also provides common genome editing approaches comprising scarless, marker-free, iterative and parallel insertions and deletions. The modular character facilitates DNA library applications, and recycling of standardized parts. Its multi-color scarless co-selection system significantly improves editing efficiency and provides visual quality controls throughout the assembly and editing process.","container-title":"Nature Communications","DOI":"10.1038/s41467-022-30843-1","ISSN":"2041-1723","issue":"1","journalAbbreviation":"Nat Commun","language":"en","license":"2022 The Author(s. CRISPR region contain the infected DNA sequence called spacer sequences and being separated by repeated sequences (22)AcrVA5, and reveal the mechanisms by which it strongly inhibits protospacer integration. Our biochemical data shows that the integration by Cas1-Cas2 was abrogated in the presence of AcrVA5. AcrVA5 exhibits low binding affinity towards Cas2 and acetylates Cas2 at Lys55 on the binding interface of the Cas2 and AcrVA5 N-terminal peptide complex to inhibit the Cas2-mediated endonuclease activity. Moreover, a detailed structural comparison between our crystal structure and homolog structure shows that binding of AcrVA5 to Cas2 causes steric hindrance to the neighboring protospacer resulting in the partial disassembly of the Cas1-Cas2 and protospacer complex, as demonstrated by electrophoretic mobility shift assay. Our study focuses on this mechanism of spacer acquisition inhibition and provides insights into the biology of CRISPR-Cas systems.","container-title":"Nature Communications","DOI":"10.1038/s41467-024-47713-7","ISSN":"2041-1723","issue":"1","journalAbbreviation":"Nat Commun","language":"en","license":"2024 The Author(s .When the bacteria detect the same foreign DNA fragment from invaded exogenous phages or plasmids (23)numerous resistance mechanisms have developed against phage. Our understanding of this defensive repertoire has recently been expanded to include the CRISPR system of Clustered, Regularly Interspaced Short Palindromic Repeats. In this remarkable pathway, short sequence tags from invading genetic elements are actively incorporated into the host's CRISPR locus, to be transcribed and processed into a set of small RNAs that guide the destruction of foreign genetic material. Here, we review the inner workings of this adaptable and heritable immune system and draw comparisons to small RNA-guided defense mechanisms in eukaryotic cells.","container-title":"Molecular cell","DOI":"10.1016/j.molcel.2009.12.033","ISSN":"1097-2765","issue":"1","journalAbbreviation":"Mol Cell","note":"PMID: 20129051\nPMCID: PMC2819186","page":"7","source":"PubMed Central","title":"The CRISPR system: small RNA-guided defense in bacteria and archaea","title-short":"The CRISPR system","volume":"37","author":[{"family":"Karginov","given":"Fedor V."},{"family":"Hannon","given":"Gregory J."}],"issued":{"date-parts":[["2010",1,15]]}}},"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} , CRISPR being activated and sgRNAs(single guide RNA) produced : SgRNAs is a combination of tracr RNA and crRNAs (24) . After transcription of CRISPR region ,pre- crRNAs or CRISPR RNAs being synthesized , and processed into crRNA that contained a single spacer sequence for complementary binding with tracrRNA (25), tracr RNA stand for the repeated sequence or remain sequence from CRISPR region , small variation or structure change in base pairing will disrupt the activity (26)it con-

sequently poses a problem for the recognition of sequences containing naturally occurring polymorphisms. The presence of genetic variance such as single nucleotide polymorphisms (SNPs . Cas 9 is a dual RNA guided DNA cleaving endonuclease which involve in this process for guiding the crRNA and tracrRNA to form the sgRNAs or RNA duplex complex , this guiding process provided the basic DNA cleavage and editing ability by using the sgRNA to complementary binding the targeting DNA and cleavage(26–29)it consequently poses a problem for the recognition of sequences containing naturally occurring polymorphisms. The presence of genetic variance such as single nucleotide polymorphisms (SNPs . Cas nucleases , like Cas 9 , need to target a locus called a protospacer adjacent motif (PAM) sequence which help to recognize DNA sequence and improve the DNA cutting efficiency(30)limiting their sequence accessibility for robust genome editing applications. In this study, we recombine the PAM-interacting domain of SpRY, a broad-targeting Cas9 possessing an NRN > NYN (R = A or G, Y = C or T .

1.2 Establish of the CRISPR therapy into glioblastoma:

Based on that, the DNA editing by Cas-9 is called RNA - guided cleavage of target DNA. For CRISPR technology, editing and design the genetic loci or the guide RNA sequence could start the DNA editing by regulating the complementary binding target sites near the PAM loci , make this tool had the ability to be involve in cancer treatment (31–33).For glioblastoma which is a notorious tumor disease, gene therapies become an ideal options , specially like Cas-9 which only require guided RNA and could leads to knock off mutated DNA for reduce cancer cell activity(34,35)however, some cells express the target gene by skipping the disrupted exon, or by using a splicing variant, thus losing the target exon. To overcome this unexpected expression of the target gene, almost the entire gene can be swapped with a selection marker. However, it is time-consuming to create a targeting vector which contains 5′ and 3′ homology arms flanked by a selection marker. Here, we developed a simple and easy method called SUCCESS (Single-strand oligodeoxynucleotides, Universal Cassette, and CRISPR/Cas9 produce Easy Simple knock-out System. Application of gene therapy to glioblastoma is flexible, with foundation of finding the proper and stable target sites. There are some new reported mechanisms of cancer formation and leads to glioblastoma development, which have potential to be use as targeting sites .Current research may target : restart antigen present process, remove immunosuppressive, inhibit angiogenesis , ecDNA, PD-1 , regulator, editing Car-T cell therapy et al . These are recent studies that reveals potential targeting sites for gene therapy.

Table 1 . Non clinical or clinical verified potential gene therapy research in glioblastoma

Potential target site	Gene therapy type	description
EGFR & IL13Rα2 bivalent chimeric antigen therapy(36)with a median overall survival of less than 1 year. Here we report the first six patients with rGBM treated in a phase 1 trial of intrathecally delivered bivalent chimeric antigen receptor (CAR	Chimeric antigen receptor (CAR)-T cell	Determine early efficacy signal , however treated patient sample size remain insufficient
Target CD97 for surface antigen(37)	Chimeric antigen receptor (CAR)-T cell	Consistent express in verified patient, available marker Experiments on mice, expanded survival rate
Engieering CARv3-TEAM-E T cells (38)	Chimeric antigen receptor (CAR)-T cell	Available to guide immune response. Applied of phase 1 clinical study in human

