Discover Oncology

Review

Unveiling the significance of cancer-testis antigens and their implications for immunotherapy in glioma

Shenghua Zhuo[1,2] · Shuo Yang[2] · Shenbo Chen[1] · Yueju Ding[1] · Honglei Cheng[1] · Liangwang Yang[1] · Kai Wang[2] · Kun Yang[1]

Received: 9 May 2024 / Accepted: 11 October 2024
Published online: 29 October 2024

Abstract
Glioma has a poor prognosis, which is attributable to its inherent characteristics and lack of specific treatments. Immunotherapy plays a pivotal role in the contemporary management of malignancies. Despite the initiation of numerous immunotherapy-based clinical trials, their effects on enhancing glioma prognosis remain limited, highlighting the need for innovative and effective therapeutic targets and strategies to address this challenge. Since the 1990s, there has been a growing interest in cancer-testis antigens (CTAs) present in normal mammalian testicular germ cells and placental trophoblast cells, which exhibit reactivated expression in various tumor types. Mechanisms such as DNA methylation, histone modification, transcriptional regulation, and alternative splicing influence the expression of CTAs in tumors. The distinct expression patterns and robust immunogenicity of CTAs are promising tumor biomarkers and optimal targets for immunotherapy. Previous reports have shown that multiple CTAs are present in gliomas and are closely related to prognosis. The expression of these antigens is also associated with the immune response in gliomas and the effectiveness of immunotherapy. Significantly, numerous clinical trials, with IL13RA2 as a representative CTA member, have assessed the immunotherapeutic potential of gliomas and have shown favorable clinical efficacy. This review provides a comprehensive overview of the regulation and function of CTAs, summarizes their expression and role in gliomas, emphasizes their importance as immunotherapy targets in gliomas, and discusses related challenges and future interventions.

Keywords Glioma · Cancer-testis antigen · Prognosis · Immunotherapy

1 Introduction

According to statistics, the mortality rate of central nervous system (CNS) tumors in China ranked among the top 10 in all age groups from 2005 to 2020 [1]. Glioblastoma (GBM), which accounts for more than half of all malignant tumors, is the most predominant histopathological malignant CNS tumor. GBM is more prevalent in males, with a 5-year survival rate of only 6.9% [2]. In the last four decades, the prevention, early detection, and treatment of GBM demonstrated minimal notable advancements. In comparison with the period of 1975–1977, the 5-year survival rate of patients with GBM exhibited a modest increase from 4 to 7% from 2009 to 2015 [3]. The prognosis remains unfavorable despite use of the

Shenghua Zhuo and Shuo Yang have contributed equally as the first author.

✉ Shenghua Zhuo, zsh1858588@muhn.edu.cn; ✉ Kai Wang, kai.wang@muhn.edu.cn; ✉ Kun Yang, chbyk1379@muhn.edu.cn | [1]Department of Neurosurgery, the First Affiliated Hospital of Hainan Medical University (Hainan Academy of Medical Sciences), Haikou, China. [2]International Center for Aging and Cancer, Hainan Medical University (Hainan Academy of Medical Sciences), Haikou, China.

comprehensive treatment methods, including surgery, radiotherapy, and chemotherapy for diffuse glioma. Patients with glioma have minimal benefit from numerous targeted drugs despite their development [4]. The field of immunotherapy research is still in its early stages. Challenges, such as the immunosuppressive microenvironment and heterogeneity within tumors, have hindered the success of several clinical trials involving checkpoint inhibitors and vaccines, causing limited advancements in the prognosis of patients with glioma [5].

Tumor antigens are potential therapeutic targets for fighting tumors because of their selective expression in tumor cells and minimal presence in normal tissues[6]. Cancer-testis antigens (CTAs) represent a distinct class of antigens associated with tumors, demonstrating a unique expression profile [7]. Functionally, CTAs are involved in gene expression regulation within tumors, contribute to tumorigenic signaling, promote tumor growth, and inhibit tumor cell apoptosis [8, 9]. This antigen holds significant promise for advancement in tumor immunotherapy, including the development of tumor vaccines, due to its typical expression in germline cells, along with various cancer activation and immunogenic properties [10–12]. Recently, numerous clinical trials focusing on CTAs have been conducted [13].

Different CTAs have diverse expression levels in glioma [14–16]. Glioma stem cells exhibit significantly higher CTA genes expression than differentiated cells [14]. Knocking down the CTAs in undifferentiated and differentiated GBM stem cell types has diverse effects, resulting in changes in neural stem cell marker expression or a reduction in cell density, possibly leading to growth arrest or cell death [15]. Five CTAs (BAGE, MAGE-A12, CASC5, DDX43, and IL-13RA2) were positively expressed in GBM tissues and cell lines [16]. Additionally, CTAs are closely associated with the prognosis of patients with glioma [17–19]. Elevated MAGE-E1, GAGE, and SOX-6 levels are correlated with poorer GBM prognosis [17]. Moreover, elevated FMR1NB [18] and F-box protein 39 (FBXO39) [19] levels are also indicative of a poorer GBM prognosis. FBXO39 boosts the invasion and migration abilities of glioma cells, along with stimulating the growth and stemness of glioma stem cells [19]. CTAs expression in gliomas correlates with immune response [20, 21] and has potential implications in immunotherapy [22, 23]. Recent clinical trials investigating IL13RA2-based immunotherapy have demonstrated favorable safety profiles and promising clinical efficacy in patients with recurrent high-grade glioma [24, 25]. These findings reveal that CTAs expression in malignant tumors has gained significant attention while conducting recent research, particularly in the context of glioma. Their unique expression patterns are potential targets of immunotherapeutic strategies to improve treatment outcomes. Understanding the regulatory mechanisms and functional roles of CTAs in glioma is crucial for advancing immunotherapy approaches. This review focuses on CTAs regulation and function, provides a summary of their expression levels, prognostic significance, and advancements as targets for immunotherapy in gliomas, and discusses the challenges and future intervention measures faced as therapeutic targets.

2 Immunotherapy of gliomas

The effectiveness of immunotherapy makes it a powerful tool for treating various malignant tumors. For example, the clinical use of PD-1 and CTLA-4 monoclonal antibodies has significantly altered the treatment paradigm for a range of malignant tumors [26]. GBM is widely recognized as an immune "cold" tumor and is distinguished by a distinct immune cell infiltration pattern and vasculature [27]. However, the majority of immunotherapies attempted to date have not succeeded in enhancing the clinical outcomes of patients with glioma. For instance, numerous phase 3 controlled trials assessing anti-PD-1 therapy have not revealed survival benefits for individuals with GBM [28]. A recent review provided a comprehensive summary of the tumor immune microenvironment and regulatory mechanisms, thoroughly examining various immunotherapy methods, including monoclonal antibodies, cytokine therapy, vaccination, and adoptive cell therapy, for gliomas [29–31]. The current search for enhanced immunotherapy targets is pivotal for tailoring treatment, identifying patients with robust responses, and selecting optimal interventions for better prognostic. To prevent repetition, this section outlines the latest therapeutic targets and treatment strategies for glioma.

The administration of oncolytic herpes simplex-1 virus (oHSV) stimulates the production and release of IGF2 into the tumor microenvironment (TME), thereby hindering the therapeutic response. Precise targeting and inhibition of IGF2 can reconfigure the TME, thereby enhancing the efficacy of oHSV [32]. In a randomized phase II clinical trial, the combination of autologous tumor lysate-pulsed dendritic cell vaccine and Toll-like receptor agonists in patients with newly diagnosed or recurrent WHO grade III–IV malignant gliomas was proven to be safe and effective in boosting systemic immune responses. Patients exhibiting increased expression of interferon-response genes have been shown to experience prolonged survival and delayed disease progression [33]. Researchers have created a second-generation chimeric antigen receptor (CAR) namely CARv3-TEAM-E T-cells, to target epidermal growth factor receptor variant III (EGFRvIII), which is capable of releasing a T-cell-engaging antibody directed against wild-type EGFR. Clinical trials have demonstrated no significant adverse events

or dose-limiting toxicities. Notably, significant and rapid radiographic tumor regression was observed in three participants with recurrent GBM, whereas transience in the responses was noted in two participants [34]. Pretreatment with the AMPK activator metformin and the mTOR inhibitor rapamycin enhances CAR-T cell metabolism and exerts enduring and potent anti-glioma cytotoxic activity under hypoxic conditions [35]. Novel FAP-CAR-T cells can induce antigen-dependent endogenous bystander killing of GBM cells [36].

Regarding immune checkpoint blockade therapy, several studies have reported that when combined with other interventions, good sensitization effects were observed. Administration of the STING agonist 8803 reshapes the immune microenvironment and improves survival in preclinical models of GBM when combined with anti-PD-1 therapy [37]. The concurrent inhibition of CCR2 and CCR5 alters the immunosuppressive microenvironment in gliomas and enhances the efficacy of anti-PD-1 therapy [38]. Bacterial photothermal therapy can induce an immunogenic TME and synergize with PD-1 blockade to enhance the efficacy of glioma immunotherapy [39]. An injectable thermogel loaded with the GLUT1 inhibitor BAY-876 and the PD-1/PD-L1 blocker BMS-1 can effectively modulate both GBM metabolism and immunity, thereby augmenting the immunotherapy effect [40]. Moreover, performing an ultrasound to transiently open the blood–brain barrier (BBB) is another crucial strategy. Low-intensity pulsed ultrasound (LIPU) combined with intravenously administered microbubbles (MB) was used to open the BBB and enhance the accumulation of liposomal doxorubicin and PD-1 blocking antibody in the brain. This approach induces immune modulation and enhances the efficacy of PD-1 blockade for treating gliomas [41]. An innovative combination immunotherapy regimen comprising Fc-enhanced anti-CTLA-4, anti-PD-1, doxorubicin, and LIPU/MB achieved a remarkable 90% cure rate in a mouse model of immunotherapy-resistant glioma. This comprehensive approach is currently being evaluated in clinical trials (NCT05864534) [42]. In summary, these studies indicate that integrating immunotherapy with novel therapeutic targets or approaches can significantly enhance the outcomes of both preclinical and clinical investigations (Fig. 1).

3 CTAs discovery and classification

The initially identified CTA includes MAGE1, which is found in melanoma in the 1990s through autologous typing techniques and confirmed by DNA cloning to be recognized by T-cells. Notably, this antigen is not detected in any normal tissues except for the testes [43, 44]. SEREX technology is used for immune screening with patient sera to identify tumor antigens using a recombinant cDNA phage display library system. This method overcomes the limitations associated with autologous typing for establishing cytotoxic T-cell lines from patients with tumors and autologous tumor cell lines. Its key advantage lies in the capacity to determine intracellular antigens, thereby facilitating the broader discovery of CTAs [45, 46]. Chen et al. successfully identified New York esophageal squamous cell carcinoma 1 (NY-ESO-1) as a prominent immunogenic target that is widely used in contemporary immunotherapeutic approaches through the application of this advanced technology. Notably, this study pioneered the use of the term "cancer/testis antigens" to characterize a distinctive protein family exclusively expressed in both testicular and cancerous cells [47]. Subsequent studies revealed CTAs expression in the immature germ and granulosa cells of the fatal ovaries [48]. In the subsequent decade, an increasing number of CTA molecules exclusively expressed in granulosa, germ, and cancer cells have been discovered [49, 50]. The advent of high-throughput polymerase chain reaction (PCR) and sequencing technologies has caused a rapid increase in the number of recognized CTA members, with > 250 CTA genes now being cataloged in public databases [51]. CTAs are categorized according to their tissue expression patterns as testis-restricted, testis/brain-restricted, and testis-selective groups that show additional expression in somatic tissues. CTAs are predominantly categorized based on their chromosomal origin of encoding into the CT-X antigen group, located on the X chromosome, and the non-X chromosome-encoded CTA group, which exhibit testis-restricted expression despite being encoded on non-X chromosomes. CT-X antigen expression is notably more limited in testes than in non-CT-X antigens [52].

