The Application of CRISPR-Cas System in Glioblastoma Research and Treatment

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Abstract: Glioblastoma is a malignant brain cancer with high mortality. Currently, there is no effective treatment that can effectively improve the survival of patients. Traditional gene editing tools, such as Zinc finger nuclease, RNAi and translational activator-like effector nucleases (TALENTS), have shown their advantages in treating glioblastoma. However, they have the disadvantages of high cost and difficult to operate. In recent years, with the discovery and development of the CRISPR-Cas system, its easy to design, inexpensive, and high flexibility make it gradually become a widely used gene editing tool and provide a strong boost for the research of glioblastoma. This article reviews the recent literature and summarizes the application of the CRISPR Cas system in the model establishment and therapy development of glioblastoma.

1 INTRODUCTION

Glioblastoma (GBM) is a highly aggressive brain cancer with a high mortality rate and no effective treatment. More than 10,000 people in the United States are diagnosed with glioblastoma each year. According to the America Association of neurological surgeons, the incidence of glioblastoma is approximately 3.22 per 100,000 people, and more than 50% of patients are diagnosed over the age of 65 years (Ostrom, 2019), and male patients are significantly more than female patients. The 5-year survival rate of glioblastoma is only 7.2% (Grochans, 2022). The average survival time of the patients is only 8 months. Therefore, there is an urgent need to find new therapeutic approaches to treat glioblastoma.

In order to better understand the pathogenesis of glioblastoma and find effective treatment options, traditional gene editing methods, such as RNA interference (RNAi). zinc finger nucleases (ZFP), translational activator-like effector nucleases (TALENTS), have been employed in the study of glioblastoma treatment (Lozada-Delgado, 2017; Marchiq, 2015; Duan, 2015). The discovery of genetic tools enables us to investigate cellular biology by manipulating gene expression. RNA interference (RNAi) is a biology process to suppress protein formation. In this process, small RNA fragments bind to target mRNA to create double-stranded RNA, which prevents the translation of target mRNA. RNAi has been widely used in the treatment of GBM. Lozada-Delgado provides a systematic summary of relevant studies in her 2017 review (Lozada-Delgado, 2017). Zinc-finger protein (ZFP) is a protein found in the human body that can interact with genes (Cassandri, 2017). Because of this characteristic, it can bind to nucleases and become a tool for gene editing. ZFP is part of the recognition gene sequence of zinc-finger nucleases (ZFN), and each Cys2-His2 tandem array can recognize base pairs arranged in triplicate. At the same time, each finger links a Fokl nuclease to achieve single-strand cleavage and double-strand break (Urnov, 2010). In a previous study, researchers found that knocking down the MCT4 and BASIGIN genes in the glioblastoma cell line U87 using ZFN slowed tumor cell growth and enhanced tumor cell sensitivity to drugs. This provides a new direction for the clinical treatment of glioblastoma (Marchiq, 2015). TALENs is mainly composed of termed repeat variable residues (RVDs) and a Fokl nucleic acids. Researchers can edit RVD to achieve different gene targeting by aim (Ousterout, 2016). TALENs have also been used as a gene editing tool to knock out PTEN gene sequence through homologous recombination to study the pathogenesis of glioblastoma (Duan, 2015)

Compared with the traditional gene editing method, CRISPR-Cas system is a more easy-to-do, time-efficient, and low-cost gene editing method. In recent years, with a large number of different CRISPR-Cas systems being developed, this emerging gene editing tool shows great potential in improving glioblastoma research and treatment. This article introduces the latest applications of CRISPR-Cas system in the research and treatment of glioblastoma.

2 CHARACTERISATION OF GBM

Glioblastoma is a highly invasive malignant brain tumor. The cause of glioblastoma is unclear, but recent studies have shown that the excessive proliferation of astrocyte, oligodendrocyte progenitor cells, and neural stem cells contributes to the develop of glioblastoma. Glioblastomas

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are classified as grade-IV tumors. Currently, the main basis for its diagnosis is the biopsy. According to 2016 WHO classification criteria, glioblastomas are mainly divided into three types: 1) Glioblastoma, isocitrate dehydrogenase (IDH) wildtype: IDH-wildtype accounts for 90% of the cases and is more common in patients over 60 years old; 2) Glioblastoma, IDH-mutant: IDH-mutant is more common in younger patients. 3) Glioblastoma not otherwise specified/NOS designations: Patients whose genotype could not be determined due to lack of biological material for testing (Louis, 2016). In recent years, not-elsewhere-classified (NEC) glioblastoma, a fourth category, has been used to classify those glioblastomas that does not matching in the existing criteria (Grochans, 2022).