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B7-H3 chimeric antigen receptor (CAR)-modified Vδ1T cells (39)a rare subset of γδT cells, hold promise for treating solid tumors. Unlike conventional T cells, they recognize tumor antigens independently of the MHC antigen presentation pathway, making them a potential \"off-the-shelf\" cell therapy product. However, isolation and activation of Vδ1T cells is challenging, which has limited their clinical investigation. Here, we developed a large-scale clinical-grade manufacturing process for Vδ1T cells and validated the therapeutic potential of B7-H3 chimeric antigen receptor (CAR	Engineering cell	mice xenograft tumor models experiments display safety profile and good effect
NXL-004 (40)tumors originating from glial cells in the brain and spinal cord, constitute 80% of all malignant primary tumors affecting the central nervous system, with glioblastomas (GBM	AAV therapy ,virus	Experiments on mice and glioblastoma organoid , observed tumor size decreased
Human epidermal growth factor receptor 2 (HER2) (41)	Chimeric antigen receptor (CAR)-T cell	In vitro and in vivo experiments display that HER2-specific CAR-T cells suppressed tumor proliferation
Anti-EGFRvIII-SGRP CAR T cells (42)	Chimeric antigen receptor (CAR)-T cell	Combination therapy of anti EGFRvIII receptor and anti- gamma-related protein (SGRP) CAR-T cell therapy, from mouse model showed concurrent therapy overcome tumor antigen escape and immune escape .
carbonic anhydrase IX (CAIX) (43)	Chimeric antigen receptor (CAR)-T cell	CAR-T cell target membrane-bound protein CAIX . CAIX being up-regulated in the hypoxia microenvironment of cancer and have a role of homeostasis regulator . In vitro and in vivo glioblastoma models experiments illustrated target the CAIX as antigen had strong potential for treatment

Table 2. Potential targets for glioblastoma pathogenic mechanism , not validated by clinical trials or in vivo experiments

Target sites/pathogenic mechanism	Type and outcome	Anti - glioblastoma mechanism / relationship with glioblastoma
long non-coding RNA (LncRNA) H19 (44)	RNA that leads to glioblastoma immune escape	Researchers suggested use cancer vaccine to target H19-IRP , no current clinical trials for glioblastoma
GTPSCS (acetyl-CoA synthetase) (45)	Enzyme ,upregulated during glioblastoma formation to increase H3K18la and GDF15 level .	showed correlation between tumor malignancy and increased GTPSCS expression in glioblastoma
CAR-NK cell therapy (46)	Engineering cells, new	Similar to CAR-T cell , express chimeric antigen receptors to anti-tumor

lymphatic endothelial-like cells (LECs) (47)	Exist in glioblastoma, secret CCL21 to support glioblastoma growth	LECs CCL21 stimulus cholesterol synthesis from glioblastoma cells and promote tumor proliferation, disrupt of the LEC paracrine may inhibit glioblastoma formation, target LEC become a potential option

2. Target angiogenesis regulator factors in glioblastoma

Angiogenesis or vascularization , benefit glioblastoma proliferation (48) ,improve cancer cell infiltrate and resistance to chemotherapy, even induce more VC- resist transition, the damage could breakthrough blood brain barrier to facilitate cancer metastasis (49,50). Understanding of how glioblastoma cell drive vessels formation' s tumor molecular mechanisms become the key . As mentioned , disrupt the pathogenic mechanism from a molecular level and genetic level is necessary for design the editing gene , and applied into CRISPR therapy or clinical trials.

Current research reveals that the epidermal growth factor receptor (EGFR) amplification may have relationship with that (51), this is one of the areas where the application of gene editing therapy is best positioned to move from being tested in a lab dish to clinical human trials. By using human glioma stem cells (GSC) to triggered the process , researcher (Spinelli, C, 2024) found out that EGFR/EGFRvIII transcript could be transferred to the extracellular vesicles (EVs) , and synthesized from GSC to endothelial cells (51,52) , which the EVs mediated the angiogenesis . Recent report demonstrated that chemotherapy, like bevacizumab (BVZ), which is a wide use anti-angiogenesis therapy (AAT) drug could be resistance from angiogenesis driven by glioblastoma cell (53) . However, a recent report evidence that there is possible to use CRISPR /Cas9 targeting the EGFR in glioblastoma to suppress cancer proliferation and restrict the angiogenesis in tumors, by experiments on lung cancer using Genome-wide CRISPR screens to knock out gene that e2xpress EGFR protein and disrupt the signaling pathway leaded to less drug resistance (54). This show that gene express EGFR protein could be a targeting sties as well as in glioblastoma for the CRISPR therapy design(55) .

Another potential gene therapy targeting sites is a transcription factor COUP-TFII, first report from Wang F and his colleges indicated COUP-TFII promotes pro-angiogenesis activity .After the team operated genetic knockdown experiments , the angiogenesis in gliomas being inhibited (56) . Angiogenesis assist in tumor metastasis (57) .Chicken ovalbumin upstream promoter transcription factor 2 (COUP-TFII) act different as both oncosuppressor and oncogene , depend on the types of tumors (58–60). For glioblastoma , the details of the pathways that being induced and leads to invasiveness and angiogenesis remain by COUP-TFII remains unknown(56), but by using in vitro and in vivo models the research teams found out that after knockdown COUP-TFII of suppression of the related gene sequence inhibit the invasion and proliferation of glioblastoma by inhibit the process of transform glioma cells to into endothelium-like cells . This reveals that COUP-TFII is a potential target sites for gene therapy by using CRISPR to remove related gene and reduce the expression of COUP-TFII (56) .

3. Develop target sites from glioblastoma and tumors microenvironment 's interaction

Immune-hostile microenvironment or deficiency of tumor-infiltrating T cells is another important factors for glioblastoma to resistance immunotherapy (48,49), leads to hypoxia and further developed to T cell exhaustion in tumor region [134] , nutrients and oxygen are deprived by the increasing number of tumor cells, fuels of aerobic respiration deficiency stimulus angiogenesis and HIF-1α expression , these conditions will up-regulated regulatory T cells, tumor- associated macrophages and myeloid-derived suppressor cells' activity , leads to the signaling of T cell exhaustion (63–67)with only around 5% of patients surviving for a period of 5 years or more after diagnosis. Despite aggressive multimodal therapy, consisting mostly of a combination of surgery, radiotherapy, and temozolomide chemotherapy, tumors nearly always recur close to the site of resection. For the past 15 years, very little progress has been made with regards to improving patient survival. Although immunotherapy represents an attractive therapy modality due to the promising pre-clinical results observed, many of these potential immunotherapeutic approaches fail during clinical trials, and to date no immunotherapeutic treatments for GBM have been approved. As for many other difficult to treat cancers, GBM combines a lack of immunogenicity with few mutations and a highly immunosuppressive tumor microenvironment (TME . Tumor microenvironment (TME) contain non-cancer cells in tumor include macrophage and the interaction with cells