4 Regulation and function of CTAs

4.1 Regulation of CTAs

4.1.1 DNA methylation

The characteristic features of cancer cells include genome-wide hypomethylation and CpG island hypermethylation associated with tumor suppressor genes and developmental regulatory factors [53]. CTAs remain inactive during differentiation into somatic cells but are expressed in germ cells and nurse cells. A phase of global DNA hypomethylation

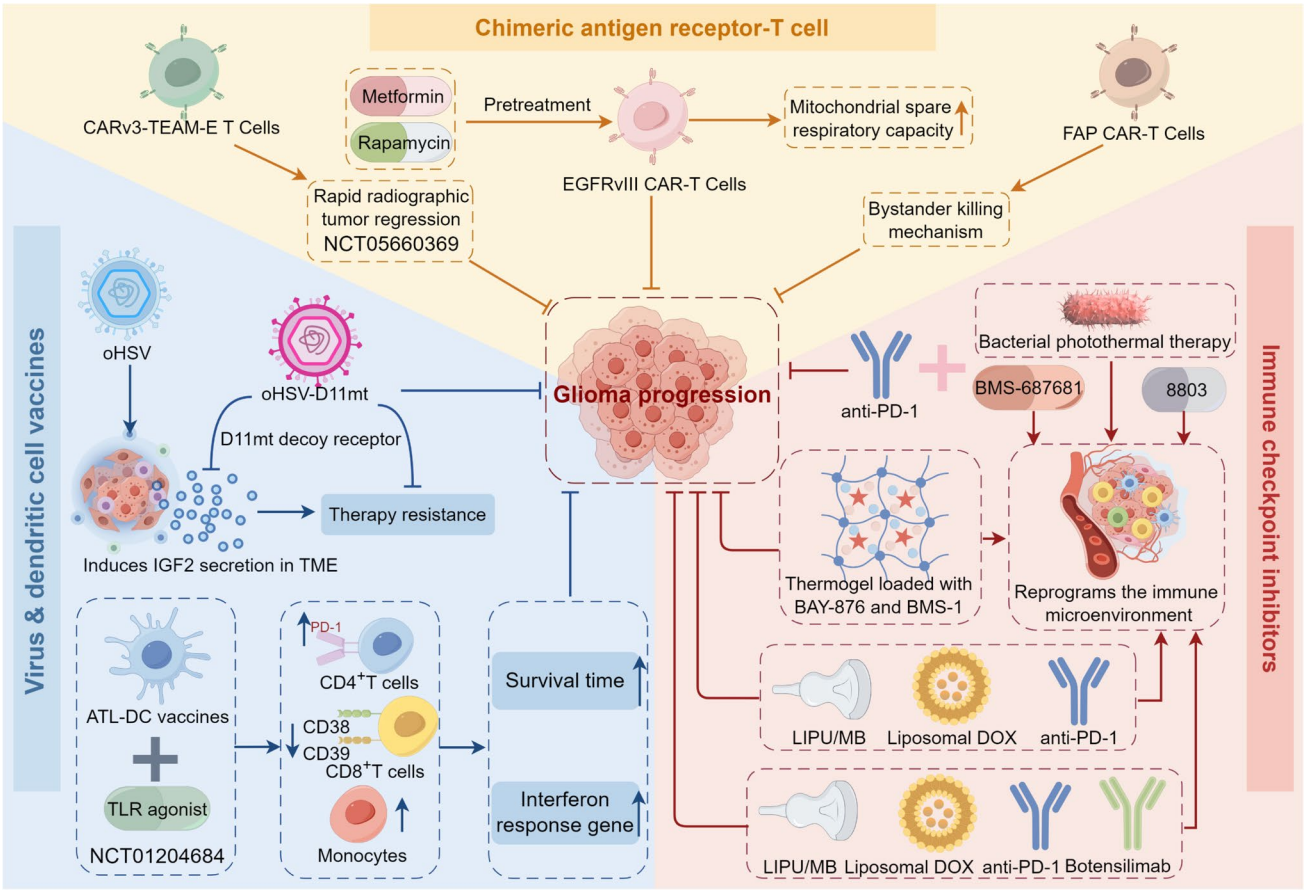

Fig. 1 The latest therapeutic targets and treatment strategies in glioma combined with immunotherapies such as oncolytic viruses, dendritic cell vaccines, chimeric antigen receptor-T cells and immune checkpoint inhibitors. *oHSV* oncolytic herpes simplex-1 virus, *IGF2* insulin like growth factor 2, *TME* tumor microenvironment, *ATL-DC* autologous tumor lysatepulsed dendritic cell, *Metformin* AMPK activator, *Rapamycin* mTOR inhibitor, *BMS-687681* CCR2/CCR5 inhibitor, *8803* STING agonist, *BAY-876* GLUT1 inhibitor, *BMS-1* PD-1/PD-L1 blocker, *LIPU/MB* low-intensity pulsed ultrasound and microbubbles, *DOX* doxorubicin, *Botensilimab* Fc-enhanced anti-CTLA-4 antibody. The Figure was drawn by Figdraw

occurs throughout gametogenesis, which is followed by DNA methylation and chromatin modification [54]. The CpG promoter of *MAGE-A1* is hypomethylated in cancer cells, and demethylation is both a necessary and sufficient condition for its expression [55, 56]. Several subsequent studies determined a pattern of DNA hypomethylation in the promoter regions of various CTAs, which are essential for maintaining their expression in tumors. *MAGE-A1* and *MAGE-A3* demethylation are frequently observed during advanced gastric cancer stages, potentially related to the aggressive biological behavior of gastric cancer [57]. Aberrant *MAGEB2* demethylation can manifest in a subset of malignant peripheral nerve sheath tumors associated with type 1 neurofibromatosis [58]. CT45 is a natural tumor antigen controlled by DNA methylation and can augment the chemosensitivity of ovarian cancer while also serving as an immunotherapeutic target [59]. DUSP22 rearrangement induces DNA hypomethylation in anaplastic large-cell lymphoma, causing CTAs overexpression [60]. Pericentromeric activation, global hypomethylation, and site-specific hypomethylation work together to enhance the *POTE* expression in ovarian cancer [61]. Elevated KK-LC-1 (CT83) expression in hepatocellular carcinoma is related to hypomethylation of CpG islands, promoting cancer progression through Notch1/Hes1 signaling pathway activation [62]. Certain CTAs are modulated by other CTAs through DNA methylation. The interaction between the transcription factors CTCF and BORIS at the *NY-ESO-1* promoter plays a crucial role in the epigenetic control of *NY-ESO-1* expression in cancer cells [63]. BORIS interacts with the demethylated promoter of *MAGEA3*, causing a transition toward more accessible chromatin conformations and upregulating its expression [64]. Moreover, MAGEA1 interacts with the *BORIS* promoter to recruit DNMT3a to improve promoter methylation and inhibit *BORIS* expression [65].

The application of DNA methyltransferase inhibitors (DNMTi) not only demonstrates CTAs regulation by DNA methylation but also induces CTAs expression. In 1994, Weber et al. revealed that the use of DNMTi decitabine (DAC) increased the expression of *MAGE1* in treated cells, marking the initial proof that CTAs are under the control of DNA methylation [66]. Subsequent research has revealed that the administration of this epigenetic modifier prompt tumors to re-establish or improve CTAs expression. Treatment of the heavily methylated cell lines SNU620 and HT29 with DAC causes *XAGE-1* promoter demethylation and subsequent gene expression [67]. The treatment of chronic lymphocytic leukemia cells with DAC prolonged NXF2 expression for multiple weeks while concomitantly improving the levels of major histocompatibility complex (MHC) and co-stimulatory molecules essential for effective antigen presentation [68]. MAGE-A3 expression was significantly increased in esophageal squamous cell carcinoma cells and tissues influenced by DNA methylation after DAC treatment [69]. The study conducted experiments in both in vivo and in vitro settings to provide evidence that DAC treatment amplifies immune therapy response. These results indicate that DAC can improve the clinical effectiveness of MAGE-A3-specific T-cell therapy by upregulating antigen expression [69]. VCX2 is exclusively detected in a minority of patients with melanoma tumors, whereas its expression in cancer cells is stimulated by the second-generation DNMTi guadecitabine [70]. DAC treatment triggers the expression of PD-L1 and NY-ESO-1 in metastatic colorectal cancer [71]. Moreover, the use of DAC may facilitate the discovery of new CTAs. A prototypical example is the serine protease PRSS56, which is a novel CTA that is reactivated in colorectal and gastric cancer cells by promoter DNA hypomethylation after DAC treatment, thereby exerting carcinogenic effects through the PI3K/AKT pathway [72]. The "stealth" antigen sperm equatorial segment protein 1 (SPESP1), which was discovered after DAC treatment, is exclusively expressed in normal tissues of the testes and placenta. Conversely, its presence is detected in the tumor cells of patients with acute myeloid leukemia or lung cancer, indicating significant immunogenicity [73]. Collectively, these studies indicate that DNA hypomethylation is a major mode of CTAs expression regulation. Notably, although CTAs' promoter DNA hypomethylation increases spontaneously or is induced by drugs, different DNA methylation patterns must be considered. In particular, a study revealed that the *CCNA1* and *TMEM108* genes are notable examples of CTAs that exhibit hypermethylation whereas most CTAs exhibit hypomethylation in colon cancer [74].

4.1.2 Histone modification

Histones are alkaline proteins located within chromosomes that are integral to DNA binding. An array of histone modification enzymes post-translationally modify the N-terminal tails of histones through mechanisms, such as methylation, acetylation, butyrylation, lactylation, and other modifications, collaboratively composing the complex "histone code." Diverse types of histone post-translational modifications manifest abnormally in tumors, where they interact either cooperatively or antagonistically. The current research focus is on investigating multifaceted molecular regulatory mechanisms to identify crucial targets for cancer therapy [75]. Histone modifications are another epigenetic mechanism involved in the regulation of CTAs expression, besides DNA methylation. Different complexes, including HDAC1-mSin3a-NCOR1, Dnmt3b-HDAC1-Egr1, and Dnmt1-PCNA-UHRF1-G9a, are consistently engaged in *NY-ESO-1* gene regulation in gliomas and meningiomas [76]. The H3K27M mutation of histone H3 is prevalent in pediatric high-grade gliomas, causing DNA hypomethylation and increasing the expression of various CTAs such as ADAMTS1, ADAM23, SPANXA1, SPANXB1/2, IL13RA2, VCY, and VCX3A. Specifically, H3K27me3 levels on the *VCX3A* promoter are decreased, whereas H3K4me3 levels are increased. Gene set enrichment analysis indicated a high abundance of CTAs containing H3.3K27M in clinical samples, with IL13RA2 upregulation playing a significant role in this enrichment [77].

Histone deacetylase inhibitors (HDACi) are extensively used to confirm the effect of acetylation on CTAs expression. Specifically, HDACi trichostatin A (TSA) reversed the silencing of the *MAGE* gene, emphasizing that the process of *MAGE* gene inhibition is associated with high methylation levels and histone deacetylation [78, 79]. Azacytidine (AZA) and sodium valproate (VPA), also known as DNMTi and HDACi, respectively, increase MAGE antigen expression in acute myeloid leukemia (AML) and myeloma cell lines. Previous AZA/VPA exposure improves the detection of MAGE-specific cytotoxic T-lymphocytes (CTLs) clones against tumor cell lines. Moreover, patients with AML or myeloma treated with both agents showed improved treatment responses [80]. The combination of DAC and HDAC inhibitor MGCD0103 stimulates MAGE-A3 expression and boosts the cytotoxic activity of MAGE-A3-specific CTLs in MAGE-A3-negative multiple myeloma (MM) [81]. HDAC inhibitor pretreatment augments the cytolytic activity of NY-ESO-1-specific CTLs against NY-ESO-1-positive soft tissue sarcoma cell line SW982 [82]. Similar findings were noted in cases of malignant pleural mesothelioma [83]. The lysine-specific demethylase 1 (LSD1) inhibitor clogylene induces global DNA demethylation, suppresses LSD1-mediated demethylation of H3K4me2 and H3K4me1, reactivates CTAs expression, and synergistically interacts with DAC [84]. The downregulation of histone methyltransferases KMT6 and KDM1, or the histone demethylase

KDM5B, markedly augmented the DAC-induced activation of *NY-ESO-1*, *MAGE-A1*, and *MAGE-A3* in lung cancer cells [85]. Allogeneic lymphocytes that express a modified T-cell receptor, which is capable of targeting NY-ESO-1 and MAGE-A3, specifically recognize and induce lung cancer cell lysis after combined treatment with DAC and the KMT6 inhibitor DZNep [85]. These studies revealed that histone modifications, particularly those involving methylation and acetylation, represent a significant regulatory mechanism for CTAs expression in tumors, demonstrating synergistic interactions with DNA methylation.

4.1.3 Transcriptional regulation

Apart from the aforementioned epigenetic controls, other regulatory mechanisms participate in CTAs regulation, including transcriptional control. The transcription factor Sp1 predominantly interacts with DNA sequences that harbor a GC box (GGGCGG or GGCGGG), thereby either enhancing or repressing gene transcription. Numerous CTAs comprise promoter regions that are abundant in CpG sites, which render these promoters potential targets for Sp1 binding and regulation. Sp1 contributes to the *MAGEA11* promoter's activity and gene expression in prostate cancer and epithelial ovarian cancer cells, whereas DNA methylation and nucleosome occupancy play contrasting roles [86]. The transcription factor BORIS (CT27) in lung cancer cells recruits Sp1 to mediate the transcriptional activity of *NY-ESO-1*, and inhibiting Sp1 expression reduces *NY-ESO-1* promoter activity [87]. Furthermore, BORIS stimulated *TSP50* expression, which is regulated by BORIS accessibility and binding to the promoter [88]. Various other transcription factors are involved in controlling the CTAs expression. Particularly, functional p53 negatively regulates the *BORIS* promoter [89]. The fusion transcription factor EWSR1-FLI1 in Ewing sarcoma enhances *FATE1* (CT43) expression [90]. The transcription factor AP-1 binds to the *MAGEC2* promoter in a Ca^{2+}-dependent manner to increase its expression in triple-negative breast cancer [91]. The meiotic transcription factor STRA8 may regulate the meiotic protein HORMAD1 (CT46) in cutaneous squamous cell carcinoma [92]. DNA methylation and histone modification affect the activity of transcription factors. Methylation of specific CpG sites may impede the interaction of transcription factors with DNA, resulting in transcriptional repression. Similarly, histone modifications influence chromatin structure and nucleosome organization, consequently modulating the binding of transcription factors and gene expression levels.