The pathogenesis of glioblastoma is complex and many researchers have found that the abnormal expression of many genes is related to the metastasis and development of tumors, includes overexpression of oncogenes, such as IDH1, and mutation of tumor suppressor genes, such as PTEN. Also, mutations in the Telomerase Reverse Transcriptase (TERT) promoter are present in 80% of GBM (Grochans, 2022). Epidermal growth factor (EGFR) is associated with 40 percent of cases (Ekstrand, 1992). Methylation and mutations in PTEN (Phosphate and tensin homolog) are associated with 60 percent of all cases (Koul, 2008). Recently, many researchers have gained break through from other angles. Zhang et al. found that the overexpression of DHHC9 localized GLUT1 Palmitoylation and suggested that the abnormal expression of DHHC9 was highly correlated with the poor prognosis of GBM patients (Zhang, 2021). Wang et al. found that Brahma-Related Gene-1(BRG-1) had little effect on GBM propagation but its expression with GBM significantly enhanced its invasion and metastasis (Wang, 2021). However, the current research is not enough to give a full picture of the pathogenesis of GBM, and more mechanisms need to be explored.

At present, surgery combined with radiotherapy is the main treatment strategy for GBM. However, the feasibility of surgery depends on the location of the tumor, such as brain stem surgery being relatively high risk. Meanwhile, there is no established standard treatment strategy for recurrent GBM (Tan, 2020). The genetic and molecular exploration of GBM can help us better understand the pathogenesis of the disease and find better diagnoses and treatment approaches.

3 THE INTRODUCTION OF CRISPR-CAS SYSTEM

CRISPR-Cas System is a very important tool in neuroblastoma-related research. CRISPR is a series of repetitive gene fragments that were first discovered in bacteria. It is considered to be an immune mechanism against viral infection (Mojica, 2005). With a deeper understanding of it, we gradually understand its inner mechanism: CRISPR locus can recognize foreign genes and guide cas protein to break foreign genes at the position of NGG (protospacer adjacent motif) by generating crRNA (Terns, 2011). It gradually shows its potential as a gene editing tool and has attracted more and more researchers' attention (Mali, 2013). Different from the traditional gene editing methods such as RNAi, TALENs, and ZFNs, CRISPR-Cas System is easy to design, which greatly improves efficiency and reduces the cost of biological research such as disease modeling.

CRISPR-Cas system-mediated homolog-directed repair and non-homologous end joining were used to knock out, correct, and insert genes. In addition, many researchers have modified Cas nuclease to regulate gene expression from three aspects: transcriptional regulation (Gilbert, 2014), gene correction, and epigenetic regulation. Researchers have made cas variants that recognize protospacer adjacent motifs (PAMs) beyond the NGG sequence, thereby improving its diversity and targeting ability. Recently, Walton from Kleinstiver's lab invented a variant of SpRY nuclease. This variant can recognize almost all PAMs, which greatly improves editing flexibility (Walton, 20200. Other researchers have generated a new variant of cas protein, dCasp, by modifying cas protein to maintain its gene targeting ability but mute its nuclease function, and combined dCasp with other effectors to mediate a finely tuned gene silencing and other genetic and epigenetic manipulation. For example, Komor and Gaudelli fused deaminase to dCas to allow single base correction (Komor, 2016; Gaudelli, 2017). The combination of CRISPR dCas with transcriptional effector can also be used to regulate gene expression and repression (Gilbert, 2014). In addition, dCas can also bind to epigenetic modifiers to mediate gene methylation, demethylation, or histone acetylation (Lei, 2017; Xu, 2016; Hilton, 2015).

4 CRISPR-CAS SYSTEM IN GLIOBLASTOMA DISEASE MODELS

Glioblastoma is a large heterogeneous group of cells. It is extremely aggressive and invasive. Mutations occur continuously during the development and progression of the disease. These mutations may directly or indirectly contribute to the worsening of the disease. Therefore, understanding the heterogeneity of tumors is an important task. The easy-to-use nature of the CRISPR Cas system allows researchers to build more complex disease models.