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facilitate tumor growth (51), even being reprogram and hijack by glioblastoma cell(52) to promote the immune escape(53).The mechanisms inside TME are still complex , previous research determine that cancer cells driven lipid metabolism to balance the TME (68,69)the oxidation of fatty acids derived from lipid droplets is essential for the survival of tumor cells that informs clinical outcome among glioblastoma patients.","container-title":"Molecular Cell","DOI":"10.1016/j.molcel.2021.06.013","ISSN":"1097-4164","issue":"13","journalAbbreviation":"Mol Cell","language":"eng","note":"PMID: 34214442","page":"2686-2687","source":"PubMed","title":"A delicate initiation: Lipolysis of lipid droplets fuels glioblastoma","title-short":"A delicate initiation","volume":"81","author":[{"family":"Yang","given":"Kailin"},{"family":"Rich","given":"Jeremy N."}],"issued":{"date-parts":[["2021",7,1]]},"label":"page"},{"id":133,"uris":["http://zotero.org/users/16059431/items/FFBJARBL"],"itemData":{"id":133,"type":"article-journal","abstract":"Many human diseases, including metabolic, immune and central nervous system disorders, as well as cancer, are the consequence of an alteration in lipid metabolic enzymes and their pathways. This illustrates the fundamental role played by lipids in maintaining membrane homeostasis and normal function in healthy cells. We reviewed the major lipid dysfunctions occurring during tumor development, as determined using systems biology approaches. In it, we provide detailed insight into the essential roles exerted by specific lipids in mediating intracellular oncogenic signaling, endoplasmic reticulum stress and bidirectional crosstalk between cells of the tumor microenvironment and cancer cells. Finally, we summarize the advances in ongoing research aimed at exploiting the dependency of cancer cells on lipids to abolish tumor progression.","container-title":"Oncogenesis","DOI":"10.1038/oncsis.2015.49","ISSN":"2157-9024","issue":"1","language":"en","license":"2016 The Author(s, but a recent research characterized tumor-associated macrophages (TAMs) involved in hypoxia of the tumor region. Governa V et al reported EV lipids originating from GBM cells promote lipid droplet (LD) formation, and the lipid scavenging by TAMs involve in the process of facilitates the process of confer the TAMs phenotype to macrophages by glioblastoma cells(70).The researchers than design experiments to targeting the enzymes that form EVs by inhibition the RNA expression , they receive observation of after terminate the EV formation , the proliferation of glioblastoma rate deceased . This indicates another potential targeting sites: inhibit the synthesize of EVs formation by gene editing of the enzyme gene expression .Reveals that more depth study into the interaction between glioblastoma and TEMs is valuable , and may harness methods for inhibit glioblastoma metastasis .

4. Epigenetics alteration of the glioblastoma

High recurrence of glioblastoma is always the major concern in the treatment (71), thus, the research on this cancer has entered the genetic level and even the epigenetic perspective in the past two years (72)often observed independently of genetic heterogeneity, raising the central question of how malignant cell states are encoded epigenetically. To address this, here we performed multiomics single-cell profiling-integrating DNA methylation, transcriptome and genotype within the same cells-of diffuse gliomas, tumors characterized by defined transcriptional cell state diversity. Direct comparison of the epigenetic profiles of distinct cell states revealed key switches for state transitions recapitulating neurodevelopmental trajectories and highlighted dysregulated epigenetic mechanisms underlying gliomagenesis. We further developed a quantitative framework to directly measure cell state heritability and transition dynamics based on high-resolution lineage trees in human samples. We demonstrated heritability of malignant cell states, with key differences in hierarchal and plastic cell state architectures in IDH-mutant glioma versus IDH-wild-type glioblastoma, respectively. This work provides a framework anchoring transcriptional cancer cell states in their epigenetic encoding, inheritance and transition dynamics.","container-title":"Nature Genetics","DOI":"10.1038/s41588-021-00927-7","ISSN":"1546-1718","issue":"10","journalAbbreviation":"Nat Genet","language":"eng","note":"PMID: 34594037\nPMCID: PMC8675181","page":"1469-1479","source":"PubMed","title":"Epigenetic encoding, heritability and plasticity of glioma transcriptional cell states","volume":"53","author":[{"family":"Chaligne","given":"Ronan"},{"family":"Gaiti","given":"Federico"},{"family":"Silverbush","given":"Dana"},{"family":"Schiffman","given":"Joshua S."},{"family":"Weisman","given":"Hannah R."},{"family":"Kluegel","given":"Lloyd"},{"family":"Gritsch","given":"Simon"},{"family":"Deochand","given":"Sunil D."},{"family":"Gonzalez Castro","given":"L. Nicolas"},{"family":"Richman","given":"Alyssa R."},{"family":"Klughammer","given":"Johanna"},{"family":"Biancalani","given":"Tommaso"},{"family":"Muus","given":"Christoph"},{"family":"Sheridan","given":"Caroline"},{"family":"Alonso","given":"Alicia"},{"family":"Izzo","given":"Franco"},{"family":"Park","given":"Jane"},{"family":"Rozenblatt-Rosen","given":"Orit"},{"family":"Regev","-

given":"Aviv"},{"family":"Suvà","given":"Mario L."},{"family":"Landau","given":"Dan A."}],"issued":{"date-parts":[["2021",10]]}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} , more and more research focus on the epigenetic modulation to glioblastoma 's metabolism and RNA mediated DNA methylation enzyme family(73). A recent research indicate a new method for glioblastoma treatment , by using genetic tools to inhibit cell cycle-regulating kinases and the epigenetic writers or erasers and test under 7 types of glioblastoma cell line, the proliferation of the cancer rapidly reduced. During their research , under hypoxia environment they observed serve mediator factors for reprogram of gene regulation for adaptive , which could be use for targeting , and proved that PRMT5 inhibitor nonmetastatic had ability to interrupt the cancer migration when elevation of the cell cycle arrest markers after targeting the regulation of the histone enzyme by nonmetastatic (74,75)and most glioblastoma patients die from the disease recurrence. Thus, systematic studies in simplified model systems are required to pinpoint the choice of targets for further exploration in clinical settings. Here, we report screening of 5 compounds targeting epigenetic writers or erasers and 6 compounds targeting cell cycle-regulating protein kinases against 3 glioblastoma cell lines following incubation under normoxic or hypoxic conditions. The viability/proliferation assay indicated that PRMT5 inhibitor onametostat was endowed with high potency under both normoxic and hypoxic conditions in cell lines that are strongly MGMT-positive (T98-G . The study of the transcriptome is genesis and incredible as it proved that gene editing onto epigenetic level is possible. In the future, CRISPR gene editing technology could induced into more aspects of the oncology drug development process, including target sites from epigenetics alterations of glioblastoma or DNA structure regulations . Here, I also call for more research on epigenetics and glioblastoma, in order to find breakthroughs in gene editing, because glioblastoma has a high degree of variability in both patients and lab based organoids. The reason for this high variability may come from the principle of epigenetics in glioblastoma. When we produce targeted drugs , specially CRISPR technology is expensive, which limited by cost or technology, designed the targeted drugs need to meet the needs of most patients as much as possible, targeting the common pathogenic mechanism and abnormal metabolic principles of this tumor in most patients.