4.1.4 Alternative splicing

Alternative splicing (AS) is another significant regulatory mechanism for CTAs. AS plays a crucial role in improving protein and functional diversity, regulating gene expression, and modulating cellular response capabilities. Dysregulation of AS represents a predominant molecular feature across diverse tumor types and facilitates tumorigenesis through multiple molecular mechanisms [93]. CTA-related genes generally demonstrate a high degree of complexity in their splicing patterns and frequently undergo AS events. The *LAGE-1* gene within a melanoma cell line exhibits the AS capacity, causing the production of two primary transcripts that encode peptides, consisting of 210 and 180 residues, respectively [94]. The *MMA-1a* gene is located on chromosome 21q22.2, where its mRNA is composed of four exons. In contrast, the splice variant *MMA-1b* is characterized by the absence of exon 3 [95]. Further investigations have revealed four more splice variants of *MMA-1a*, designated as *MMA1C*, *MMA1D*, *MMA1E*, and *MMA1F*, with *MMA1C*, *MMA1D*, and *MMA1E* belonging to the CTAs family [96]. The *SSX2*, *SSX4*, *SSX5*, and *SSX7* genes within the SSX gene family of CTAs demonstrate AS variants [97]. L552S in pulmonary adenocarcinoma is identified as an AS variant of XAGE-1, characterized by excessive expression and immunogenic properties [98]. Both *CSAGE* and *TRAG-3* exhibit AS isoform in chondrosarcoma cell lines [99]. LIPI (CT17) in Ewing tumor cells demonstrates several transcript variants, all of which were derived via AS [100]. Additionally, the control of AS leads to the formation of various isoforms of *BORIS* [101]. Other than the main regulatory mechanisms discussed above, pathways, such as proteasomal degradation, may affect the expression and function of CTAs. In particular, the stability, solubility, and cellular localization of NY-ESO-1 and MAGE-C1 are regulated by proteasomal degradation [102]. Collectively, the induction and integration of these expression regulatory patterns facilitate CTAs reactivation in cancer cells.

4.2 Function of CTAs

According to the previous review, CTAs are involved in a variety of cellular processes related to cancer, including transcription network regulation in tumor cells, protein degradation modulation to counter tumor suppression mechanisms,

and critical cellular function participation such as cell division, genomic instability, DNA damage response, apoptosis evasion, metastasis, stemness maintenance, autophagy, and TME. Extensive evidence highlights the multifaceted roles of CTAs in human cancer and their pivotal contribution to the initiation or reactivation of key cancer hallmarks [8, 13]. These effects represent the pathways by which cancer develops diverse biological capabilities, thereby manifesting the cancer phenotype, which is specifically characterized by the acquisition of distinct cancer hallmarks [103–105]. This section does not extensively categorize their functions but instead expounds on their roles through representative studies, considering a comprehensive summary of CTA roles in the previous review, to prevent repetition.

PRAME serves as a prognostic indicator for various cancers, including melanoma, neuroblastoma, serous ovarian adenocarcinoma, and breast cancer. RRAMEF2 (PRAME family member 2) is a CTA that belongs to the distinctive PRAME multigene family, demonstrating considerable homology and similar expression patterns to PRAME. PRAMEF2 levels decrease FOXP3-dependently under metabolic stress in breast cancer cells. PRAMEF2 facilitates the polyubiquitination of the LATS1 in Hippo/YAP pathway, causing its proteasomal breakdown. LATS1 degradation promotes the nuclear accumulation of the transcriptional coactivator YAP, thereby amplifying malignant characteristics and indicating the pivotal role of PRAMEF2 in YAP-driven oncogenic signaling [106]. ATPase family AAA domain-containing protein 2 (ATAD2) is a member of the CTA family. ATAD2, which functions as a transcription factor coactivator, is involved in epigenetic modifications and plays a role in regulating the expression of downstream oncogenes or tumor suppressor genes [107]. Accumulated research evidence indicates that ATAD2 drives carcinogenesis by modulating chromatin, inducing uncontrolled cancer cell proliferation, inhibiting apoptosis, accelerating cancer cell cycle progression, facilitating cancer epithelial–mesenchymal transition and metastasis, and potentially serves as a prognostic and predictive marker for clinical outcomes and drug responses in cancer [107]. Importantly, *ATAD2* is a crucial gene that regulates melanoma formation. *ATAD2* knockout in a zebrafish model of melanoma susceptibility prevented cell transformation into tumors, even in the presence of oncogenic and tumor suppressor mutations. Conversely, *ATAD2* reintroduction restored the cells' tumorigenic potential. Targeting ATAD2 has the potential to be a novel cancer treatment approach [108].

Apart from exerting pro-cancer effects, certain CTAs play significant roles in treatment responses. Studies have revealed a high expression of GAGE in cervical cancer that is resistant to radiotherapy. In particular, the GAGE12 protein variant interacts with the intermediate filament synemin and localizes to chromatin, thereby promoting radiotherapy resistance. This variant facilitates the association of HDAC1/2 with the inhibitor, actin, thereby elevating histone 3 lysine 56 acetylation (H3K56Ac) levels, improving chromatin accessibility, and enhancing DNA repair efficiency. Disruption of GAGE-linked complexes can potentially restore radiosensitivity [109]. An additional study demonstrated that CT45 acts as an independent prognostic factor in high-grade serous ovarian cancer, with its elevated expression significantly related to prolonged disease-free survival in advanced patients. CT45 directly interacts with the PP4 phosphatase complex, thereby modulating its activity. Increased CT45 expression causes DNA damage and increases sensitivity to platinum-based chemotherapy drugs. Human leukocyte antigen (HLA) class I peptides derived from CT45, identified through immunopeptidomics, can activate patient-derived CTLs and improve tumor cell destruction [59].

The unique expression patterns of CTAs frequently position them as highly promising targets for immunotherapy, bolstered by a wealth of clinical trial data that validate this perspective. A clinical trial involving patients with NY-ESO-1-positive metastatic melanoma or metastatic synovial sarcoma refractory to standard therapies administered an adoptively transferred therapy using autologous T cells transduced with a T-cell receptor (TCR) directed against NY-ESO-1. Out of 6 patients with synovial sarcoma, 4 demonstrated objective clinical responses, and 5 of 11 patients with melanoma exhibited positive responses. Notably, 2 patients with melanoma achieved complete regression and maintained it for 1 year, whereas 1 patient with synovial sarcoma experienced partial relief for 1.5 years [110]. A phase I/II trial involving patients with MM will evaluate the safety and efficacy of autologous T-cells transduced with a naturally processed peptide TCR targeting NY-ESO-1 and LAGE-1. The results revealed that the infusion was well-tolerated without any clinically significant cytokine release syndrome. The engineered T cells not only expanded and persisted but also trafficked to the bone marrow, demonstrating a cytotoxic phenotype. Notably, 16 of 20 (80%) patients in the late stage demonstrated positive clinical responses, with a median progression-free survival of > 19 months [111].

A multicenter dose-escalation trial (NCT03132922) evaluated afami-cel, a TCR-T-cell therapy designed with improved autologous peptide affinity, in patients with recurrent/refractory solid tumors expressing MAGE-A4. The trial revealed that afami-cel effectively infiltrated tumors, highlighting a favorable benefit-risk profile and providing early and durable responses. Particularly noteworthy were the outcomes for patients with metastatic synovial sarcoma, who experienced a median response duration of 28.1 weeks [112]. The phase 2 open-label trial (NCT04044768), conducted on a larger scale, evaluated the efficacy, safety, and tolerability of afami-cel in patients with advanced/metastatic synovial sarcoma or myxoid/round cell liposarcoma. The safety profile revealed positivity, mainly manifesting as mild cytokine release

syndrome and manageable, reversible hematologic toxicity. Out of 25 assessable patients, 2 attained complete remission, 8 experienced partial remission and 11 achieved disease stabilization, with 9 out of 10 responders maintaining their response until the data cut-off [113]. In summary, these studies indicate that CTAs play a crucial role in the initiation, progression, and therapeutic responses of different tumor types, and they serve as a significant asset for combating and treating cancer.

5 Expression and function of CTAs in gliomas

Over the last 30 years, various CTAs have been identified in gliomas, providing evidence of their oncogenic properties. Ectopic lineage characteristics are essential for Drosophila brain tumor growth, indicating that their inactivation could exert tumor-suppressive effects in other species. Moreover, several lineage genes that are upregulated in the Drosophila brain tumor model exhibit direct homology to CTA-related genes, including PIWIL1/*piwi* and SYCP1/*c(3)G* [114]. In 2012, comprehensive reviews consolidated the expression and function of CTAs in brain tumors, encompassing the MAGE and SSX families, SOX-6, TSGA10, TCSAG1, CSAG2, CXorf48, XAGE1, and other related proteins [115]. Hence, this review predominantly focuses on studies that directly address the expression and function of CTAs in gliomas over the last 12 years.

5.1 CTA expression in gliomas

A study investigated the mRNA and protein expression of ACTL8, CTCFL, OIP5, and XAGE3 in 108 glioma specimens using reverse transcription PCR (RT-PCR) and immunohistochemical staining techniques. The results revealed that 61.11% of glioma tissues expressed at least one type of CTA mRNA, whereas 58.33% expressed at least one type of CTA protein. Positive CTCFL protein expression was associated with a poor prognosis, indicating that glioma is a CTA-rich tumor and highlighting the prognostic relevance of specific CTAs [116]. Another study used a similar methodology to evaluate the mRNA and protein expression of the FMR1NB in glioma specimens. The mRNA and protein expression levels in glioma tissues were 58.8% and 46.34%, respectively. Notably, FMR1NB protein expression is an independent prognostic indicator of unfavorable outcomes [18]. IL13RA1 and IL13RA2 are two additional CTA types, both of which are associated with an adverse prognosis of GBM [117, 118]. Additionally, other CTAs are expressed in gliomas and have prognostic implications. Table 1 shows the expression patterns of representative CTAs in glioma samples or cell lines and their prognostic relevance. The prevalence of positive expression in gliomas may be underestimated, potentially causing the oversight of certain CTAs with independent prognostic or therapeutic value, given the limited CTA detection in these studies.

5.2 Function of CTAs in gliomas

Centrosomal protein 55 (CEP55), also known as c10orf3 and CT111, plays a crucial role in cytokinesis, demonstrating its overexpression in various cancer types. Cep55 is involved in modulating the PI3K/AKT pathway, facilitating invasion and metastasis, augmenting cancer cell stemness, and fostering tumorigenesis [134, 135]. CEP55 modulates glucose

Table 1 Expression of representative CTAs in glioma specimens and cell lines and their effect on prognosis

CTAs	Detection methods	Prognostic value	References
FAM133A	IHC	High expression indicates good outcomes	[119]
TMEFF2	IHC		[120]
IGSF11	IHC	High expression indicates poor outcomes	[121]
KIF2C	RT-qPCR, WB		[122]
PBK	RT-qPCR, IHC		[123, 124]
PIWIL2	WB, IHC		[125]
SPA17	WB		[126]
SPAG4	RT-qPCR, WB		[127]
TTK	RT-qPCR		[128]
ACRBP-V5a	RT-qPCR	Prognostic value not evaluated	[129]
NUF2	RT-qPCR, WB		[130, 131]
SPAG9	WB, IHC		[132, 133]

metabolism, proliferation, and apoptosis of glioma cells via the Akt/mTOR signaling pathway in gliomas [136]. Similar studies revealed that CEP55 stimulates proliferation and suppresses the apoptosis of glioma cells [137, 138]. A previous review summarized the expression and function of NY-ESO-1 in gliomas [115]. The epigenetic *NY-ESO-1* regulation involves the sequential recruitment of three epigenetic regulatory complexes in gliomas[76]. Insulin-like growth factor 2 mRNA-binding protein 3 (IGF2BP3), also known as CT98, serves as a prognostic indicator of unfavorable outcomes in gliomas [139, 140]. IGF2BP3 enhances glioma cell proliferation by modulating IGF-2 to activate the PI3K/MAPK pathway [141]. Moreover, it facilitates glioma cell migration by increasing the translation of RELA/p65 [142]. Lymphocyte Antigen 6 Family Member K (LY6K), also known as CT97, promotes the tumorigenicity of GBM through the CAV-1-mediated improvement of the ERK1/2 signaling pathway [143]. A previous study considered *ATAD2* as an essential gene crucial for melanoma development [108]. The expression of polo-like kinase 4 (PLK4) in GBM cells is significantly upregulated caused by the exogenous overexpression of ATAD2, and PLK4 exerts a pro-cancer effect [144]. Opa-interacting protein 5 (OIP5, CT86) is upregulated in patients with GBM, and it is correlated with a negative prognosis and aggressive cell proliferation. Mechanistically, the transcription factor E2F1 triggers OIP5 expression to ensure the stability and continuity of the E2F1 signaling process [145]. Furthermore, OIP5 may be related to the responsiveness of GBM cells to Lomustine therapy [146]. FBXO39 referred to as CT144, is expressed at a higher level in high-grade gliomas compared to low-grade gliomas. It can improve the migration and invasion of glioma cells while preserving the stemness of glioma stem cells [19]. Immunoglobulin superfamily 11 (IGSF11), also known as CT119, in patients with glioma with high IGSF11 expression, demonstrates significant immune cell infiltration and a strong immunosuppressive microenvironment [121]. Sperm-associated antigen 9 (SPAG9), alternatively recognized as CT89, provides evidence that SPAG9 reduction in GBM cells diminishes cell proliferation and invasion abilities [133]. In summary, these studies revealed the diverse restricted expression of CTAs in gliomas, which correlates with patient prognosis and serves specific functions (Fig. 2). However, many upstream and downstream CTAs expression mechanisms remain unclear, emphasizing the necessity to prioritize core CTAs identification and conduct in-depth studies on their mechanisms.

6 Immunotherapeutic significance of CTAs in gliomas

CTA-targeted antibodies, vaccines, and CAR T-cell therapy have recently been used in cancer treatment, achieving promising results in preclinical and early clinical trials [10, 110–113]. The exploration of a large number of CTA peptide-based vaccines indicated the research on widely expressed CTAs (such as MAGE-A3, MAGE-A4, and NY-ESO-1) as a noteworthy example, and personalized cancer treatment has become a trend after demonstrating the feasibility, safety, and immunotherapeutic activity of CTA peptide vaccines tailored to individual tumors [147].