4.1 Organoid

As an innovative biological model in recent years, organoids have been used to study many diseases. Organoid is a 3D cell culture using stem cell-derived cells. It represents the original physiological and architecture characteristics of the organs. Using gene editing, researchers have developed many organoid disease models that resembles the actual features of the diseases in the original organ (Lancaster, 2014). GBM organoid disease model is also a great system for studying GBM (Linkous, 2019). A research team has designed a neoplastic cerebral organoid (neoCOR). The team delivered plasmids encoding CRISPR Cas, gRNA, Sleepy Beauty transpose, and oncogenes and fluorescent reporters into cells by electroporation. These cells are then

used to generate neoCOR. This approach successfully induced tumorigenesis and found MYC overexpression and several combinations of tumor suppressor gene knockdown (CDKN2A-/-/CDKN2B-/-/EGFROE/EGFRvIIIOE; NF1-/-/ PTEN-/-/p53-/-; EGFRvIIIOE/CDKN2A-/-/PTEN-/-) are responsible for the tumorigenesis (Bian, 2018). Another team used 3D printing to design a 3D organoid model with four different cell lines. This model partially recapitulates the tumor microenvironment and explores the role of macrophages in GBM growth and metastasis. Using CRISPR Cas9 genome-wide screen, they found that stem cell marker genes such as SOX2 and OLIG2 were significantly upregulated. The whole genome gene screen was used to compare the difference in gene expression between the sphere culture and the 3D model. The 3D model is more sensitive to a hypoxia environment while sphere culture is more frequent in ion transport-related expression. They also found that the knockdown of PAG1 and ZNF830 delayed glioblastoma development in 3D models, suggesting their potential as therapeutic targets (Tang, 2020).

4.2 Mouse Model

With the development of the CRISPR-Cas system, researchers more tends to use the CRISPR-Cas system to establish mouse models. Chen et al. combined piggyBac tranposase and CRISPR-Cas9 system to achieve gene mutation in neocortical neural progenitors. Transposase is mainly used for the expression of fluorescent proteins, while other parts of the construction include sgRNAs targeting multiple oncogenes such as NF1, PTEN, and CRISPR cas system (Chen, 2015). Oldrini et al. combined the RCAS-TVA system with the CRISPR-Cas9 system to precisely control human tumor formation. The team demonstrated that the knockdown of known tumor suppressor genes (PTEN/p53/Cdkn2a etc.) greatly promoted the formation of glioblastoma. They also found that by introducing a pair of sgRNAs targeting two genes simultaneously and by chromosomal deletion and chromosomal translocation, they could achieve gene fusions with oncogenic potential (Bcan-Ntrk1; Myb-Qk) (Oldrini, 2018). In a similar study, researchers used CRISPR-Cas9 to knock out a single Ptch1 or multiple genes (Trp53, Pten, Nf1) to generate medulloblastoma and glioblastoma. By whole genome sequencing, it has confirmed that the Ptch1 gene knockout model generated by CRISPR-Cas9 showed no off-target mutant (Zuckermann, 2015). In conclusion, the CRISPR-Cas system is a fast and convenient gene editing tool and enables the development of genetically engineering mouse GBM disease models.

5 CRISPR-CAS SYSTEM IN GLIOBLASTOMA GENE THERAPY

5.1 Identify Genes Regulate the Development and Progression of GBM

In recent years, CRISPR-Cas9 has been widely used in the study of GBM treatment. Programmed Cell Death protein ligand 1 (PD-L1) is considered to be an immunity co-inhibitory factor. A team designed a dual-sgRNA/homolog-directed repair template transfection system to knock out PD-L1 in a human U87 glioma cell line in vitro. In the experimental group that successfully knocked out PD-1L, there was a significant reduction in tumor cell proliferation and metastasis and a shift of tumor-associated macrophages toward the M1 type (Fierro, 2022). These results suggest that PD-L1 is a potential therapeutical target for glioblastoma treatment. Similarly, a team from Uppsala University used CRISPR-Cas9 to knock out CD44 in the U251M cell line. CD44-/- glioblastoma cells showed reduction of growth and stemness, and induction of senescence (Kolliopoulos, 2022). Cancer-specific insertion-deletions attacker (CINDELA) system designed by the University of British Columbia can specifically target tumor cells (Kwon, 2022). The specificity of sgRNA is mainly determined based on the mutations in cancer cells, which are primarily small insertions and deletions, and whole-genome nucleotide sequences. In patient-derived glioblastoma cultures, AAV-CRISPR transfected tumor cells significantly reduced in size.