The heterogeneity of the glioblastoma have a strong role in resistant drugs treatment , due to the numerous subtypes, glioma stem cell or variety TME (76–78)composition of cells, and phenotypical characteristics makes it difficult to accurately classify GBM into distinct subtypes and find effective therapies. The advancement of sequencing technologies in recent years has further corroborated the heterogeneity of GBM at the single cell level. Recent studies have only begun to elucidate the different cell states present in GBM and how they correlate with sensitivity to therapy. Furthermore, it has become clear that GBM heterogeneity not only depends on intrinsic factors but also strongly differs between new and recurrent GBM, and treatment naïve and experienced patients. Understanding and connecting the complex cellular network that underlies GBM heterogeneity will be indispensable in finding new ways to tackle this deadly disease. Here, we present an overview of the multiple layers of GBM heterogeneity and discuss novel findings in the age of single cell technologies.","container-title":"Oncogene","DOI":"10.1038/s41388-023-02738-y","ISSN":"1476-5594","issue":"27","journalAbbreviation":"Oncogene","language":"eng","note":"PMID: 37277603\nPMCID: PMC10913075","page":"2155-2165","source":"PubMed","title":"Glioblastoma heterogeneity at single cell resolution","volume":"42","author":[{"family":"Eisenbarth","given":"David"},{"family":"Wang","given":"Y. Alan"}],"issued":{"date-parts":[["2023",6]]}},"label":"page"},{"id":154,"uris":["http://zotero.org/users/16059431/items/GXMWRPHN"],"itemData":{"id":154,"type":"article-journal","abstract":"Glioblastoma multiforme (GBM. Previous research find out that DNA methylation facilitates in the heterogeneity of glioblastomas (79), reveals that epigenetics mutation may promote the cancer development (80)yet little is known about the role of the epigenome in glioblastoma disease progression. Here, we present genome-scale maps of DNA methylation in matched primary and recurring glioblastoma tumors, using data from a highly annotated clinical cohort that was selected through a national patient registry. We demonstrate the feasibility of DNA methylation mapping in a large set of routinely collected FFPE samples, and we validate bisulfite sequencing as a multipurpose assay that allowed us to infer a range of different genetic, epigenetic, and transcriptional characteristics of the profiled tumor samples. On the basis of these data, we identified subtle differences between primary and recurring tumors, links between DNA methylation and the tumor microenvironment, and an association of epigenetic tumor heterogeneity with patient survival. In summary, this study establishes an open resource for dissecting DNA methylation heterogeneity in a genetically diverse and heterogeneous cancer, and it demonstrates the feasibility of integrating epigenomics, radiology, and digital pathology for a national cohort, thereby leveraging existing samples and data collected as part of routine clinical practice.","container-title":"Nature Medicine","DOI":"10.1038/s41591-0

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18-0156-x","ISSN":"1546-170X","issue":"10","journalAbbreviation":"Nat Med","language":"eng","note":"PMID: 30150718\nPMCID: PMC6181207","page":"1611-1624","source":"PubMed","title":"The DNA methylation landscape of glioblastoma disease progression shows extensive heterogeneity in time and space","volume":"24","author":[{"family":"Klughammer","given":"Johanna"},{"family":"Kiesel","given":"Barbara"},{"family":"Roetzer","given":"Thomas"},{"family":"Fortelny","given":"Nikolaus"},{"family":"Nemc","given":"Amelie"},{"family":"Nenning","given":"Karl-Heinz"},{"family":"Furtner","given":"Julia"},{"family":"Sheffield","given":"Nathan C."},{"family":"Datlinger","given":"Paul"},{"family":"Peter","given":"Nadine"},{"family":"Nowosielski","given":"Martha"},{"family":"Augustin","given":"Marco"},{"family":"Mischkulnig","given":"Mario"},{"family":"Ströbel","given":"Thomas"},{"family":"Alpar","given":"Donat"},{"family":"Ergüner","given":"Bekir"},{"family":"Senekowitsch","given":"Martin"},{"family":"Moser","given":"Patrizia"},{"family":"Freyschlag","given":"Christian F."},{"family":"Kerschbaumer","given":"Johannes"},{"family":"Thomé","given":"Claudius"},{"family":"Grams","given":"Astrid E."},{"family":"Stockhammer","given":"Günther"},{"family":"Kitzwoegerer","given":"Melitta"},{"family":"Oberndorfer","given":"Stefan"},{"family":"Marhold","given":"Franz"},{"family":"Weis","given":"Serge"},{"family":"Trenkler","given":"Johannes"},{"family":"Buchroithner","given":"Johanna"},{"family":"Pichler","given":"Josef"},{"family":"Haybaeck","given":"Johannes"},{"family":"Krassnig","given":"Stefanie"},{"family":"Mahdy Ali","given":"Kariem"},{"family":"Campe","given":"Gord","non-dropping-particle":"von"},{"family":"Payer","given":"Franz"},{"family":"Sherif","given":"Camillo"},{"family":"Preiser","given":"Julius"},{"family":"Hauser","given":"Thomas"},{"family":"Winkler","given":"Peter A."},{"family":"Kleindienst","given":"Waltraud"},{"family":"Würtz","given":"Franz"},{"family":"Brandner-Kokalj","given":"Tanisa"},{"family":"Stultschnig","given":"Martin"},{"family":"Schweiger","given":"Stefan"},{"family":"Dieckmann","given":"Karin"},{"family":"Preusser","given":"Matthias"},{"family":"Langs","given":"Georg"},{"family":"Baumann","given":"Bernhard"},{"family":"Knosp","given":"Engelbert"},{"family":"Widhalm","given":"Georg"},{"family":"Marosi","given":"Christine"},{"family":"Hainfellner","given":"Johannes A."},{"family":"Woehrer","given":"Adelheid"},{"family":"Bock","given":"Christoph"}],"issued":{"date-parts":[["2018",10]]}}},"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} . In this field, circular Extrachromosomal DNA (ecDNA) brought to the attention, as it is driven a lot of oncogenic gene expression (81)evolution of drug resistance and poor patient outcomes. Applying computational methods for the detection and reconstruction of ecDNA across a retrospective cohort of 481 medulloblastoma tumors from 465 patients, we identify circular ecDNA in 82 patients (18%, by amplifies the oncogenes(82,83)we detected and classified focal amplifications in 8,060 newly diagnosed primary cancers, untreated metastases and heavily pretreated tumors. The ecDNAs were detected at significantly higher frequency in untreated metastatic and pretreated tumors compared to newly diagnosed cancers. Tumors from chemotherapy-pretreated patients showed significantly higher ecDNA frequency compared to untreated cancers. In particular, tubulin inhibition associated with ecDNA increases, suggesting a role for ecDNA in treatment response. In longitudinally matched tumor samples, ecDNAs were more likely to be retained compared to chromosomal amplifications. EcDNAs shared between time points, and ecDNAs in advanced cancers were more likely to harbor localized hypermutation events compared to private ecDNAs and ecDNAs in newly diagnosed tumors. Relatively high variant allele fractions of ecDNA localized hypermutations implicated early ecDNA mutagenesis. Our findings nominate ecDNAs to provide tumors with competitive advantages during cancer progression and metastasis.","container-title":"Nature Genetics","DOI":"10.1038/s41588-024-01949-7","ISSN":"1546-1718","issue":"11","journalAbbreviation":"Nat Genet","language":"en","license":"2024 The Author(s, maintain different lineages(84)which poses a major hurdle to effective treatment. Evidence indicates a key role for oncogene amplification on extrachromosomal DNA (ecDNA, altering p53 (85)and is associated with poor outcomes for patients with cancer1–6. At present, it is unclear whether ecDNA is a later manifestation of genomic instability, or whether it can be an early event in the transition from dysplasia to cancer. Here, to better understand the development of ecDNA, we analysed whole-genome sequencing (WGS . EcDNA also accelerate tumor evolution as it have enhanced mutagenesis ability , high amplification ability during mitosis and disturb among daughter cells leads to the glioblastoma strong heterogeneity and high drug resistance(71).As a hypothesis , targeting the amplicon of the ecDNA 's production may be one of the options as part of gene therapy' s targeting sites. A recent experiment verify this hypothesis : the team designed a inhibitor CHK1 that inhibit ecDNA by localize the double strands DNA and broken the

double strands, and in the experimented gastric cancer model after inhibitor given , extensive and preferential tumor cell death . This transcription–replication conflict could be used as a target sites (86) for glioblastoma as well(71).A recent study also provided strong evidence for the potential of combining CRISPR advantages into epigenetic therapy , the research team verified the epigenetic modulators inhibit the transposable elements (TEs) , by using designed CRISPR-mediated drugs to inhibit the epigenetic modulators the TEs reproduce specific peptide which being found out used as antigen for immune cell and leads to immune response (87), the research also highlighted that using CRISPR technology to target the activation strategies could minimize the side effect, however, the translational effect of induce epigenetic elements into target sites require more research (8)

5. PD-1 potential for glioblastoma CRIPSR therapy

Another contribution factor of glioblastoma high recurrence is the tumor immune escape mechanism (88,89), current research determined multiple pathways that assist glioblastoma immune suppression ability. For example, Watson et al recent report that by inhibit colony-stimulating factor-1 receptor (CSF-1R) the fibrosis of glioblastoma constantly reduced and lead to tumor proliferation failure , in this process researchers determine that the inhibitors influence the fibrosis response inside the TME , downregulated the PDFL signaling pathway and disturb gene sets expression (90), confirmed the targetable pathways for gene therapy .