In gliomas, CTAs expression is related to improved antitumor immune responses. Specifically, elevated MAGE-D4 levels were observed in glioma, causing a humoral reaction against MAGE-D4 in the serum of 17% (7/41) of patients, but were absent in 77 healthy donors [20]. Anti-OY-TES-1 antibodies were found in the serum of 5 out of 36 (14%) patients with glioma but were absent in all serum samples from 107 healthy donors, and the OY-TES-1 protein was expressed in all glioma tissues from patients with positive serum antibodies against OY-TES-1 [21]. Activated $CD4^+$ T helper cells treated with DAC express endogenous CTAs, which serve as antigen-presenting cells that stimulate autologous CTL and natural killer cell production [22]. Phase I clinical trials on recurrent GBM revealed efficacy, with tumor regression lasting over 20 months in two patients, and no observed treatment-related adverse reactions [22]. Another study further revealed that DAC upregulated novel antigens and CTAs mRNA expression by inducing DNA hypomethylation, thereby increasing the presentation of new antigens in MHC class I molecules on tumor cells. This process improves T-cell activation specific to these new antigens and CTAs, thereby eliminating DAC-treated GBM cells [23]. Moreover, changes in multiple CTA proteins and HLA peptides were observed in GBM cell lines after DAC treatment [148].

The presence of NY-ESO-1 in gliomas represents a promising target for the use of anticancer T-cells during treatment [149]. DAC markedly reactivates NY-ESO-1 expression in glioma cells but not in normal cells [150]. Systemic DAC administration significantly reduced tumor volume and extended the survival of animals after adoptive transfer therapy with NY-ESO-1-specific CTLs in an orthotopic xenograft model of GBM. This indicates that DAC induces epigenetic silencing of CTAs expression in immunogenetically unresponsive GBM, thereby presenting a novel strategy for tumor immunotherapy targeting DAC-induced CTAs expression [150]. DAC improves the susceptibility of GBM to NY-ESO-1-specific T-lymphocyte-targeted immunotherapy via the Fas/Fas ligand pathway [151]. Furthermore, DAC post-treatment in mice bearing intracranial gliomas consistently and effectively triggered the expression of the immunogenic tumor rejection

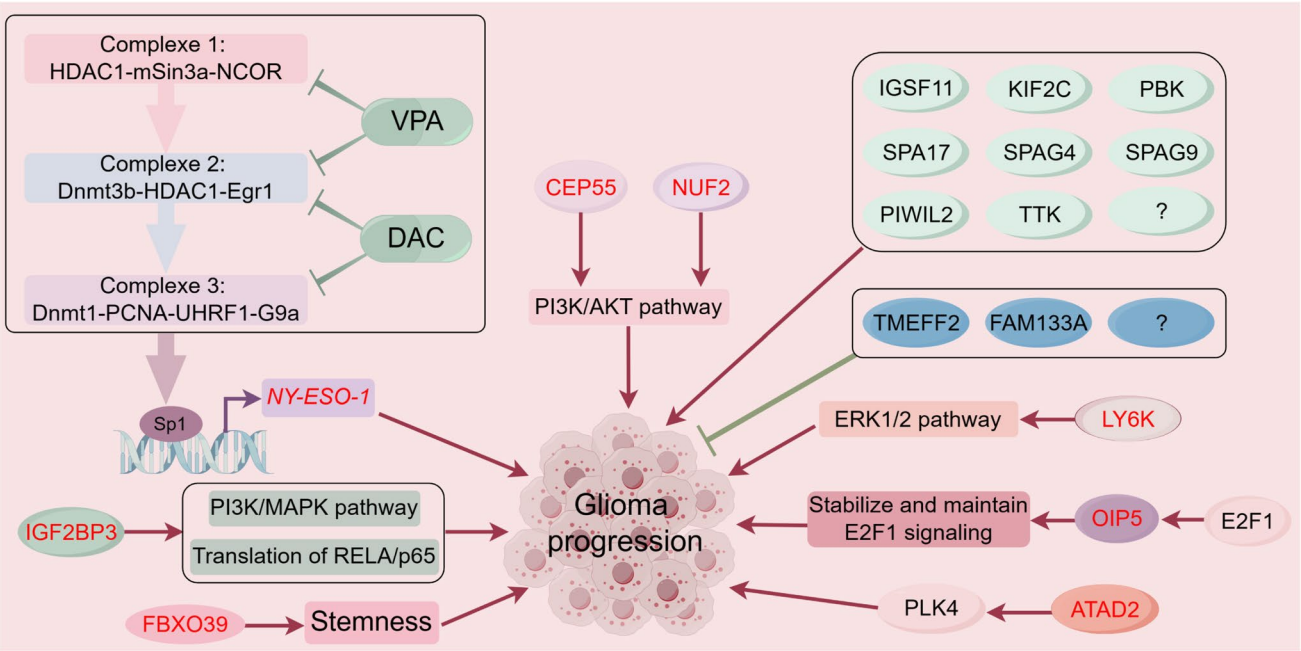

Fig. 2 Summary diagram of cancer-testis antigens' function in gliomas. Cancer-testis antigens: *NY-ESO-1* New York esophageal squamous cell carcinoma 1, *IGF2BP3* insulin-like growth factor 2 mRNA-binding protein 3, *FBXO39* F-box protein 39, *CEP55* centrosomal protein 55, *NUF2* NUF2 component of NDC80 kinetochore complex, *IGSF11* immunoglobulin superfamily member 11, *KIF2C* kinesin family member 2C, *PBK* PDZ-binding kinase, *SPA17* sperm autoantigenic protein 17, *SPAG4* sperm-associated antigen 4, *SPAG9* sperm-associated antigen 9, *PIWIL2* PIWI-like protein 2, *TTK* TTK protein kinase, *TMEFF2* transmembrane protein with EGF-like and two follistatin-like domains 2, *FAM133A* family with sequence similarity 133 member A, *LY6K* lymphocyte antigen 6 family member K, *OIP5* opa-interacting protein 5, *ATAD2* ATPase family AAA-domain containing 2, *DAC* decitabine, *VPA* valproate, *Sp1* Sp1 transcription factor, *E2F1* E2F transcription factor 1, *PLK4* polo-like kinase 4. The Figure was drawn by Figdraw

antigen NY-ESO-1. NY-ESO-1-specific adoptive T-cell therapy demonstrated antitumor effects and provided substantial survival advantages in mice with intracranial xenografts of human gliomas after DAC treatment [152].

Another more in-depth study of CTA is IL13RA2. IL-13RA2-induced CTLs killed U251 glioma cells in vitro [153]. The pseudomonas exotoxin, designed to target IL13RA2 and EGFR, amplifies TRAIL-induced cell death in GBM cells [154]. Dendritic cells transfected with *Il13ra2* mRNA for vaccine therapy demonstrated significantly extended survival in mice with glioma, than in the control group [155]. The IL13RA2-targeting peptide Pep-1L conjugated with Actinium-225 ([225Ac]Pep-1L) was used for initial in vivo safety and therapeutic investigations by administering it to mice with orthotopic GBM. Mice treated with [225Ac]Pep-1L demonstrated notably higher overall survival (OS), median, and average survival rates compared to the control group [156]. YYB-103 CAR-T cells, which selectively bind to IL13RA2, significantly reduced tumor volume and prolonged OS in tumor-bearing mice [157]. Bi-specific T-cell engagers, including the EGFR and IL13RA2-targeted antibodies, which are secreted by monovalent or multivalent T cells, demonstrate robust antitumor activity both in vitro and in vivo, indicating notable sensitivity and specificity [158]. The bi-specific IL-13Rα2/TGF-β CAR-T cells disrupt TGF-β-induced immune suppression and boost antitumor reactions in GBM [159]. A phase I dose-escalation trial following a 3 + 3 design administered cytotoxin infusions through convection-enhanced delivery to dogs presenting with gliomas that express IL13RA2 or EPHA2 receptors. Notably, objective tumor responses were documented in half of the experimental dogs (8 out of 16), resulting in tumor volume reductions of as much as 94%. These results constitute crucial preclinical evidence that supports the translation of this multi-receptor targeted therapy approach to human clinical trials [160]. Several ongoing clinical trials are actively recruiting participants for IL13RA2-targeted CAR-T-cell therapy (NCT05540873, NCT05752877, NCT04661384, NCT05168423, and NCT02208362). Notably, the phase I trial result of NCT02208362 revealed both the safety and promising clinical efficacy of intracranial region-targeted IL13RA2 CAR-T therapy in selected patients with recurrent high-grade glioma [24, 25].

The combination of targeted CTAs with other tumor antigens in immunotherapy strategies is noteworthy. A group of researchers investigated a novel vaccine therapy that targets both the tumor vascular system and tumor cells in patients with HLA-A2402^{+} recurrent/progressive high-grade glioma, using multiple glioma oncology antigens (GOAs) and glioma angiogenesis-associated antigens (GAAA) peptides. This vaccine consisted of peptide epitopes from four GOAs (LY6K,

CT97, DEPDC1, KIF20A, and FOXM1) and two GAAA (VEGFR1 and VEGFR2). The median OS for all participants was 9.2 months, with 5 individuals achieving a minimum of 6 months without disease progression, 2 with recurrent GBM maintaining stable conditions, and 1 with anaplastic astrocytoma achieving complete remission 9 months after vaccination [161]. In summary, these studies underscore the significant role of CTAs in glioma immunotherapy, emphasizing the necessity of conducting larger-scale and more advanced clinical trials to confirm the therapeutic effectiveness of immunotherapy strategies that target CTAs (Fig. 3).

7 Challenges and future interventions regarding CTAs as therapeutic targets for gliomas

Several studies have emphasized the expression of CTAs in gliomas, highlighting their immunogenicity and potential for significant advancement in immunotherapy. However, the use of CTAs as an effective target is challenging despite its promising aspects. The limited efficacy of glioma therapy is attributed to obstacles such as the blood–brain tumor barrier (BBTB) [162] and immunosuppressive microenvironment [30], which pose inevitable challenges. The BBTB can restrict the delivery of CTA-based immunotherapeutic agents to gliomas, posing challenges in achieving optimal therapeutic levels. Gliomas establish a complex immunosuppressive microenvironment that may impair the efficacy of immunotherapy directed at CTAs. Conquering this immune evasion is a significant challenge.

One pressing challenge that must be addressed is the heterogeneity of tumors [163]. Tumor heterogeneity causes the expression of various antigens within tumor cells, both spatially and temporally, casting doubt on the specificity of CTAs as antigens in gliomas. One important challenge related to CTAs expression in gliomas is the variability and complexity of CTAs expression patterns within individual tumors and among different glioma subtypes. Gliomas exhibit diverse molecular profiles and genetic alterations that can influence the expression and patterns of CTAs. This heterogeneity hampers the prediction of target specific CTAs that are consistently expressed among all patients with glioma. Moreover, diverse CTAs expression exist within the same solid tumor, each with distinct functions, complicating targeted treatment design and introducing uncertainty. Additionally, the dynamic nature of CTAs expression in response to treatment or disease progression further complicates the development of effective CTA-targeted immunotherapies for gliomas. Another crucial challenge arises from the tumor-promoting potential of CTAs themselves. Treatment-induced CTAs expression improves tumor cell destruction while concurrently accelerating tumor progression, potentially compromising the long-term

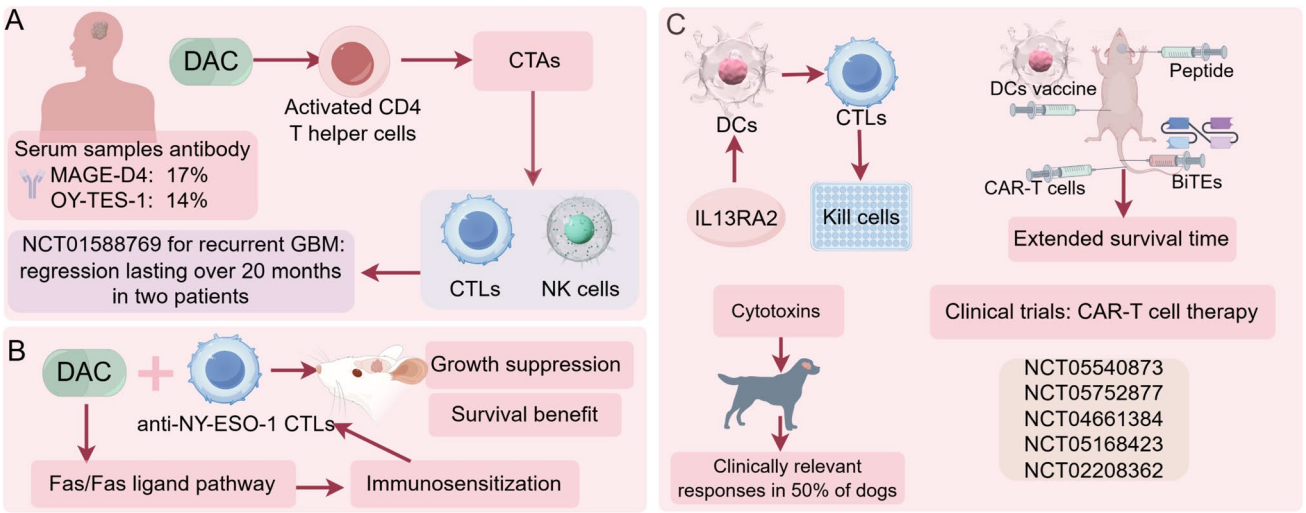

Fig. 3 Summary diagram of the cancer-testis antigens' immunotherapeutic significance in gliomas, including improved antitumor immune responses and potential applications in immunotherapy. **A** Specific CTAs, including MAEG-D4 and OY-TES-1, were detected in the serum of patients with glioma. Additionally, $CD4^+$ T helper cells treated with decitabine (DAC) expressed endogenous CTAs and could function as antigen-presenting cells to stimulate the generation of autologous cytotoxic T lymphocytes and natural killer cells, thereby enhancing immunotherapy responses. **B** DAC improves the susceptibility of mice with intracranial xenografts of human gliomas to NY-ESO-1-specific T-lymphocyte-targeted immunotherapy. **C** Various studies, encompassing in vitro cell experiments, mouse models, and numerous clinical trials involving dogs and human patients with glioma, have indicated the significant promise of IL13RA2 as a target for immunotherapy. The Figure was drawn by Figdraw

quality of patient survival. Understanding and addressing the expression of this variable CTAs within gliomas is crucial for optimizing the efficacy of immunotherapeutic strategies targeting CTAs in this challenging disease context.