5.2 CRISPR Screening in GBM

In addition, the CRISPR-Cas9 system with barcode integrated sgRNA has been used to perform high-throughput screening in GBM, which effectively improves the efficiency of finding novel therapeutic targets to treat GBM. In a in vivo CRISPR screening, the investigators designed an AAV-SB-crisp system to identify genes that can improve the immunotherapeutic potential of CD8+ T cell in GBM treatment. This system integrates 6628 sgRNAs into CD8+ T cells in mice. These sgRNAs targeted the elimination of 1658 genes related to "Cell Surface". The transfected T cell population was injected into mice transplanted with glioblastoma. By screening the barcode corresponding to each sgRNA (depending on the specific AAV-SB100x) and further RNAi gene enrichment ranking (RIGER), they found that T cells carrying lag3, Pdia3, Mgat5, or Emp1 knockout mutations exhibited better proliferation ability at the tumor region. Furthermore, both syngeneic orthotopic and antigen-specific transgenic glioblastoma mice that transplanted with T cells knocked out Lag3, Mgat5, Pdia3, and emp1 have a longer survival time. Pdia3-knockout CAR-T cells were more potent to kill human U87 GBM cells. These results indicate the potential of Lag3, Mgat5, Pdia3, and emp1 as targeted genes in GBM immunotherapy (Ye, 2019). Bernareggi et al. used whole genome CRISPR-Cas9 system to transduced the Brunello short guide (sgRNA) library into four cell lines GSC1517,

387, CW468, and D456 to explore the mechanism of tumor immune responses to natural killer cells. In glioblastoma stem cells (GSCs), the team demonstrated that the knockdown of CHMP2A could increase the sensitivity of GSC to NK cell-mediated cytotoxicity and activate NF-kB to enable NK cell migration to the tumor site (Bernareggi, 2022). In addition, the expression of CHMP2A caused NK cell apoptosis and its anti-tumor ability. Similarly, Prolo use lentivirus to transduce sgRNA and cas9 protein into the U138 cell line to identify novel genes that are essential for glioblastoma invasion. The team combined sgRNA with mcherry and cas9 with blue fluorescence protein to directly observe successfully transduced cells. The successfully transduced cells were placed in a transwell. The cells in upper and bottom wells were collected and integrated sgRNAs were isolated and identified. The results showed that knockdown or inhibit MAP4K4 could significantly reduce tumor migration and invasion (Prolo, 2019).

5.3 CRISPR-Cas System and CAR-T in GBM Treatment

As an emerging tumor treatment method, CAR-T cells have showed a great treatment result in B-cell-resistant leukemia and lymphoma. However, due to local immunosuppression, CAR-T cell has limited treatment effect in solid tumors such as glioblastoma. For example, EFGR targeting CAR-T cells is a commonly used CAR-T cell in the treatment of non-solid tumors but its therapeutical function is limited by local immunosuppression in glioblastoma. To solve this problem, Choi et al. used CRISPR-Cas9 system to disrupt the expression of genes related to T-cell receptor (TCR) and beta-2-microglobulin (B2M) to suppress the activation of the PD1 pathway. The results showed that PD-1 knockout CAR-T cells had higher activity against tumors in vitro. Mice receiving CART-EGFRviiΔPD-1 had significantly increased survival time in vivo (Choi, 2019). In Another study, researcher used CRISPR-Cas9 to target the exons of PD-1. In contrast experiments with U-251MG (non-EGFRvIII expressing) and Ev-DKMG (EGFRvIII expressing), This mode of PD-1 knockdown did not independently affect tumor growth but could be combined with EFGR targeting CAR-T cells to increase the ability to inhibit tumor growth (Nakazawa, 2020). Diacylglycerol kinase can bind proteins such as protein kinase and Ras to affect multiple signaling pathways in tumor growth (Purow, 2015). So, it has received attention from other researchers. A team used lentivirus-loaded CRISPR cas9 to knock out DGK. In vitro results showed that CAR-T cells had increased resistance to, for example, TGFβ and were found to promote tumor regression in a mouse model of allogeneic transplantation (Jung, 2018). In another recent study, researchers found that inhibition of IFNγR expression in solid tumors, including glioblastoma, was associated with increased resistance to CAR-T cell therapy in a genetic screen using CRISPR cas9. Studies in-vivo and in-vitro models have shown that Mutations in proteins involved in IFNγR-mediated pathways (IFNGR1, JAK1, or JAK2) can block CAR-T cell adhesion and thus reduce CAR-T cell cytotoxicity to tumor cells (Larson, 2022). The findings of this study provide a new idea for CAR-T cells in the treatment of glioblastoma.