Another potential target site is PD-1 and PD-L1, which received serve success research of CRISPR gene-editing in glioblastoma in the past two years (91). Programmed cell death protein 1 (PD-1) had important role in cell cycle Checkpoint inhibition (CPI)of T cell , PD-1 is normally found on CD8+ T cell, γδ T cells and suppressed glycolysis, phagocytosis and T cell stimulatory potential to suppress immune capacity and reduce T cell proliferation by cell cycle regulation, there are overexpress of PD-1 in tumors specially in glioblastoma (92–97)2, yet can in some cases enhance survival3-5 and responses to immune checkpoint blockade therapies, including anti-PD-1, which targets PD-1 (encoded by PDCD1. The overpress by glioblastoma leads to ineffective T cell migration and infiltration in the tumor region(98,99), promote the glioblastoma immune escape ability.PD-L1 is the ligand for binding PD-1 and the combination results in blockage anti-tumor immune responses(100), both of the PD-1 and PD-L1 have ability to be target sites : A recent report present the possibility of apply PD-1 as target sites for CRISPR gene editing therapy for anti glioblastoma by deletion the PD-L1 on both cell membrane and intracellular PD-L1 , the team first discover two single guide DNA for Cas-9 to editing and found out that using homology-directed repair template (HDR) with dual sgRNA under Cas9 modification could deletion the PD-L1 more efficiency , the results showed successful inhibition of glioblastoma growth and polarized tumor-associated macrophages (TAM) toward an M1 phenotype(101)vitality, proliferation, and migration, and is therefore a promising target for treating GBM. CRISPR/Cas9-mediated genomic editing can delete both cell surface and intracellular PD-L1. This systemic deliverable genomic PD-L1 deletion system can be used as an effective anti-GBM therapy by inhibiting tumor growth and migration, and overcoming immunosuppression. To target PD-L1 for CRISPR/Cas9 gene editing, we first identified two single guide RNA (sgRNA, activated M1 - like TAM could start anti tumor response and reduce tumor growth (102,103) . This study greatly proved that using CRISPR involved technique for glioblastoma gene editing of PD-L1 is available . There is also an recent research on PD-1 in immune checkpoint blocked inhibition , study the recurrent glioblastoma treatment towards PD-1 and determined that ERK1/2 phosphorylation (p-ERK) has direct relationship with glioblastoma mutated , confirmed that testing p-ERK activation could check the blockage inhibitor of PD-1 treatment successful rate (104)we reported enrichment of BRAF/PTPN11 mutations in 30% of rGBM that responded to PD-1 blockade. Given that BRAF and PTPN11 promote MAPK/ERK signaling, we investigated whether activation of this pathway is associated with response to PD-1 inhibitors in rGBM, including patients that do not harbor BRAF/PTPN11 mutations. Here we show that immunohistochemistry for ERK1/2 phosphorylation (p-ERK, this research had the accessible of using CRISPR therapy to eliminate glioblastoma ‘s recurrent point. On the other hand, research on PD-L1 is representative, with clear and distinct pathogenic mechanisms and molecular metabolism combined with the flexibility of CRISPR targeted gene design, making clinical drug production possible and hopeful

6. Combination of immune therapy and gene therapy in glioblastoma

Now, the immune therapy strategies’ development for glioblastoma are focusing more on genetic therapy , ,specially in using CRISPR technique for gene editing of the target sites , not just focus on the mutated gene sequence of glioblastoma stem cell but also included TME (105),

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TAM, CAR-T cell (106), MYC etc., a number of mechanistic pathways have been excavated that can be used for gene targeting in the past two years, for example in October a research reported that in glioblastoma stem cell 's oncogene MYC function is translate upstream open reading frames' RNA into pre-mRNA encoded protein (MPEP) ,and induces downstream AKT-mTOR signaling and promotes tumor growth ,the team than verify this by using gene editing tool to ablation the MYC , the researchers observed the death of glioblastoma increased, demonstrated the potential in application of gene editing therapy from in vivo to in vitro (108).Multiple researches in different types of cancer involved CRISPR treatment towards MYC and the showed the potential of future in gene therapy(109)based on precision CRISPR editing using template libraries with either the original or altered sequence, and a sequence tag, enabling direct comparison between original and mutated cells. Using the example of the MYC oncogene, we identify important transcriptional targets and show that E-box mutations at MYC target gene promoters reduce cellular fitness.",”container-title”:”Nature Biotechnology”,”DOI”:”10.1038/s41587-022-01444-6”,”ISSN”:”1546-1696”,”issue”:”2”,”journalAbbreviation”:”Nat Biotechnol”,”language”:”en”,”license”:”2022 The Author(s.

Another gene therapy that is approaching mature leverage into clinical treatment but still require more discover of target sites for CRISPR technology and convert the editing tools into drug design of glioblastoma is CAR - T cell (98,106). The mechanisms of apply Car - T cell therapy into glioblastoma, still contain a part of the unexplored research gap, and new research is coming out all the time, however, chimeric antigen receptor (CAR) T cell therapy did stands out for some cancer ' s treatment (110)and the transition toward glioblastoma is highly possible(111) with a median overall survival of less than 1 year. Here we report the first six patients with rGBM treated in a phase 1 trial of intrathecally delivered bivalent chimeric antigen receptor (CAR (figure 1) . In the past six months, several new technologies for boosting engineered T cells have emerged, raising the possibility of targeting glioblastoma . CARs reprogram receptors of T cells or tumor-associated antigens (TAA) for recognize the specific target antigen on tumor cells and create anti tumor immune response , so the target usually located on immune cell and majorly T cells, epidermal growth factor receptor (HER2) are usually associated with the ability of attractive target tumor antigens(112). Due to the blood brain barrier and hostile tumor microenvironment , enlarge the Car- T cell therapy effect and enhance the anti-solid tumor of glioblastoma in brain became a challenge(113) .