Additional challenges include off-target effects, the emergence of treatment resistance, and stringent regulatory constraints on clinical translation. Targeting CTAs with immunotherapy can trigger off-target effects and unintended immune responses, increasing the risk of autoimmune reactions and other adverse events. Glioma cells might acquire resistance to CTA-targeting immunotherapies, which could impair their sustained efficacy. Transitioning from preclinical investigations to clinical studies poses challenges regarding the safety, efficacy, and regulatory approval of CTA-targeted immunotherapies in patients with glioma.

Overcoming these challenges is crucial for the advancement of CTA-based treatment strategies for gliomas. Continued research efforts are needed to overcome the challenges associated with the variability and complexity of CTAs expression in gliomas. A pivotal strategy for unraveling these intricacies has been the extensive use of single-cell sequencing, spatial transcriptomics, next-generation sequencing, and proteomics. Future interventions should focus on identifying and validating reliable biomarkers that can accurately predict CTAs expression profiles in individual patients and on developing innovative strategies to target multiple CTAs simultaneously to enhance the efficacy of immunotherapies. Overall, addressing the challenges and limitations related to CTAs expression in gliomas is essential for advancing the field of immunotherapy and realizing the full potential of CTAs as a therapeutic target in this challenging disease setting.

It is crucial to conduct in-depth investigations of the interplay among CTAs, immune cytokines, and immune cells to assess the significance of CTA-focused therapeutic approaches targeting TME. Currently, diverse drug design methodologies and the use of nanomedicine provide promising solutions to overcome BBTB and its off-target effects. Conducting reliable preclinical animal studies or employing patient-derived organoid models is vital for mitigating off-target effects, halting the emergence of treatment resistance, and preventing tumor progression linked to treatment-induced CTAs expression. Additionally, exploring the use of combination therapies that combine CTA-targeted approaches with other treatment modalities in the future, such as chemotherapy or radiotherapy may offer synergistic benefits and improve outcomes for patients with glioma. Collective efforts are crucial for determining effective therapeutic targets for gliomas. Thus, future studies must investigate the functions of CTAs and scrutinize the individual patterns of CTAs expression and their implications for patient care.

8 Conclusion

The rapid advancement of immunotherapy has instilled optimism for achieving a complete remission of malignant tumors. Given its unique expression profile and immunogenic properties, CTAs is a promising focal point for antitumor immunotherapy. In the context of current research, this review outlines the most recent advancements in the expression, functionality, and immunotherapeutic applications of CTAs in glioma. Specifically, predominantly gliomas express CTAs, with certain CTAs expression associated with patient prognosis and specific immunogenicity, thereby potentially serving as promising targets for immunotherapy. Numerous clinical trials focusing on immunotherapy have been conducted or are currently ongoing to investigate the association between CTA member IL13RA2 and glioma treatment. These trials have consistently demonstrated the safety, feasibility, and tolerability of this approach in patients with glioma. These encouraging clinical trial outcomes suggest that CTA-based immunotherapy could become a cornerstone in the future management of this disease.

However, similar to other cancer treatment strategies, immunotherapy approaches for CTAs encounter numerous challenges and obstacles that require additional time to address the adverse effects of BBTB, tumor heterogeneity, and the immunosuppressive microenvironment. Nonetheless, the future outlook of this treatment strategy remains promising due to the emergence of omics technologies, such as single-cell sequencing, and advanced delivery methods, such as nanomedicine. It is essential to identify personalized optimal CTA targets, develop precise dosing schedules, and use dependable preclinical models to overcome off-target effects and treatment resistance, ultimately enhancing the efficiency of clinical translation. Further investigation of the distinct mechanisms of CTAs in gliomas, coupled with immunotherapy targeting CTAs or in combination with radiotherapy, chemotherapy, and other modalities, is crucial for identifying personalized and precision therapeutic strategies that maximize patient outcomes.

Acknowledgements The authors sincerely thank the ZYEdit team for the language polishment and the drawing tools provided by Figdraw (www.figdraw.com).

Author contributions K.Y., K.W. and S.Z. conceived and designed the study. S.Z. and S.Y. provided equal contributions to article writing and figure drawing. S.C., Y.D., H.C., and L.Y. revised the manuscript. The final manuscript has been read and approved by all authors.

Funding This work was supported by the Finance science and technology project of Hainan province (Grant No. ZDYF2022SHFZ088), the National Nature Science Foundation of China (Grant No. 82060456), the Hainan Province "South China Sea Rising Star" Science and Technology Innovation Talent Platform Project (NHXXRCXM202351), the Hainan Provincial Natural Science Foundation of China (Grant No. 821MS137), and project supported by Hainan Province Clinical Medical Center.

Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate Not applicable.

Competing interests The authors declare no competing interests.

References

1. Qi J, Li M, Wang L, Hu Y, Liu W, Long Z, et al. National and subnational trends in cancer burden in China, 2005–20: an analysis of national mortality surveillance data. Lancet Public Health. 2023;8(12):e943–55. https://doi.org/10.1016/s2468-2667(23)00211-6.
2. Ostrom QT, Price M, Neff C, Cioffi G, Waite KA, Kruchko C, et al. CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2016–2020. Neuro Oncol. 2023;25:iv1–99. https://doi.org/10.1093/neuonc/noad149.
3. Miller KD, Ostrom QT, Kruchko C, Patil N, Tihan T, Cioffi G, et al. Brain and other central nervous system tumor statistics, 2021. CA Cancer J Clin. 2021;71(5):381–406. https://doi.org/10.3322/caac.21693.
4. Yang K, Wu Z, Zhang H, Zhang N, Wu W, Wang Z, et al. Glioma targeted therapy: insight into future of molecular approaches. Mol Cancer. 2022;21(1):39. https://doi.org/10.1186/s12943-022-01513-z.
5. van den Bent MJ, Geurts M, French PJ, Smits M, Capper D, Bromberg JEC, et al. Primary brain tumours in adults. Lancet. 2023;402(10412):1564–79. https://doi.org/10.1016/s0140-6736(23)01054-1.
6. Leko V, Rosenberg SA. Identifying and targeting human tumor antigens for T cell-based immunotherapy of solid tumors. Cancer Cell. 2020;38(4):454–72. https://doi.org/10.1016/j.ccell.2020.07.013.
7. Salmaninejad A, Zamani MR, Pourvahedi M, Golchehre Z, Hosseini Bereshneh A, Rezaei N. Cancer/testis antigens: expression, regulation, tumor invasion, and use in immunotherapy of cancers. Immunol Invest. 2016;45(7):619–40. https://doi.org/10.1080/08820139.2016.1197241.
8. Gibbs ZA, Whitehurst AW. Emerging contributions of cancer/testis antigens to neoplastic behaviors. Trends Cancer. 2018;4(10):701–12. https://doi.org/10.1016/j.trecan.2018.08.005.
9. Whitehurst AW. Cause and consequence of cancer/testis antigen activation in cancer. Annu Rev Pharmacol Toxicol. 2014;54:251–72. https://doi.org/10.1146/annurev-pharmtox-011112-140326.
10. Yang P, Meng M, Zhou Q. Oncogenic cancer/testis antigens are a hallmarker of cancer and a sensible target for cancer immunotherapy. Biochim Biophys Acta, Rev Cancer. 2021;1876(1):188558. https://doi.org/10.1016/j.bbcan.2021.188558.
11. Gordeeva O. Cancer-testis antigens: Unique cancer stem cell biomarkers and targets for cancer therapy. Semin Cancer Biol. 2018;53:75–89. https://doi.org/10.1016/j.semcancer.2018.08.006.
12. O'Donnell L, Smith LB, Rebourcet D. Sperm-specific proteins: new implications for diagnostic development and cancer immunotherapy. Curr Opin Cell Biol. 2022;77:102104. https://doi.org/10.1016/j.ceb.2022.102104.
13. Nin DS, Deng LW. Biology of cancer-testis antigens and their therapeutic implications in cancer. Cells. 2023;12(6):926. https://doi.org/10.3390/cells12060926.
14. Yawata T, Nakai E, Park KC, Chihara T, Kumazawa A, Toyonaga S, et al. Enhanced expression of cancer testis antigen genes in glioma stem cells. Mol Carcinog. 2010;49(6):532–44. https://doi.org/10.1002/mc.20614.
15. Low J, Dowless M, Shiyanova T, Rowlinson S, Ricci-Vitiani L, de Maria R, et al. Knockdown of cancer testis antigens modulates neural stem cell marker expression in glioblastoma tumor stem cells. J Biomol Screen. 2010;15(7):830–9. https://doi.org/10.1177/1087057110374983.
16. Akiyama Y, Komiyama M, Miyata H, Yagoto M, Ashizawa T, Iizuka A, et al. Novel cancer-testis antigen expression on glioma cell lines derived from high-grade glioma patients. Oncol Rep. 2014;31(4):1683–90. https://doi.org/10.3892/or.2014.3049.