6 CONCLUSION

As a powerful gene editing tool, CRISPR-Cas system has widely applied to all aspects of glioblastoma research. It has great potential in establishing disease model, screening for therapeutical target, and employing as a gene therapy. However, so far, there are limited research focus on using the CRISPR-Cas system to regulate genes at the RNA level to treat tumors, which is safer and more controllable. Therefore, future research are required in such direction.

REFERENCES

1. Bernareggi, D., Xie, Q., Prager, B., Yun, J., Cruz, L., & Pham, T. et al. (2022). CHMP2A regulates tumor sensitivity to natural killer cell-mediated cytotoxicity. Nature Communications, 13(1). DOI: 10.1038/s41467-022-29469-0
2. Bian, S., Repic, M., Guo, Z., Kavirayani, A., Burkard, T., & Bagley, J. et al. (2018). Genetically engineered cerebral organoids model brain tumor formation. Nature Methods, 15(8), 631-639. DOI: 10.1038/s41592-018-0070-7
3. Cassandri, M., Smirnov, A., Novelli, F., Pitolli, C., Agostini, M., & Malewicz, M. et al. (2017). Zinc-finger proteins in health and disease. Cell Death Discovery, 3(1). DOI: 10.1038/cddiscovery.2017.71
4. Chen, F., Rosiene, J., Che, A., Becker, A., & LoTurco, J. (2015). Tracking and transforming neocortical progenitors by CRISPR/Cas9 gene targeting and PiggyBac transposase lineage labeling. Development. DOI: 10.1242/dev.118836
5. Choi BD, Yu X, Castano AP, Darr H, Henderson DB, Bouffard AA, Larson RC, Scarfò I, Bailey SR, Gerhard GM, Frigault MJ, Leick MB, Schmidts A, Sagert JG, Curry WT, Carter BS, Maus MV. CRISPR-Cas9 disruption of PD-1 enhances activity of universal EGFRvIII CAR T cells in a preclinical model of human glioblastoma. J Immunother Cancer. 2019 Nov 14;7(1):304. doi: 10.1186/s40425-019-0806-7. PMID: 31727131; PMCID: PMC6857271.
6. Duan, S., Yuan, G., Liu, X., Ren, R., Li, J., & Zhang, W. et al. (2015). PTEN deficiency reprogrammes human neural stem cells towards a glioblastoma stem cell-like phenotype. Nature Communications, 6(1). DOI: 10.1038/ncomms10068
7. Ekstrand, A.J.; Sugawa, N.; James, C.D.; Collins, V.P. Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. Proc. Natl. Acad. Sci. USA 1992, 89, 4309–4313.