Thus, a recent study provide further engineering design towards the differences and heterogeneity among glioblastomas, the team localized a T and NK cell costimulatory protein transmembrane and immunoglobulin domain–containing 2 (TMIGD2) which is part of the costimulatory domain that have an important role of stimulus Car-T cell immune activity like T cell activation and differentiation, except during the process the TMIGD2 expression decreased and ends the response, the researcher targeted the TMIGD2 in vivo and block the inhibit affect of TMIGD2 signaling and harvested enlargement of Car-T cell antitumor responses and the persistence ability(114)we developed a TMIGD2 optimized potent/persistent (TOP, this is a promising therapeutic strategy for target the solid tumor glioblastoma , and the combination of CRISPR and immune therapy is possible(115)only SRPK1/2 specific inhibitors and small interfering RNA (siRNA.CRISPR is the pillar of the editing and genetic therapy.

There is a steady flow of research on combining crispr gene editing technology and the treatment of glioblastoma , and the core is to find the appropriate target point. Because the malignant tumor is different among different patient, there is a very large gap between individuals in the carcinogenesis reaction and heterogeneity, so it is necessary to reserve and study enough target sites. At present, there is no summarize for the relationship between glioblastoma targeting point and the differences or occurred mutations. It is hoped that in the future, more and more studies can summarize the different heterogeneity and the relative matching targeting points of the tumor in different patients, which could allow complementary editing gene sites and alteration signaling pathways for CRISPR /Cas 9 technology into drug design , integrates the cancer surfaceome atlas and phenotypic states of glioblastoma could help us to understand the network of tumor driven factors and related regulation pathway, promote the prediction of the potential target sites(116,117).

7. Study of pediatric glioblastoma CRISPR therapy becomes necessary

The clinical treatment for glioblastoma remain major focusing on improve survival period(118), complete cure may require completion of the genetic profile and phenotype of glioblastoma 's research to link oncogenic factors and identification of a sufficient number of pathways to be targeted by CRISPR - mediated technique . Beyond the discussion and research of glioblastoma, a special aspect worth to pay more attention is the driven factors of pediatric glioblastomas. Considering the high degree of intracellular heterogeneity and plasticity of glioblastoma(119) the molecular determinants defining GSCs in their native

state in patients remain poorly understood. Here we used single cell datasets and identified GSCs at the apex of the differentiation hierarchy of GBM. By reconstructing the GSCs' regulatory network, we identified the YAP/TAZ coactivators as master regulators of this cell state, irrespectively of GBM subtypes. YAP/TAZ are required to install GSC properties in primary cells downstream of multiple oncogenic lesions, and required for tumor initiation and maintenance in vivo in different mouse and human GBM models. YAP/TAZ act as main roadblock of GSC differentiation and their inhibition irreversibly lock differentiated GBM cells into a non-tumorigenic state, preventing plasticity and regeneration of GSC-like cells. Thus, GSC identity is linked to a key molecular hub integrating genetics and microenvironmental inputs within the multifaceted biology of GBM.","container-title":"Nature Cancer","DOI":"10.1038/s43018-020-00150-z","ISSN":"2662-1347","issue":"2","journalAbbreviation":"Nat Cancer","language":"eng","note":"PMID: 33644767\nPMCID: PMC7116831","page":"174-188","source":"PubMed","title":"Single-cell analyses reveal YAP/TAZ as regulators of stemness and cell plasticity in Glioblastoma","volume":"2","author":[{"family":"Castellan","given":"Martina"},{"family":"Guarnieri","given":"Alberto"},{"family":"Fujimura","given":"Atsushi"},{"family":"Zanconato","given":"Francesca"},{"family":"Battilana","given":"Giusy"},{"family":"Panciera","given":"Tito"},{"family":"Sladitschek","given":"Hanna Lucie"},{"family":"Contessotto","given":"Paolo"},{"family":"Citron","given":"Anna"},{"family":"Grilli","given":"Andrea"},{"family":"Romano","given":"Oriana"},{"family":"Bicciato","given":"Silvio"},{"family":"Fassan","given":"Matteo"},{"family":"Porcù","given":"Elena"},{"family":"Rosato","given":"Antonio"},{"family":"Cordenonsi","given":"Michelangelo"},{"family":"Piccolo","given":"Stefano"}],"issued":{"date-parts":[["2021",2]]}}},"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} , it is necessary to study the differences between pediatric tumors and adult tumors separately. Unfortunately, pediatric glioblastoma 's survival time only ranges from 13 to 73 months(120). The difference between pediatric glioblastoma and adults' glioblastoma leads to barriers of applying normal immunotherapy to child failed, as the features of genetic and epigenetic altered in child , and current research concluded the tumor microenvironment also different(121)and aggressive therapy often leads to long-term sequelae in survivors, making these tumors challenging to treat. Immunotherapy has revolutionized prospects for many cancer types in adults, but the intrinsic complexity of treating pediatric patients and the scarcity of clinical studies of children to inform effective approaches have hampered the development of effective immunotherapies in pediatric settings. Here, we review recent advances and ongoing challenges in pediatric brain cancer immunotherapy, as well as considerations for efficient clinical translation of efficacious immunotherapies into pediatric settings.","container-title":"Nature Cancer","DOI":"10.1038/s43018-021-00319-0","ISSN":"2662-1347","issue":"1","journalAbbreviation":"Nat Cancer","language":"eng","note":"PMID: 35121998","page":"11-24","source":"PubMed","title":"The current landscape of immunotherapy for pediatric brain tumors","volume":"3","author":[{"family":"Hwang","given":"Eugene I."},{"family":"Sayour","given":"Elias J."},{"family":"Flores","given":"Catherine T."},{"family":"Grant","given":"Gerald"},{"family":"Wechsler-Reya","given":"Robert"},{"family":"Hoang-Minh","given":"Lan B."},{"family":"Kieran","given":"Mark W."},{"family":"Salcido","given":"Joanne"},{"family":"Prins","given":"Robert M."},{"family":"Figg","given":"John W."},{"family":"Platten","given":"Michael"},{"family":"Candelario","given":"Kate M."},{"family":"Hale","given":"Paul G."},{"family":"Blatt","given":"Jason E."},{"family":"Governale","given":"Lance S."},{"family":"Okada","given":"Hideho"},{"family":"Mitchell","given":"Duane A."},{"family":"Pollack","given":"Ian F."}],"issued":{"date-parts":[["2022",1]]}}},"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} .As well as the tumor structural variants also drive different mechanisms for pediatric glioma genesis , normally causing by disruption of topologically associated domains (TADs) and gene-enhancer interactions due to chromosome damage, one recent report indicated that structural variants are often outside the exome and results recurrent single-nucleotide variants , indicate the potential therapeutic targets and suggested the screening of pediatric glioblastoma should contain the whole genome(122).There is no denying that CRISPR has advantages in genome modification(123), a complete understanding of the whole genome is the icing on the cake for the establishment of CRISPR-based gene editing therapies .