17. Tabatabaei Yazdi SA, Safaei M, Gholamin M, Abdollahi A, Nili F, Jabbari Nooghabi M, et al. Expression and prognostic significance of cancer/testis antigens, MAGE-E1, GAGE, and SOX-6, in glioblastoma: an immunohistochemistry evaluation. Iran J Pathol. 2021;16(2):128–36. https://doi.org/10.30699/ijp.2020.125038.2368.
18. Bi SQ, Peng Y, Wei ZD, Yao SZ, Luo B, Ge YY, et al. FMR1NB involved in glioma tumorigenesis is a promising target for prognosis and therapy. Curr Med Sci. 2022;42(4):803–16. https://doi.org/10.1007/s11596-022-2586-4.
19. Wu J, Yao F, Li Y, Zhao Z, Liu J, Xu T, et al. The cancer-testis antigen FBXO39 predicts poor prognosis and is associated with stemness and aggressiveness in glioma. Pathol Res Pract. 2022;239:154168. https://doi.org/10.1016/j.prp.2022.154168.
20. He SJ, Gu YY, Yu L, Luo B, Fan R, Lin WZ, et al. High expression and frequently humoral immune response of melanoma-associated antigen D4 in glioma. Int J Clin Exp Pathol. 2014;7(5):2350–60.
21. Li X, Yan J, Fan R, Luo B, Zhang Q, Lin Y, et al. Serum immunoreactivity of cancer/testis antigen OY-TES-1 and its tissues expression in glioma. Oncol Lett. 2017;13(5):3080–6. https://doi.org/10.3892/ol.2017.5799.
22. Kirkin AF, Dzhandzhugazyan KN, Guldberg P, Fang JJ, Andersen RS, Dahl C, et al. Adoptive cancer immunotherapy using DNA-demethylated T helper cells as antigen-presenting cells. Nat Commun. 2018;9(1):785. https://doi.org/10.1038/s41467-018-03217-9.
23. Ma R, Rei M, Woodhouse I, Ferris K, Kirschner S, Chandran A, et al. Decitabine increases neoantigen and cancer testis antigen expression to enhance T-cell-mediated toxicity against glioblastoma. Neuro Oncol. 2022;24(12):2093–106. https://doi.org/10.1093/neuonc/noac107.
24. Brown CE, Alizadeh D, Starr R, Weng L, Wagner JR, Naranjo A, et al. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. N Engl J Med. 2016;375(26):2561–9. https://doi.org/10.1056/NEJMoa1610497.
25. Brown CE, Hibbard JC, Alizadeh D, Blanchard MS, Natri HM, Wang D, et al. Locoregional delivery of IL-13Rα2-targeting CAR-T cells in recurrent high-grade glioma: a phase 1 trial. Nat Med. 2024;30(4):1001–12. https://doi.org/10.1038/s41591-024-02875-1.
26. Oladejo M, Paulishak W, Wood L. Synergistic potential of immune checkpoint inhibitors and therapeutic cancer vaccines. Semin Cancer Biol. 2023;88:81–95. https://doi.org/10.1016/j.semcancer.2022.12.003.
27. Quail DF, Joyce JA. The microenvironmental landscape of brain tumors. Cancer Cell. 2017;31(3):326–41. https://doi.org/10.1016/j.ccell.2017.02.009.
28. Arrieta VA, Dmello C, McGrail DJ, Brat DJ, Lee-Chang C, Heimberger AB, et al. Immune checkpoint blockade in glioblastoma: from tumor heterogeneity to personalized treatment. J Clin Invest. 2023;133(2):e163447. https://doi.org/10.1172/jci163447.
29. Aghajani M, Jalilzadeh N, Aghebati-Maleki A, Yari A, Tabnak P, Mardi A, et al. Current approaches in glioblastoma multiforme immunotherapy. Clin Transl Oncol. 2024;26(7):1584–612. https://doi.org/10.1007/s12094-024-03395-7.
30. Lin H, Liu C, Hu A, Zhang D, Yang H, Mao Y. Understanding the immunosuppressive microenvironment of glioma: mechanistic insights and clinical perspectives. J Hematol Oncol. 2024;17(1):31. https://doi.org/10.1186/s13045-024-01544-7.
31. Karimi-Shahri M, Khorramdel M, Zarei S, Attarian F, Hashemian P, Javid H. Glioblastoma, an opportunity T cell trafficking could bring for the treatment. Mol Biol Rep. 2022;49(10):9863–75. https://doi.org/10.1007/s11033-022-07510-1.
32. Noh MH, Kang JM, Miller AA, Nguyen G, Huang M, Shim JS, et al. Targeting IGF2-IGF1R signaling to reprogram the tumor microenvironment for enhanced viro-immunotherapy. Neuro Oncol. 2024. https://doi.org/10.1093/neuonc/noae105.
33. Everson RG, Hugo W, Sun L, Antonios J, Lee A, Ding L, et al. TLR agonists polarize interferon responses in conjunction with dendritic cell vaccination in malignant glioma: a randomized phase II Trial. Nat Commun. 2024;15(1):3882. https://doi.org/10.1038/s41467-024-48073-y.
34. Choi BD, Gerstner ER, Frigault MJ, Leick MB, Mount CW, Balaj L, et al. Intraventricular CARv3-TEAM-E T cells in recurrent glioblastoma. N Engl J Med. 2024;390(14):1290–8. https://doi.org/10.1056/NEJMoa2314390.
35. Hatae R, Kyewalabye K, Yamamichi A, Chen T, Phyu S, Chuntova P, et al. Enhancing CAR-T cell metabolism to overcome hypoxic conditions in the brain tumor microenvironment. JCI Insight. 2024;9(7):e177141. https://doi.org/10.1172/jci.insight.177141.
36. Yu W, Truong NT, Polara R, Gargett T, Tea MN, Pitson SM, et al. Endogenous bystander killing mechanisms enhance the activity of novel FAP-specific CAR-T cells against glioblastoma. Clin Transl Immunology. 2024;13(7):e1519. https://doi.org/10.1002/cti2.1519.
37. Najem H, Lea ST, Tripathi S, Hurley L, Chen CH, William I, et al. STING agonist 8803 reprograms the immune microenvironment and increases survival in preclinical models of glioblastoma. J Clin Invest. 2024;134(12):e175033. https://doi.org/10.1172/jci175033.
38. Pant A, Hwa-Lin Bergsneider B, Srivastava S, Kim T, Jain A, Bom S, et al. CCR2 and CCR5 co-inhibition modulates immunosuppressive myeloid milieu in glioma and synergizes with anti-PD-1 therapy. Oncoimmunology. 2024;13(1):2338965. https://doi.org/10.1080/2162402x.2024.2338965.
39. Chen Q, Zheng Y, Chen X, Xing Y, Zhang J, Yan X, et al. Bacteria synergized with PD-1 blockade enhance positive feedback loop of cancer cells-M1 macrophages-T cells in glioma. Adv Sci (Weinh). 2024;11(20):e2308124. https://doi.org/10.1002/advs.202308124.
40. Li T, Xu D, Ruan Z, Zhou J, Sun W, Rao B, et al. Metabolism/Immunity dual-regulation thermogels potentiating immunotherapy of glioblastoma through lactate-excretion inhibition and PD-1/PD-L1 blockade. Adv Sci (Weinh). 2024;11(18):e2310163. https://doi.org/10.1002/advs.202310163.
41. Arrieta VA, Gould A, Kim KS, Habashy KJ, Dmello C, Vázquez-Cervantes GI, et al. Ultrasound-mediated delivery of doxorubicin to the brain results in immune modulation and improved responses to PD-1 blockade in gliomas. Nat Commun. 2024;15(1):4698. https://doi.org/10.1038/s41467-024-48326-w.
42. Kim KS, Habashy K, Gould A, Zhao J, Najem H, Amidei C, et al. Fc-enhanced anti-CTLA-4, anti-PD-1, doxorubicin, and ultrasound-mediated BBB opening: a novel combinatorial immunotherapy regimen for gliomas. Neuro Oncol. 2024. https://doi.org/10.1093/neuonc/noae135.
43. Coulie PG, Weynants P, Lehmann F, Herman J, Brichard V, Wölfel T, et al. Genes coding for tumor antigens recognized by human cytolytic T lymphocytes. J Immunother Emphas Tumor Immunol. 1993;14(2):104–9. https://doi.org/10.1097/00002371-199308000-00004.
44. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science. 1991;254(5038):1643–7. https://doi.org/10.1126/science.1840703.
45. Sahin U, Türeci O, Schmitt H, Cochlovius B, Johannes T, Schmits R, et al. Human neoplasms elicit multiple specific immune responses in the autologous host. Proc Natl Acad Sci U S A. 1995;92(25):11810–3. https://doi.org/10.1073/pnas.92.25.11810.
46. Chen YT. The journey from autologous typing to SEREX, NY-ESO-1, and cancer/testis antigens. Cancer Immun. 2012;12:8.

47. Chen YT, Scanlan MJ, Sahin U, Türeci O, Gure AO, Tsang S, et al. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc Natl Acad Sci U S A. 1997;94(5):1914–8. https://doi.org/10.1073/pnas.94.5.1914.
48. Nelson PT, Zhang PJ, Spagnoli GC, Tomaszewski JE, Pasha TL, Frosina D, et al. Cancer/testis (CT) antigens are expressed in fetal ovary. Cancer Immun. 2007;7:1.
49. Scanlan MJ, Simpson AJ, Old LJ. The cancer/testis genes: review, standardization, and commentary. Cancer Immun. 2004;4:1.
50. Simpson AJ, Caballero OL, Jungbluth A, Chen YT, Old LJ. Cancer/testis antigens, gametogenesis and cancer. Nat Rev Cancer. 2005;5(8):615–25. https://doi.org/10.1038/nrc1669.
51. Almeida LG, Sakabe NJ, deOliveira AR, Silva MC, Mundstein AS, Cohen T, et al. CTdatabase: a knowledge-base of high-throughput and curated data on cancer-testis antigens. Nucleic Acids Res. 2009;37:D816-9. https://doi.org/10.1093/nar/gkn673.
52. Hofmann O, Caballero OL, Stevenson BJ, Chen YT, Cohen T, Chua R, et al. Genome-wide analysis of cancer/testis gene expression. Proc Natl Acad Sci U S A. 2008;105(51):20422–7. https://doi.org/10.1073/pnas.0810777105.
53. Nishiyama A, Nakanishi M. Navigating the DNA methylation landscape of cancer. Trends Genet. 2021;37(11):1012–27. https://doi.org/10.1016/j.tig.2021.05.002.
54. Kimmins S, Sassone-Corsi P. Chromatin remodelling and epigenetic features of germ cells. Nature. 2005;434(7033):583–9. https://doi.org/10.1038/nature03368.
55. De Smet C, De Backer O, Faraoni I, Lurquin C, Brasseur F, Boon T. The activation of human gene MAGE-1 in tumor cells is correlated with genome-wide demethylation. Proc Natl Acad Sci U S A. 1996;93(14):7149–53. https://doi.org/10.1073/pnas.93.14.7149.
56. De Smet C, Lurquin C, Lethé B, Martelange V, Boon T. DNA methylation is the primary silencing mechanism for a set of germ line- and tumor-specific genes with a CpG-rich promoter. Mol Cell Biol. 1999;19(11):7327–35. https://doi.org/10.1128/mcb.19.11.7327.
57. Honda T, Tamura G, Waki T, Kawata S, Terashima M, Nishizuka S, et al. Demethylation of MAGE promoters during gastric cancer progression. Br J Cancer. 2004;90(4):838–43. https://doi.org/10.1038/sj.bjc.6601600.
58. Nobeyama Y, Nakagawa H. Aberrant demethylation and expression of MAGEB2 in a subset of malignant peripheral nerve sheath tumors from neurofibromatosis type 1. J Dermatol Sci. 2016;81(2):118–23. https://doi.org/10.1016/j.jdermsci.2015.11.004.
59. Coscia F, Lengyel E, Duraiswamy J, Ashcroft B, Bassani-Sternberg M, Wierer M, et al. Multi-level proteomics identifies CT45 as a chemosensitivity mediator and immunotherapy target in ovarian cancer. Cell. 2018;175(1):159-70.e16. https://doi.org/10.1016/j.cell.2018.08.065.
60. Luchtel RA, Dasari S, Oishi N, Pedersen MB, Hu G, Rech KL, et al. Molecular profiling reveals immunogenic cues in anaplastic large cell lymphomas with DUSP22 rearrangements. Blood. 2018;132(13):1386–98. https://doi.org/10.1182/blood-2018-03-838524.
61. Sharma A, Albahrani M, Zhang W, Kufel CN, James SR, Odunsi K, et al. Epigenetic activation of POTE genes in ovarian cancer. Epigenetics. 2019;14(2):185–97. https://doi.org/10.1080/15592294.2019.1581590.
62. Chen Z, Zuo X, Pu L, Zhang Y, Han G, Zhang L, et al. Hypomethylation-mediated activation of cancer/testis antigen KK-LC-1 facilitates hepatocellular carcinoma progression through activating the Notch1/Hes1 signalling. Cell Prolif. 2019;52(3):e12581. https://doi.org/10.1111/cpr.12581.
63. Hong JA, Kang Y, Abdullaev Z, Flanagan PT, Pack SD, Fischette MR, et al. Reciprocal binding of CTCF and BORIS to the NY-ESO-1 promoter coincides with derepression of this cancer-testis gene in lung cancer cells. Cancer Res. 2005;65(17):7763–74. https://doi.org/10.1158/0008-5472.Can-05-0823.
64. Bhan S, Negi SS, Shao C, Glazer CA, Chuang A, Gaykalova DA, et al. BORIS binding to the promoters of cancer testis antigens, MAGEA2, MAGEA3, and MAGEA4, is associated with their transcriptional activation in lung cancer. Clin Cancer Res. 2011;17(13):4267–76. https://doi.org/10.1158/1078-0432.Ccr-11-0653.
65. Zhao J, Wang Y, Liang Q, Xu Y, Sang J. MAGEA1 inhibits the expression of BORIS via increased promoter methylation. J Cell Sci. 2019;132(1):jcs218628. https://doi.org/10.1242/jcs.218628.
66. Weber J, Salgaller M, Samid D, Johnson B, Herlyn M, Lassam N, et al. Expression of the MAGE-1 tumor antigen is up-regulated by the demethylating agent 5-aza-2'-deoxycytidine. Cancer Res. 1994;54(7):1766–71.
67. Lim JH, Kim SP, Gabrielson E, Park YB, Park JW, Kwon TK. Activation of human cancer/testis antigen gene, XAGE-1, in tumor cells is correlated with CpG island hypomethylation. Int J Cancer. 2005;116(2):200–6. https://doi.org/10.1002/ijc.21007.
68. Dubovsky JA, McNeel DG, Powers JJ, Gordon J, Sotomayor EM, Pinilla-Ibarz JA. Treatment of chronic lymphocytic leukemia with a hypomethylating agent induces expression of NXF2, an immunogenic cancer testis antigen. Clin Cancer Res. 2009;15(10):3406–15. https://doi.org/10.1158/1078-0432.Ccr-08-2099.
69. Shi X, Chen X, Fang B, Ping Y, Qin G, Yue D, et al. Decitabine enhances tumor recognition by T cells through upregulating the MAGE-A3 expression in esophageal carcinoma. Biomed Pharmacother. 2019;112:108632. https://doi.org/10.1016/j.biopha.2019.108632.
70. Jakobsen MK, Traynor S, Stæhr M, Duijf PG, Nielsen AY, Terp MG, et al. The cancer/testis antigen gene VCX2 is rarely expressed in malignancies but can be epigenetically activated using DNA methyltransferase and histone deacetylase inhibitors. Front Oncol. 2020;10:584024. https://doi.org/10.3389/fonc.2020.584024.
71. Taib N, Merhi M, Inchakalody V, Mestiri S, Hydrose S, Makni-Maalej K, et al. Treatment with decitabine induces the expression of stemness markers, PD-L1 and NY-ESO-1 in colorectal cancer: potential for combined chemoimmunotherapy. J Transl Med. 2023;21(1):235. https://doi.org/10.1186/s12967-023-04073-y.
72. Li D, Xia L, Huang P, Wang Z, Guo Q, Huang C, et al. Serine protease PRSS56, a novel cancer-testis antigen activated by DNA hypomethylation, promotes colorectal and gastric cancer progression via PI3K/AKT axis. Cell Biosci. 2023;13(1):124. https://doi.org/10.1186/s13578-023-01060-0.
73. Kosaka A, Yajima Y, Hatayama M, Ikuta K, Sasaki T, Hirai N, et al. A stealth antigen SPESP1, which is epigenetically silenced in tumors, is a suitable target for cancer immunotherapy. Cancer Sci. 2021;112(7):2705–13. https://doi.org/10.1111/cas.14973.
74. Abu N, Rus Bakarurraini NAA, Nasir SN, Ishak M, Baharuddin R, Jamal R, et al. Methylation profile of cancer testis antigens in colorectal cancer. Iran J Immunol. 2023;20(1):83–91. https://doi.org/10.22034/iji.2023.92600.2171.
75. Liu R, Wu J, Guo H, Yao W, Li S, Lu Y, et al. Post-translational modifications of histones: mechanisms, biological functions, and therapeutic targets. MedComm (2020). 2023;4(3):e292. https://doi.org/10.1002/mco2.292.