8. Fierro, J., DiPasquale, J., Perez, J., Chin, B., Chokpapone, Y., Tran, A. M., . . . Dou, H. (2022). Dual-SGRNA CRISPR/cas9 knockout of Pd-L1 in human U87 glioblastoma tumor cells inhibits proliferation, invasion, and tumor-associated macrophage polarization. Scientific Reports, 12(1). doi:10.1038/s41598-022-06430-1
9. Gaudelli, N., Komor, A., Rees, H., Packer, M., Badran, A., Bryson, D., & Liu, D. (2017). Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature, 551(7681), 464-471. DOI: 10.1038/nature24644
10. Gilbert, L., Horlbeck, M., Adamson, B., Villalta, J., Chen, Y., & Whitehead, E. et al. (2014). Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation. Cell, 159(3), 647-661. DOI: 10.1016/j.cell.2014.09.029
11. Grochans, S., Cybulska, A., Simińska, D., Korbecki, J., Kojder, K., Chlubek, D., & Baranowska-Bosiacka, I. (2022). Epidemiology of Glioblastoma Multiforme–Literature Review. Cancers, 14(10), 2412. DOI: 10.3390/cancers14102412
12. Hilton, I., D'Ippolito, A., Vockley, C., Thakore, P., Crawford, G., Reddy, T., & Gersbach, C. (2015). Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. Nature Biotechnology, 33(5), 510-517. DOI: 10.1038/nbt.3199
13. Jung, I. Y., Kim, Y. Y., Yu, H. S., Lee, M., Kim, S., & Lee, J. (2018). CRISPR/Cas9-Mediated Knockout of DGK Improves Antitumor Activities of Human T Cells. Cancer research, 78(16), 4692–4703. https://doi.org/10.1158/0008-5472.CAN-18-0030
14. Koul D. (2008). PTEN signaling pathways in glioblastoma. Cancer biology & therapy, 7(9), 1321–1325. https://doi.org/10.4161/cbt.7.9.6954
15. Kolliopoulos, C., Ali, M., Castillejo-Lopez, C., Heldin, C., & Heldin, P. (2022). CD44 Depletion in Glioblastoma Cells Suppresses Growth and Stemness and Induces Senescence. Cancers, 14(15), 3747. DOI: 10.3390/cancers14153747
16. Komor, A., Kim, Y., Packer, M., Zuris, J., & Liu, D. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature, 533(7603), 420-424. DOI: 10.1038/nature17946
17. Kwon, T., Ra, J., Lee, S., Baek, I., Khim, K., & Lee, E. et al. (2022). Precision targeting tumor cells using cancer-specific InDel mutations with CRISPR-Cas9. Proceedings Of the National Academy of Sciences, 119(9). DOI: 10.1073/pnas.2103532119
18. Lancaster, M., & Knoblich, J. (2014). Organogenesis in a dish: Modeling development and disease using organoid technologies. Science, 345(6194). DOI: 10.1126/science.1247125
19. Larson, R. C., Kann, M. C., Bailey, S. R., Haradhvala, N. J., Llopis, P. M., Bouffard, A. A., Scarfó, I., Leick, M. B., Grauwet, K., Berger, T. R., Stewart, K., Anekal, P. V., Jan, M., Joung, J., Schmidts, A., Ouspenskaia, T., Law, T., Regev, A., Getz, G., & Maus, M. V. (2022). CAR T cell killing requires the IFNγR pathway in solid but not liquid tumours. Nature, 604(7906), 563–570. https://doi.org/10.1038/s41586-022-04585-5
20. Lei, Y., Zhang, X., Su, J., Jeong, M., Gundry, M., & Huang, Y. et al. (2017). Targeted DNA methylation in vivo using an engineered dCas9-MQ1 fusion protein. Nature Communications, 8(1). DOI: 10.1038/ncomms16026
21. Linkous, A., Balamatsias, D., Snuderl, M., Edwards, L., Miyaguchi, K., & Milner, T. et al. (2019). Modeling Patient-Derived Glioblastoma with Cerebral Organoids. Cell Reports, 26(12), 3203-3211.e5. DOI: 10.1016/j.celrep.2019.02.063
22. Louis, D., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., & Cavenee, W. et al. (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathologica, 131(6), 803-820. DOI: 10.1007/s00401-016-1545-1
23. Lozada-Delgado, E. L., Grafals-Ruiz, N., & Vivas-Mejía, P. E. (2017). RNA interference for glioblastoma therapy: Innovation ladder from the bench to clinical trials. Life sciences, 188, 26–36. https://doi.org/10.1016/j.lfs.2017.08.027
24. Marchiq, I., Le Floch, R., Roux, D., Simon, M. P., & Pouyssegur, J. (2015). Genetic disruption of lactate/H+ symporters (MCTs) and their subunit CD147/BASIGIN sensitizes glycolytic tumor cells to phenformin. Cancer Research, 75(1), 171–180. https://doi.org/10.1158/0008-5472.CAN-14-2260
25. Mali, P., Yang, L., Esvelt, K., Aach, J., Guell, M., & DiCarlo, J. et al. (2013). RNA-Guided Human Genome Engineering via Cas9. Science, 339(6121), 823-826. DOI: 10.1126/science.1232033
26. Mojica, F., Diez-Villasenor, C., Garcia-Martinez, J., & Soria, E. (2005). Intervening Sequences of Regularly Spaced Prokaryotic Repeats Derive from Foreign Genetic Elements. Journal Of Molecular Evolution, 60(2), 174-182. DOI: 10.1007/s00239-004-0046-3
27. Nakazawa, T., Natsume, A., Nishimura, F., Morimoto, T., Matsuda, R., Nakamura, M., Yamada, S., Nakagawa, I., Motoyama, Y., Park, Y. S., Tsujimura, T., Wakabayashi, T., & Nakase, H. (2020). Effect of CRISPR/Cas9-Mediated PD-1-Disrupted Primary Human Third-Generation CAR-T Cells Targeting EGFRvIII on In Vitro Human Glioblastoma Cell Growth. Cells, 9(4), 998. https://doi.org/10.3390/cells9040998
28. Oldrini, B., Curiel-García, Á., Marques, C., Matia, V., Uluçkan, Ö., & Graña-Castro, O. et al. (2018). Somatic genome editing with the RCAS-TVA-CRISPR-Cas9 system for precision tumor modeling. Nature Communications, 9(1). DOI: 10.1038/s41467-018-03731-w
29. Ostrom Q, Cioffi G, Gittleman H, Patil N, Waite K, Kruchko C. Barnholtz-Sloan JJN-o: CBTRUS

Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2012–2016. Neuro-Oncol. 2019; 21: v1–v100.

30. Ousterout, D., & Gersbach, C. (2016). The Development of TALE Nucleases for Biotechnology. Talens, 27-42. DOI: 10.1007/978-1-4939-2932-0_3
31. Prolo, L., Li, A., Owen, S., Parker, J., Foshay, K., & Nitta, R. et al. (2019). Targeted genomic CRISPR-Cas9 screen identifies MAP4K4 as essential for glioblastoma invasion. Scientific Reports, 9(1). DOI: 10.1038/s41598-019-50160-w
32. Purow, B. (2015). Molecular Pathways: Targeting Diacylglycerol Kinase Alpha in Cancer. Clinical Cancer Research, 21(22), 5008-5012. DOI: 10.1158/1078-0432.cc-15-0413
33. Tan, A., Ashley, D., López, G., Malinzak, M., Friedman, H., & Khasraw, M. (2020). Management of glioblastoma: State of the art and future directions. CA: A Cancer Journal for Clinicians, 70(4), 299-312. DOI: 10.3322/caac.21613
34. Tang, M., Xie, Q., Gimple, R., Zhong, Z., Tam, T., & Tian, J. et al. (2020). Three-dimensional bioprinted glioblastoma microenvironments model cellular dependencies and immune interactions. Cell Research, 30(10), 833-853. DOI: 10.1038/s41422-020-0338-1
35. Terns, M., & Terns, R. (2011). CRISPR-based adaptive immune systems. Current Opinion In Microbiology, 14(3), 321-327. DOI: 10.1016/j.mib.2011.03.005
36. Urnov, F., Rebar, E., Holmes, M., Zhang, H., & Gregory, P. (2010). Genome editing with engineered zinc finger nucleases. Nature Reviews Genetics, 11(9), 636-646. DOI: 10.1038/nrg2842
37. Wang, Y., Yang, C. H., Schultz, A. P., Sims, M. M., Miller, D. D., & Pfeffer, L. M. (2021). Brahma-related gene-1 (BRG1) promotes the malignant phenotype of glioblastoma cells. Journal of Cellular and Molecular Medicine, 25(6), 2956-2966. doi:10.1111/jcmm.16330
38. Walton, R. T., Christie, K. A., Whittaker, M. N. & Kleinstiver, B. P. (2020) Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants. Science 368 (6488), 290–296.
39. Xu, X., Tao, Y., Gao, X., Zhang, L., Li, X., & Zou, W. et al. (2016). A CRISPR-based approach for targeted DNA demethylation. Cell Discovery, 2(1). doi: 10.1038/celldisc.2016.9
40. Ye, L., Park, J., Dong, M., Yang, Q., Chow, R., & Peng, L. et al. (2019). In vivo CRISPR screening in CD8 T cells with AAV–Sleeping Beauty hybrid vectors identify membrane targets for improving immunotherapy for glioblastoma. Nature Biotechnology, 37(11), 1302-1313. DOI: 10.1038/s41587-019-0246-4
41. Zhang, Z., Li, X., Yang, F., Chen, C., Liu, P., Ren, Y., Sun, P., Wang, Z., You, Y., Zeng, Y. X., & Li, X. (2021). DHHC9-mediated GLUT1 S-palmitoylation promotes glioblastoma glycolysis and tumorigenesis. Nature communications, 12(1), 5872. https://doi.org/10.1038/s41467-021-26180-4
42. Zuckermann, M., Hovestadt, V., Knobbe-Thomsen, C., Zapatka, M., Northcott, P., & Schramm, K. et al. (2015). Somatic CRISPR/Cas9-mediated tumour suppressor disruption enables versatile brain tumour modelling. Nature Communications, 6(1). DOI: 10.1038/ncomms8391