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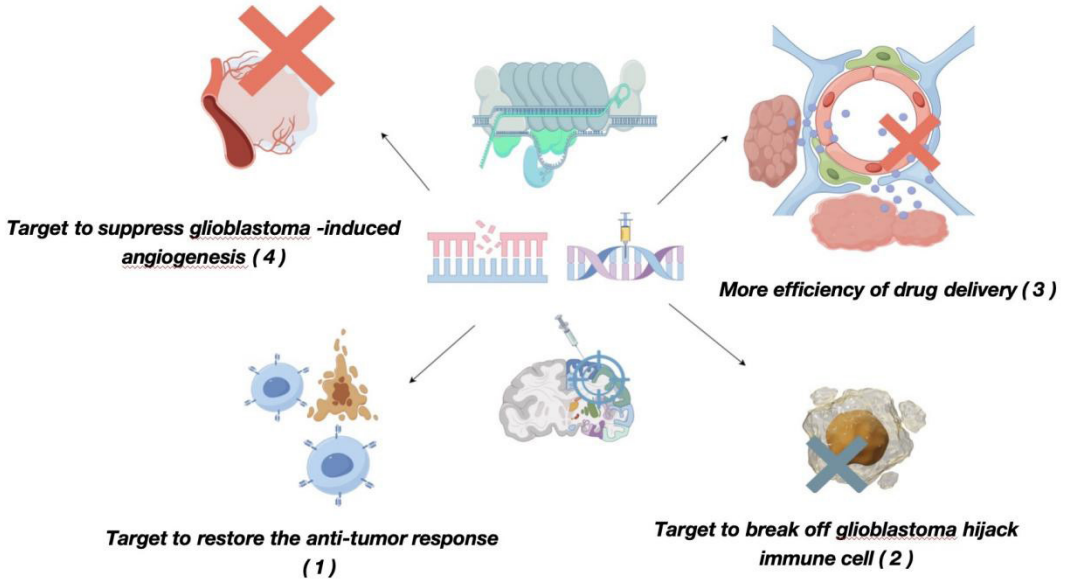

Figure 1. Potential CRISPR therapy . (a) Induce T cell filtration and enhance the MYC present, increase anti-tumor response , related with CRISPR - immune therapy; (b) Target to improve the tumor microenvironment as glioblastoma could induce T cell autophagy , suppress this process could restore anti-tumor response; (c) variety delivery methods for transfer drugs that contain CRISPR editing gene into the target region , proper delivery methods have ability to reduce off - target ; (d) target to inhibit the activities like angiogenesis that benefit glioblastoma proliferation to avoid tumor development .

8. Breakthrough the physical barrier of brain :

When we step into the discussion about gene therapy, one unavoidable point is to deliver the edited gene vector to the targeted cells (124), even for the CRISPR experiments against glioblastoma upload a suitable drug delivery method has also become a important field (8) . The idea of modular viral vector design is closely related to the role of the target drug, and even can be said to determine which target drug to edit and which target point, not just the simple delivery of edited genes into glioblastoma, It is based on the design of the vector virus that decides whether to deliver it into the tumor microenvironment to increase lymphocyte infiltration or directly induce autophagy(125–127)the use of adeno-associated virus (AAV.There are many options for viral vector, however ,there are pros and corns for the deliver of edited substance , base on the high heterogeneity ,infiltration of T cells, notorious microenvironment , invasiveness , made the transport method facing challenges and the self toxin of virus , numbers of recent research focus on the deliver method of the adeno-associated virus (AAV) as more research disclosed AAV have more transduction efficiency and broad cellular tropism in CNS(128). For example, the glioblastoma synthesis immune-suppressive chemokines to suppress immune cell , which leads to expression of lymphocyte chemotaxis decrease, a team report using recombinant AAV therapy that contained gene-edited CXCL9 transgene to stimulus the lymphocyte filtration against glioblastoma by express chemotaxis more ,enhancing T cell recruitment , offer evidence for AAV had ability to transfer edited gene enter TME.

In fact , consideration of apply AAV delivery of edited genes requires consideration of the three dimensions of the complex tumor microenvironment combined with the ability of the virus to penetrate the blood-brain barrier, and AAV did not have a high penetration ability across blood brain barrier(129), even compare with other potential virus AAV have advantages of low immunogenicity and less immune response against the virus delivery(130)and has attracted a significant amount of attention in the field, especially in clinical-stage experimental therapeutic strategies. The ability to generate recombinant AAV particles lacking any viral genes and containing DNA sequences of interest for various therapeutic applications has thus far

proven to be one of the safest strategies for gene therapies. This review will provide an overview of some important factors to consider in the use of AAV as a vector for gene therapy.","container-title":"BioDrugs: Clinical Immunotherapeutics, Biopharmaceuticals and Gene Therapy","-DOI":"10.1007/s40259-017-0234-5","ISSN":"1179-190X","issue":"4","journalAbbreviation":"BioDrugs","language":"eng","note":"PMID: 28669112\nPMCID: PMC5548848","page":"317-334","source":"PubMed","title":"Adeno-Associated Virus (AAV, design methods for assist AAV cross blood brain barrier become requirement for gene therapy target CNS tumor .Mediated the virus to reduce the immunogenicity could improve the safety and reduced side effect .

Consider the risks associated with virus delivery , the use of different materials for encapsulation of the edited genes to transmembrane diffuse is a second option , except electroporation only used in vivo(131)and the Cas9 nuclease of the system acts as a pair of scissors to cleave the double strands of DNA. Since its discovery, CRISPR-Cas9 has become the most robust platform for genome engineering in eukaryotic cells. Recently, the CRISPR-Cas9 system has triggered enormous interest in therapeutic applications. CRISPR-Cas9 can be applied to correct disease-causing gene mutations or engineer T cells for cancer immunotherapy. The first clinical trial using the CRISPR-Cas9 technology was conducted in 2016. Despite the great promise of the CRISPR-Cas9 technology, several challenges remain to be tackled before its successful applications for human patients. The greatest challenge is the safe and efficient delivery of the CRISPR-Cas9 genome-editing system to target cells in human body. In this review, we will introduce the molecular mechanism and different strategies to edit genes using the CRISPR-Cas9 system. We will then highlight the current systems that have been developed to deliver CRISPR-Cas9 in vitro and in vivo for various therapeutic purposes.","container-title":"Journal of Controlled Release: Official Journal of the Controlled Release Society","DOI":"10.1016/j.jconrel.2017.09.012","ISSN":"1873-4995","journalAbbreviation":"J Control Release","language":"eng","note":"PMID: 28911805\nPMCID: PMC5723556","page":"17-26","source":"PubMed","title":"Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications","volume":"266","author":[{"family":"Liu","given":"Chang"},{"family":"Zhang","given":"Li"},{"family":"Liu","given":"Hao"},{"family":"Cheng","given":"Kun"}],"issued":{"date-parts":[["2017",11,28]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} . An incredible CRISPR-Cas9 brain delivery platform being developed from Yan Zou 's team : a unique nanocapsule designed by simply fabricated by encapsulating the single Cas9/sgRNA complex within a glutathione-sensitive polymer shell, which show great ability in high BBB penetration, targeting tumor cell precisely, and Cas9/sgRNA selective release , the team target the low-density lipoprotein receptor–related protein-1 (LRP-1) which s highly expressed in glioblastoma and designed a thin, disulfide–cross-linked polymeric shell decorated with angiopep-2 peptide which complementary with the LRP-1, this manufiure platform is carefully designed with each points to overcome challenges:

Table 3.

Designed details of the nano capsule	Overcome difficulties
Angiopep-2 peptide	Complementary to LRP-1 , Avoid off target
N early neutral surface charge	Avoid degradation by ribonuclease (RNase)
Small size of angiopep-2 peptide	BBB penetration and intracellular delivery , Reach GBM-specific targeting
Disulfide–cross-linking	Use higher intracellular glutathione (GSH) conditions in glioblastoma for cleavage nanocapsule and release edited gene
anti–Polo-like kinase 1 (PLK1) inside	decreases expression of the cellular mitosis protein PLK1 in GBM to reduce off target

This study demonstrated that the nanocapsules could be designed to meet the delivery conditions and improve the efficiency, providing a stable and efficient treatment for cripsr gene editing therapy into glioblastoma(132).