76. Cartron PF, Blanquart C, Hervouet E, Gregoire M, Vallette FM. HDAC1-mSin3a-NCOR1, Dnmt3b-HDAC1-Egr1 and Dnmt1-PCNA-UHRF1-G9a regulate the NY-ESO1 gene expression. Mol Oncol. 2013;7(3):452–63. https://doi.org/10.1016/j.molonc.2012.11.004.
77. Deng H, Zeng J, Zhang T, Gong L, Zhang H, Cheung E, et al. Histone H3.3K27M mobilizes multiple cancer/testis (CT) antigens in pediatric glioma. Mol Cancer Res. 2018;16(4):623–33. https://doi.org/10.1158/1541-7786.Mcr-17-0460.
78. Wischnewski F, Pantel K, Schwarzenbach H. Promoter demethylation and histone acetylation mediate gene expression of MAGE-A1, -A2, -A3, and -A12 in human cancer cells. Mol Cancer Res. 2006;4(5):339–49. https://doi.org/10.1158/1541-7786.Mcr-05-0229.
79. Hou SY, Sang MX, Geng CZ, Liu WH, Lü WH, Xu YY, et al. Expressions of MAGE-A9 and MAGE-A11 in breast cancer and their expression mechanism. Arch Med Res. 2014;45(1):44–51. https://doi.org/10.1016/j.arcmed.2013.10.005.
80. Goodyear O, Agathanggelou A, Novitzky-Basso I, Siddique S, McSkeane T, Ryan G, et al. Induction of a CD8+ T-cell response to the MAGE cancer testis antigen by combined treatment with azacitidine and sodium valproate in patients with acute myeloid leukemia and myelodysplasia. Blood. 2010;116(11):1908–18. https://doi.org/10.1182/blood-2009-11-249474.
81. Moreno-Bost A, Szmania S, Stone K, Garg T, Hoerring A, Szymonifka J, et al. Epigenetic modulation of MAGE-A3 antigen expression in multiple myeloma following treatment with the demethylation agent 5-azacitidine and the histone deacetlyase inhibitor MGCD0103. Cytotherapy. 2011;13(5):618–28. https://doi.org/10.3109/14653249.2010.529893.
82. Gong W, Wang L, Schubert ML, Kleist C, Neuber B, Wang S, et al. HDAC inhibition for optimized cellular immunotherapy of NY-ESO-1-positive soft tissue sarcoma. Biomedicines. 2022;10(2):373. https://doi.org/10.3390/biomedicines10020373.
83. Bensaid D, Blondy T, Deshayes S, Dehame V, Bertrand P, Grégoire M, et al. Assessment of new HDAC inhibitors for immunotherapy of malignant pleural mesothelioma. Clin Epigenet. 2018;10:79. https://doi.org/10.1186/s13148-018-0517-9.
84. Han H, Yang X, Pandiyan K, Liang G. Synergistic re-activation of epigenetically silenced genes by combinatorial inhibition of DNMTs and LSD1 in cancer cells. PLoS ONE. 2013;8(9):e75136. https://doi.org/10.1371/journal.pone.0075136.
85. Rao M, Chinnasamy N, Hong JA, Zhang Y, Zhang M, Xi S, et al. Inhibition of histone lysine methylation enhances cancer-testis antigen expression in lung cancer cells: implications for adoptive immunotherapy of cancer. Cancer Res. 2011;71(12):4192–204. https://doi.org/10.1158/0008-5472.Can-10-2442.
86. James SR, Cedeno CD, Sharma A, Zhang W, Mohler JL, Odunsi K, et al. DNA methylation and nucleosome occupancy regulate the cancer germline antigen gene MAGEA11. Epigenetics. 2013;8(8):849–63. https://doi.org/10.4161/epi.25500.
87. Kang Y, Hong JA, Chen GA, Nguyen DM, Schrump DS. Dynamic transcriptional regulatory complexes including BORIS, CTCF and Sp1 modulate NY-ESO-1 expression in lung cancer cells. Oncogene. 2007;26(30):4394–403. https://doi.org/10.1038/sj.onc.1210218.
88. Kosaka-Suzuki N, Suzuki T, Pugacheva EM, Vostrov AA, Morse HC 3rd, Loukinov D, et al. Transcription factor BORIS (Brother of the Regulator of Imprinted Sites) directly induces expression of a cancer-testis antigen, TSP50, through regulated binding of BORIS to the promoter. J Biol Chem. 2011;286(31):27378–88. https://doi.org/10.1074/jbc.M111.243576.
89. Renaud S, Pugacheva EM, Delgado MD, Braunschweig R, Abdullaev Z, Loukinov D, et al. Expression of the CTCF-paralogous cancer-testis gene, brother of the regulator of imprinted sites (BORIS), is regulated by three alternative promoters modulated by CpG methylation and by CTCF and p53 transcription factors. Nucleic Acids Res. 2007;35(21):7372–88. https://doi.org/10.1093/nar/gkm896.
90. Gallegos ZR, Taus P, Gibbs ZA, McGlynn K, Gomez NC, Davis I, et al. EWSR1-FLI1 activation of the cancer/testis antigen FATE1 promotes ewing sarcoma survival. Mol Cell Biol. 2019;39(14):e00138-e219. https://doi.org/10.1128/mcb.00138-19.
91. Beasley HK, Widatalla SE, Whalen DS, Williams SD, Korolkova OY, Namba C, et al. Identification of MAGEC2/CT10 as a high calcium-inducible gene in triple-negative breast cancer. Front Endocrinol (Lausanne). 2022;13:816598. https://doi.org/10.3389/fendo.2022.816598.
92. Gantchev J, Messina-Pacheco J, Martínez Villarreal A, Ramchatesingh B, Lefrançois P, Xie P, et al. Ectopically expressed meiosis-specific cancer testis antigen HORMAD1 promotes genomic instability in squamous cell carcinomas. Cells. 2023;12(12):1627. https://doi.org/10.3390/cells12121627.
93. Bradley R, Anczuków O. RNA splicing dysregulation and the hallmarks of cancer. Nat Rev Cancer. 2023;23(3):135–55. https://doi.org/10.1038/s41568-022-00541-7.
94. Lethé B, Lucas S, Michaux L, De Smet C, Godelaine D, Serrano A, et al. LAGE-1, a new gene with tumor specificity. Int J Cancer. 1998;76(6):903–8. https://doi.org/10.1002/(sici)1097-0215(19980610)76:6%3c903::aid-ijc22%3e3.0.co;2-1.
95. de Wit NJ, Weidle UH, Ruiter DJ, van Muijen GN. Expression profiling of MMA-1a and splice variant MMA-1b: new cancer/testis antigens identified in human melanoma. Int J Cancer. 2002;98(4):547–53. https://doi.org/10.1002/ijc.10241.
96. de Wit NJ, Cornelissen IM, Diepstra JH, Weidle UH, Ruiter DJ, van Muijen GN. The MMA1 gene family of cancer-testis antigens has multiple alternative splice variants: characterization of their expression profile, the genomic organization, and transcript properties. Genes Chromosomes Cancer. 2005;42(1):10–21. https://doi.org/10.1002/gcc.20107.
97. Güre AO, Wei IJ, Old LJ, Chen YT. The SSX gene family: characterization of 9 complete genes. Int J Cancer. 2002;101(5):448–53. https://doi.org/10.1002/ijc.10634.
98. Wang T, Fan L, Watanabe Y, McNeill P, Fanger GR, Persing DH, et al. L552S, an alternatively spliced isoform of XAGE-1, is over-expressed in lung adenocarcinoma. Oncogene. 2001;20(53):7699–709. https://doi.org/10.1038/sj.onc.1204939.
99. Lin C, Mak S, Meitner PA, Wolf JM, Bluman EM, Block JA, et al. Cancer/testis antigen CSAGE is concurrently expressed with MAGE in chondrosarcoma. Gene. 2002;285(1–2):269–78. https://doi.org/10.1016/s0378-1119(02)00395-5.
100. Schmiedel BJ, Hutter C, Hesse M, Staege MS. Expression of multiple membrane-associated phospholipase A1 beta transcript variants and lysophosphatidic acid receptors in Ewing tumor cells. Mol Biol Rep. 2011;38(7):4619–28. https://doi.org/10.1007/s11033-010-0595-z.
101. Novak Kujundžić R, Grbeša I, Ivkić M, Krušlin B, Konjevoda P, Gall TK. Possible prognostic value of BORIS transcript variants ratio in laryngeal squamous cell carcinomas - a pilot study. Pathol Oncol Res. 2014;20(3):687–95. https://doi.org/10.1007/s12253-014-9749-y.
102. Pagotto A, Caballero OL, Volkmar N, Devalle S, Simpson AJ, Lu X, et al. Centrosomal localisation of the cancer/testis (CT) antigens NY-ESO-1 and MAGE-C1 is regulated by proteasome activity in tumour cells. PLoS ONE. 2013;8(12):e83212. https://doi.org/10.1371/journal.pone.0083212.
103. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57–70. https://doi.org/10.1016/s0092-8674(00)81683-9.
104. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74. https://doi.org/10.1016/j.cell.2011.02.013.
105. Hanahan D. Hallmarks of cancer: new dimensions. Cancer Discov. 2022;12(1):31–46. https://doi.org/10.1158/2159-8290.Cd-21-1059.