Perspective and conclusion:

Glioblastoma , is despicable and vicious , with highly invasive growth and a high level of malignancy . It has the ability to invade different brain lobes even deep brain

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structures such as the corpus callosum during carcinogenesis . The damage of brain structure by glioblastoma had an wide negative effect on patients , as the growth of glioblastoma is violent, the intracranial pressure of the patient is high and leads to localized symptoms like severe headache , visual and language barriers, on the other hand, hemiplegia , epilepsy, and psychiatric disorders such as focus attention decline occurred as well(133). The universal of new technologies' application, the gene therapy technology, bring hope to patients . In terms of the characterization of internationally widely researched , gene therapy has gone through three generations, from Zinc finger at the beginning, to TALENS in the middle, and currently the CRISPR technology with the most advantages and mature clinical trial experience . Numerous studies have shown that the intracellular metabolic activity and related gene expression in glioblastoma are extremely complex. Therefore, corresponding gene editing techniques require stable and high gene sequence coverage, which is also the advantages of CRISPR technology : multifunctionality, not only can be used for gene knockout, but also for gene insertion and replacement(134)quality, and tolerance to various environmental stresses. The discovery and modification of CRISPR/Cas system, a nature-occurred gene editing tool, opens an era for studying gene function and precision crop breeding.\nAIM OF REVIEW: In this review, we first introduce the brief history of CRISPR/Cas discovery followed the mechanism and application of CRISPR/Cas system on gene function study and crop improvement. Currently, CRISPR/Cas genome editing has been becoming a mature cutting-edge biotechnological tool for crop improvement that already used in many different traits in crops, including pathogen resistance, abiotic tolerance, plant development and morphology and even secondary metabolism and fiber development. Finally, we point out the major issues associating with CRISPR/Cas system and the future research directions.Key Scientific Concepts of Review: CRISPR/Cas9 system is a robust and powerful biotechnological tool for targeting an individual DNA and RNA sequence in the genome. It can be used to target a sequence for gene knockin, knockout and replacement as well as monitoring and regulating gene expression at the genome and epigenome levels by binding a specific sequence. Agrobacterium-mediated method is still the major and efficient method for delivering CRISPR/Cas regents into targeted plant cells. However, other delivery methods, such as virus-mediated method, have been developed and enhanced the application potentials of CRISPR/Cas9-based crop improvement. PAM requirement offers the CRISPR/Cas9-targted genetic loci and also limits the application of CRISPR/Cas9. Discovering new Cas proteins and modifying current Cas enzymes play an important role in CRISPR/Cas9-based genome editing. Developing a better CRISPR/Cas9 system, including the delivery system and the methods eliminating off-target effects, and finding key/master genes for controlling crop growth and development is two major directions for CRISPR/Cas9-based crop improvement.","container-title":"Journal of Advanced Research","DOI":"10.1016/j.jare.2020.10.003","ISSN":"2090-1224","journalAbbreviation":"J Adv Res","language":"eng","note":"PMID: 33842017\nPMCID: PMC8020163","page":"207-221","source":"PubMed","title":"CRISPR/Cas: A powerful tool for gene function study and crop improvement","title-short":"CRISPR/Cas","volume":"29","author":[{"family":"Zhang","given":"Dangquan"},{"family":"Zhang","given":"Zhiyong"},{"family":"Unver","given":"Turgay"},{"family":"Zhang","given":"Baohong"}],"issued":{"date-parts":[["2021",3]]}}},"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} , CRISPR has the potential to develop multiple therapies, such as immunotherapy, viral therapy etc.(135)a set of distinct challenges remains. Consequently, the quest for novel strategies has become imperative to safeguard and more effectively release the full functions of engineered T cells. These factors are intricately linked to the success of adoptive cell therapy. Recently, CRISPR-based technologies have emerged as a major breakthrough for maintaining T cell functions. These technologies have allowed the discovery of T cells' negative regulators such as specific cell-surface receptors, cell-signaling proteins, and transcription factors that are involved in the development or maintenance of T cell dysfunction. By employing a CRISPR-genic invalidation approach to target these negative regulators, it has become possible to prevent the emergence of hypofunctional T cells. This review revisits the establishment of the dysfunctional profile of T cells before delving into a comprehensive summary of recent CRISPR-gene invalidations, with each invalidation contributing to the enhancement of engineered T cells' antitumor capacities. The narrative unfolds as we explore how these advancements were discovered and identified, marking a significant advancement in the pursuit of superior adoptive cell therapy.","container-title":"Cancer Gene Therapy","DOI":"10.1038/s41417-024-00771-x","ISSN":"1476-5500","issue":"8","journalAbbreviation":"Cancer Gene Ther","language":"eng","note":"PMID: 38609574","page":"1124-1134","source":"PubMed","title":"CRISPR-Cas gene knockouts to optimize engineered T cells for cancer immunotherapy","volume":"31","author":[{"family":"De Castro","given":"Valentine"},{"family":"Galaine","given":"Jeanne"},{"family":"Loyon","given":"Romain"},{"family":"Godet","given":"Yann"}],"issued":{"date-parts":[["2024",8]]}}}

],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} . The products of CRISPR technology are not directly invested in anti-cancer mechanisms against glioblastoma, but are invested in various therapies, which not only have high efficiency but also minimize side effects(136). By using gene therapy to target the pathogenic molecular mechanism of glioblastoma, cancer cell growth restriction, and breaking the hijacking of immune cells by cancer cells, it has become a new treatment method for anti glioblastoma . Another point that can be involved into the gene therapy research, is the cancer metastasis of glioblastoma, and related studies and experiments can also be conducted , as genomic alterations became the factors of glioblastoma metastasis(137). Glioblastoma 's metastasis is rare with few reports like glioblastoma-lung metastasis , glioblastoma-spine metastasis(138)local relapse was found and the patient received a second surgery. After another 4 months, we found a hard mass in the right posterior neck when she admitted to our department for fourth cycle of adjuvant chemotherapy. Immunohistochemical investigation supported the diagnosis of glioblastoma metastases to the neck after resection of the right neck mass. A few days later, spinal vertebral magnetic resonance imaging (MRI, cracking the cancer metastasis dynamics of glioblastoma has the potential to promote precise and comprehensive research and development of anti-cancer treatments . Not omitting the pathogenic factors of every glioblastoma, the expression of mutated genes, and the function of over-expressed proteins are important factors in improving the accuracy of targeted therapy, as there are significant differences between glioblastomas .

The tenacious resistance of glioblastoma to chemotherapy and the complex invasiveness of cancer cells made surgery and other therapies difficult to carry out, beyond that, children's glioblastoma are differences from adults. Therefore, gene therapy technology from CRISPR technology with advantages of individually designed functions, targeting, minimal damage, low off target rate, high precision, and low probability of damaging non cancer cells has become the next choice and research direction for medical clinical therapy development. The existence of glioblastoma is despair, but the appear of gene therapy technology which can target oncogenes or factors that promote cancer development, brings hope.

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