106. Ghosh M, Das S. PRAMEF2-mediated dynamic regulation of YAP signaling promotes tumorigenesis. Proc Natl Acad Sci U S A. 2021;118(40):e2105523118. https://doi.org/10.1073/pnas.2105523118.
107. Fu J, Zhang J, Chen X, Liu Z, Yang X, He Z, et al. ATPase family AAA domain-containing protein 2 (ATAD2): From an epigenetic modulator to cancer therapeutic target. Theranostics. 2023;13(2):787–809. https://doi.org/10.7150/thno.78840.
108. Baggiolini A, Callahan SJ, Montal E, Weiss JM, Trieu T, Tagore MM, et al. Developmental chromatin programs determine oncogenic competence in melanoma. Science. 2021;373(6559):eabc1048. https://doi.org/10.1126/science.abc1048.
109. Nin DS, Wujanto C, Tan TZ, Lim D, Damen JMA, Wu KY, et al. GAGE mediates radio resistance in cervical cancers via the regulation of chromatin accessibility. Cell Rep. 2021;36(9):109621. https://doi.org/10.1016/j.celrep.2021.109621.
110. Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, Dudley ME, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol. 2011;29(7):917–24. https://doi.org/10.1200/jco.2010.32.2537.
111. Rapoport AP, Stadtmauer EA, Binder-Scholl GK, Goloubeva O, Vogl DT, Lacey SF, et al. NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma. Nat Med. 2015;21(8):914–21. https://doi.org/10.1038/nm.3910.
112. Hong DS, Van Tine BA, Biswas S, McAlpine C, Johnson ML, Olszanski AJ, et al. Autologous T cell therapy for MAGE-A4(+) solid cancers in HLA-A*02(+) patients: a phase 1 trial. Nat Med. 2023;29(1):104–14. https://doi.org/10.1038/s41591-022-02128-z.
113. D'Angelo SP, Van Tine BA, Attia S, Blay J-Y, Strauss SJ, Valverde Morales CM, et al. SPEARHEAD-1: a phase 2 trial of afamitresgene autoleucel (Formerly ADP-A2M4) in patients with advanced synovial sarcoma or myxoid/round cell liposarcoma. J Clin Oncol. 2021;39:11504.
114. Janic A, Mendizabal L, Llamazares S, Rossell D, Gonzalez C. Ectopic expression of germline genes drives malignant brain tumor growth in Drosophila. Science. 2010;330(6012):1824–7. https://doi.org/10.1126/science.1195481.
115. Ghafouri-Fard S, Modarressi MH. Expression of cancer-testis genes in brain tumors: implications for cancer immunotherapy. Immunotherapy. 2012;4(1):59–75. https://doi.org/10.2217/imt.11.145.
116. Li X, Ning L, Zhang Q, Ge Y, Liu C, Bi S, et al. Expression profile of ACTL8, CTCFL, OIP5 and XAGE3 in glioma and their prognostic significance: a retrospective clinical study. Am J Transl Res. 2020;12(12):7782–96.
117. Zeng J, Zhang J, Yang YZ, Wang F, Jiang H, Chen HD, et al. IL13RA2 is overexpressed in malignant gliomas and related to clinical outcome of patients. Am J Transl Res. 2020;12(8):4702–14.
118. Han J, Puri RK. Analysis of the cancer genome atlas (TCGA) database identifies an inverse relationship between interleukin-13 receptor α1 and α2 gene expression and poor prognosis and drug resistance in subjects with glioblastoma multiforme. J Neurooncol. 2018;136(3):463–74. https://doi.org/10.1007/s11060-017-2680-9.
119. Huang GH, Du L, Li N, Zhang Y, Xiang Y, Tang JH, et al. Methylation-mediated miR-155-FAM133A axis contributes to the attenuated invasion and migration of IDH mutant gliomas. Cancer Lett. 2018;432:93–102. https://doi.org/10.1016/j.canlet.2018.06.007.
120. Xie S, Zhang Y, Peng T, Guo J, Cao Y, Guo J, et al. TMEFF2 promoter hypermethylation is an unfavorable prognostic marker in gliomas. Cancer Cell Int. 2021;21(1):148. https://doi.org/10.1186/s12935-021-01818-x.
121. Ghouzlani A, Rafii S, Karkouri M, Lakhdar A, Badou A. The promising IgSF11 immune checkpoint is highly expressed in advanced human gliomas and associates to poor prognosis. Front Oncol. 2020;10:608609. https://doi.org/10.3389/fonc.2020.608609.
122. Bie L, Zhao G, Wang YP, Zhang B. Kinesin family member 2C (KIF2C/MCAK) is a novel marker for prognosis in human gliomas. Clin Neurol Neurosurg. 2012;114(4):356–60. https://doi.org/10.1016/j.clineuro.2011.11.005.
123. Kruthika BS, Jain R, Arivazhagan A, Bharath RD, Yasha TC, Kondaiah P, et al. Transcriptome profiling reveals PDZ binding kinase as a novel biomarker in peritumoral brain zone of glioblastoma. J Neurooncol. 2019;141(2):315–25. https://doi.org/10.1007/s11060-018-03051-5.
124. Joel M, Mughal AA, Grieg Z, Murrell W, Palmero S, Mikkelsen B, et al. Targeting PBK/TOPK decreases growth and survival of glioma initiating cells in vitro and attenuates tumor growth in vivo. Mol Cancer. 2015;14:121. https://doi.org/10.1186/s12943-015-0398-x.
125. Li J, Xu L, Bao Z, Xu P, Chang H, Wu J, et al. High expression of PIWIL2 promotes tumor cell proliferation, migration and predicts a poor prognosis in glioma. Oncol Rep. 2017;38(1):183–92. https://doi.org/10.3892/or.2017.5647.
126. Tu Z, Peng J, Long X, Li J, Wu L, Huang K, et al. Sperm autoantigenic protein 17 predicts the prognosis and the immunotherapy response of cancers: a pan-cancer analysis. Front Immunol. 2022;13:844736. https://doi.org/10.3389/fimmu.2022.844736.
127. Zhao J, Liu B, Yang JA, Tang D, Wang X, Chen Q. Human sperm-associated antigen 4 as a potential biomarker of glioblastoma progression and prognosis. NeuroReport. 2019;30(6):446–51. https://doi.org/10.1097/wnr.0000000000001226.
128. Kessler AF, Feldheim J, Schmitt D, Feldheim JJ, Monoranu CM, Ernestus RI, et al. Monopolar spindle 1 kinase (MPS1/TTK) mRNA expression is associated with earlier development of clinical symptoms, tumor aggressiveness and survival of glioma patients. Biomedicines. 2020;8(7):192. https://doi.org/10.3390/biomedicines8070192.
129. Zheng B. Expression and clinical importance of a newly discovered alternative splice variant of the gene for acrosin binding protein found in human brain tumors. Asian Biomed (Res Rev News). 2020;14(6):243–52. https://doi.org/10.1515/abm-2020-0033.
130. Guo L, Wu Z. FOXM1-mediated NUF2 expression confers temozolomide resistance to human glioma cells by regulating autophagy via the PI3K/AKT/mTOR signaling pathway. Neuropathology. 2022;42(5):430–46. https://doi.org/10.1111/neup.12824.
131. Huang SK, Qian JX, Yuan BQ, Lin YY, Ye ZX, Huang SS. SiRNA-mediated knockdown against NUF2 suppresses tumor growth and induces cell apoptosis in human glioma cells. Cell Mol Biol. 2014;60(4):30–6.
132. Jiang J, Liu Y, Fang W, Liu F. Sperm-associated antigen 9 promotes astrocytoma cell invasion through the upregulation of podocalyxin. Mol Med Rep. 2014;10(1):417–22. https://doi.org/10.3892/mmr.2014.2168.
133. Yi F, Ni W, Liu W, Pan X, Han X, Yang L, et al. SPAG9 is overexpressed in human astrocytoma and promotes cell proliferation and invasion. Tumor Biol. 2013;34(5):2849–55. https://doi.org/10.1007/s13277-013-0845-5.
134. Tandon D, Banerjee M. Centrosomal protein 55: a new paradigm in tumorigenesis. Eur J Cell Biol. 2020;99(5):151086. https://doi.org/10.1016/j.ejcb.2020.151086.
135. Jeffery J, Sinha D, Srihari S, Kalimutho M, Khanna KK. Beyond cytokinesis: the emerging roles of CEP55 in tumorigenesis. Oncogene. 2016;35(6):683–90. https://doi.org/10.1038/onc.2015.128.
136. Wang G, Liu M, Wang H, Yu S, Jiang Z, Sun J, et al. Centrosomal protein of 55 regulates glucose metabolism, proliferation and apoptosis of glioma cells via the Akt/mTOR signaling pathway. J Cancer. 2016;7(11):1431–40. https://doi.org/10.7150/jca.15497.
137. Li F, Jin D, Tang C, Gao D. CEP55 promotes cell proliferation and inhibits apoptosis via the PI3K/Akt/p21 signaling pathway in human glioma U251 cells. Oncol Lett. 2018;15(4):4789–96. https://doi.org/10.3892/ol.2018.7934.

138. Xu Y, Lu T, Xu W, Chen W, Liang W, Jin W. Expression of centrosomal protein 55 in glioma tissue and the influence on glioma cell functions. Transl Cancer Res. 2019;8(1):228–37. https://doi.org/10.21037/tcr.2019.01.33.
139. Sun C, Zheng X, Sun Y, Yu J, Sheng M, Yan S, et al. Identification of IGF2BP3 as an adverse prognostic biomarker of gliomas. Front Genet. 2021;12: 743738. https://doi.org/10.3389/fgene.2021.743738.
140. Johnson RM, Phillips HS, Bais C, Brennan CW, Cloughesy TF, Daemen A, et al. Development of a gene expression-based prognostic signature for IDH wild-type glioblastoma. Neuro Oncol. 2020;22(12):1742–56. https://doi.org/10.1093/neuonc/noaa157.
141. Suvasini R, Shruti B, Thota B, Shinde SV, Friedmann-Morvinski D, Nawaz Z, et al. Insulin growth factor-2 binding protein 3 (IGF2BP3) is a glioblastoma-specific marker that activates phosphatidylinositol 3-kinase/mitogen-activated protein kinase (PI3K/MAPK) pathways by modulating IGF-2. J Biol Chem. 2011;286(29):25882–90. https://doi.org/10.1074/jbc.M110.178012.
142. Bhargava S, Visvanathan A, Patil V, Kumar A, Kesari S, Das S, et al. IGF2 mRNA binding protein 3 (IMP3) promotes glioma cell migration by enhancing the translation of RELA/p65. Oncotarget. 2017;8(25):40469–85. https://doi.org/10.18632/oncotarget.17118.
143. Sastry NG, Wan X, Huang T, Alvarez AA, Pangeni RP, Song X, et al. LY6K promotes glioblastoma tumorigenicity via CAV-1-mediated ERK1/2 signaling enhancement. Neuro Oncol. 2020;22(9):1315–26. https://doi.org/10.1093/neuonc/noaa032.
144. Wang J, Zuo J, Wang M, Ma X, Gao K, Bai X, et al. Polo-like kinase 4 promotes tumorigenesis and induces resistance to radiotherapy in glioblastoma. Oncol Rep. 2019;41(4):2159–67. https://doi.org/10.3892/or.2019.7012.
145. He J, Zhao Y, Zhao E, Wang X, Dong Z, Chen Y, et al. Cancer-testis specific gene OIP5: a downstream gene of E2F1 that promotes tumorigenesis and metastasis in glioblastoma by stabilizing E2F1 signaling. Neuro Oncol. 2018;20(9):1173–84. https://doi.org/10.1093/neuonc/noy037.
146. Rodrigues-Junior DM, Biassi TP, Carlin V, Buri MV, Torrecilhas AC, Bortoluci KR, et al. OIP5 expression sensitize glioblastoma cells to lomustine treatment. J Mol Neurosci. 2018;66(3):383–9. https://doi.org/10.1007/s12031-018-1184-1.
147. Wei X, Chen F, Xin K, Wang Q, Yu L, Liu B, et al. Cancer-testis antigen peptide vaccine for cancer immunotherapy: progress and prospects. Transl Oncol. 2019;12(5):733–8. https://doi.org/10.1016/j.tranon.2019.02.008.
148. Shraibman B, Kadosh DM, Barnea E, Admon A. Human leukocyte antigen (HLA) peptides derived from tumor antigens induced by inhibition of DNA methylation for development of drug-facilitated immunotherapy. Mol Cell Proteomics. 2016;15(9):3058–70. https://doi.org/10.1074/mcp.M116.060350.
149. Liu Z, Poiret T, Persson O, Meng Q, Rane L, Bartek J Jr, et al. NY-ESO-1- and survivin-specific T-cell responses in the peripheral blood from patients with glioma. Cancer Immunol Immunother. 2018;67(2):237–46. https://doi.org/10.1007/s00262-017-2066-z.
150. Natsume A, Wakabayashi T, Tsujimura K, Shimato S, Ito M, Kuzushima K, et al. The DNA demethylating agent 5-aza-2′-deoxycytidine activates NY-ESO-1 antigenicity in orthotopic human glioma. Int J Cancer. 2008;122(11):2542–53. https://doi.org/10.1002/ijc.23407.
151. Konkankit VV, Kim W, Koya RC, Eskin A, Dam MA, Nelson S, et al. Decitabine immunosensitizes human gliomas to NY-ESO-1 specific T lymphocyte targeting through the Fas/Fas ligand pathway. J Transl Med. 2011;9:192. https://doi.org/10.1186/1479-5876-9-192.
152. Everson RG, Antonios JP, Lisiero DN, Soto H, Scharnweber R, Garrett MC, et al. Efficacy of systemic adoptive transfer immunotherapy targeting NY-ESO-1 for glioblastoma. Neuro Oncol. 2016;18(3):368–78. https://doi.org/10.1093/neuonc/nov153.
153. Yan X, Su Z, Zhang J, Wu Z, Ye S, Lu X, et al. Killing effect of interleukin-13 receptor alpha 2 (IL-13Ralpha2) sensitized DC-CTL cells on human glioblastoma U251 cells. Cell Immunol. 2010;263(2):172–5. https://doi.org/10.1016/j.cellimm.2010.03.013.
154. Karakaş N, Stuckey D, Revai-Lechtich E, Shah K. IL13Rα2- and EGFR-targeted pseudomonas exotoxin potentiates the TRAIL-mediated death of GBM cells. Int J Mol Med. 2021;48(1):145. https://doi.org/10.3892/ijmm.2021.4978.
155. Saka M, Amano T, Kajiwara K, Yoshikawa K, Ideguchi M, Nomura S, et al. Vaccine therapy with dendritic cells transfected with Il13ra2 mRNA for glioma in mice. J Neurosurg. 2010;113(2):270–9. https://doi.org/10.3171/2009.9.Jns09708.
156. Sattiraju A, Solingapuram Sai KK, Xuan A, Pandya DN, Almaguel FG, Wadas TJ, et al. IL13RA2 targeted alpha particle therapy against glioblastomas. Oncotarget. 2017;8(26):42997–3007. https://doi.org/10.18632/oncotarget.17792.
157. Kim K, Gwak HS, Han N, Hong EK, Choi BK, Lee S, et al. Chimeric antigen receptor t cells with modified interleukin-13 preferentially recognize IL13Rα2 and suppress malignant glioma: a preclinical study. Front Immunol. 2021;12:715000. https://doi.org/10.3389/fimmu.2021.715000.
158. Yin Y, Rodriguez JL, Li N, Thokala R, Nasrallah MP, Hu L, et al. Locally secreted BiTEs complement CAR T cells by enhancing killing of antigen heterogeneous solid tumors. Mol Ther. 2022;30(7):2537–53. https://doi.org/10.1016/j.ymthe.2022.05.011.
159. Hou AJ, Shih RM, Uy BR, Shafer A, Chang ZNL, Comin-Anduix B, et al. IL-13Rα2/TGF-β bispecific CAR-T cells counter TGF-β-mediated immune suppression and potentiate anti-tumor responses in glioblastoma. Neuro Oncol. 2024. https://doi.org/10.1093/neuonc/noae126.
160. Rossmeisl JH, Herpai D, Quigley M, Cecere TE, Robertson JL, D'Agostino RB, et al. Phase I trial of convection-enhanced delivery of IL13RA2 and EPHA2 receptor targeted cytotoxins in dogs with spontaneous intracranial gliomas. Neuro Oncol. 2021;23(3):422–34. https://doi.org/10.1093/neuonc/noaa196.
161. Kikuchi R, Ueda R, Saito K, Shibao S, Nagashima H, Tamura R, et al. A pilot study of vaccine therapy with multiple glioma oncoantigen/glioma angiogenesis-associated antigen peptides for patients with recurrent/progressive high-grade glioma. J Clin Med. 2019;8(2):263. https://doi.org/10.3390/jcm8020263.
162. van Tellingen O, Yetkin-Arik B, de Gooijer MC, Wesseling P, Wurdinger T, de Vries HE. Overcoming the blood-brain tumor barrier for effective glioblastoma treatment. Drug Resist Updat. 2015;19:1–12. https://doi.org/10.1016/j.drup.2015.02.002.
163. Lauko A, Lo A, Ahluwalia MS, Lathia JD. Cancer cell heterogeneity & plasticity in glioblastoma and brain tumors. Semin Cancer Biol. 2022;82:162–75. https://doi.org/10.1016/j.semcancer.2021.02.014.