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REVIEW ARTICLE

The IL13α 2R paves the way for anti-glioma nanotherapy

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Abstract Glioblastoma (GBM) is one of the most aggressive (grade IV) gliomas characterized by a high rate of recurrence, resistance to therapy and a grim survival prognosis. The long-awaited improvement in GBM patients' survival rates essentially depends on advances in the development of new therapeutic approaches. Recent preclinical studies show that nanoscale materials could greatly contribute to the improvement of diagnosis and management of brain cancers. In the current review, we will discuss how specific features of glioma pathobiology can be employed for designing efficient targeting approaches. Moreover, we will summarize the main evidence for the potential of the IL-13R alpha 2 receptor (IL13α2R) targeting in GBM early diagnosis and experimental therapy.

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Introduction

Glioblastoma multiforme (GBM), also known as grade IV astrocytoma, is the most common form of primary brain cancer, accounting for ~52% of all primary brain tumors. Arising from brain's glia cells and their progenitors, GBM manifests itself either as a slowly-growing malignant tumor or as an aggressive neoplasm that develops rapidly and leads to early mortality. Recent studies on GBM pathobiology identified multiple signal transduction pathways determining prognosis and treatment strategies for this type of cancer.[1] The most important signaling molecules implicated in GBM pathology include insulin-like growth factor (IGF)-binding protein-3 (IGFBP3), RECK, and TIMP3. The TGF-β/SMAD signaling pathway also plays a crucial role in GBM progression by mediating the miR-21 regulation.[2] The most critical and specific for the glioma tumor progression is the NOTCH pathway, which represents a point of convergence of many important endogenous signaling pathways as well as those activated by exogenous factors (radiation, hypoxia, etc). Activation of the cancer stem cell proliferation is usually regulated by BMP, Notch, Wnt and NF-κB signaling pathways.[3]

An advanced treatment strategy utilizing a combination of radiation and chemotherapy protocols still results in poor clinical outcomes for patients with median survival standing at 14 months, mostly due to a high rate of the disease recurrence. In the past decade, studies on the mechanisms of GBM tumor cell survival in the course of therapeutic intervention became relevant to and increasingly important for the success of glioblastoma treatment. There is a large body of evidence indicating that the mechanisms of GBM cell survival are one of the major factors behind the origin of the glioblastoma therapeutic resistance acquired as a result of conventional GBM therapy. Currently, the standard of care (a conventional treatment strategy) for GBM includes radiotherapy followed by chemotherapy with Temozolomide (TMZ). Taking into consideration the high degree of tumor infiltration and the effect of the tumor on its microenvironment, surgical resection is usually not recommended.[4] It has been shown that the surviving cells trigger a robust activation of the cellular programs that mediate a non-canonical tumor stress response, such as UPR-induced autophagy.[5] The survival and drug resistance of glioblastoma cells may play a pivotal role in the clinical outcome of the patients' treatment, since some experimental agents may exert a dual effect by inducing cell death, on the one hand, and the tumor cell resistance, on the other. Therefore, in spite of the encouraging clinical efficacy demonstrated by some GBM combinational therapies, a further improvement of the patients' long-term survival remains both the ultimate goal and the first priority task for novel experimental therapies of this deadly disease.

From GBM pathobiology and the molecular mechanisms of tumor recurrence to new therapeutic strategies

The median survival of GBM patients after surgical intervention, unlike that of patients with other solid tissue malignancies, is only approximately 6 months, while addition of radio/chemotherapy combinational treatment can prolong it up to 14.6 months.[2] Resection of the tumor mass in combination with TMZ and ionizing radiation treatments significantly increases GBM patients' survival, but is unable to prevent tumor recurrence. Moreover, recent data obtained in glioma cell lines demonstrate an upregulated expression of genes responsible for angiogenesis and stemness.[3] Several proteins have been implicated in glioma cell plasticity, intracellular communication and resistance to the therapy,[4] although their role in tumor progression and survival is controversial. Most recently, data from the rapidly evolving field of sequencing- and array-based gene expression profiling, and molecular/genetic characterization of tumors allowed to classify GBM tumors into various molecular subtypes that differ with regard to TERT and ATRX[5] promoter mutations as well as MGMT promoter methylation and IDH1/2 mutation status. A robust gene expression-based molecular classification of GBM tumors from 206 patient samples by Verhaak et al., revealed an 840-gene cluster signature defining 3 distinct GBM subtypes (proneural, neural, and mesenchymal).[6] Despite the recent advancements in GBM classification, the precise molecular mechanism regulating the survival and resistance of glioma cells remains elusive and is pending investigation. It has been shown that: *i)* accumulation of IDH1/2 mutations[7] in genomic DNA leads to the production of D-2-hydroxyglutarate followed by an epigenetic shift (IDH mutations impair histone demethylation, block cell differentiation and promote genomic instability[8,9]); *ii)* the lack of MGMT promoter methylation[10] protects the tumor cells via the expression of O^6-alkylguanine DNA alkyltransferase that removes the O^6-methyl groups from methylguanine modification of DNA, caused by the alkylating activity of TMZ, thereby repairing the TMZ-induced damage[11]; *iii)* transcription and accumulation of human cytomegalovirus IE1 protein mediates intracellular persistence of the human cytomegalovirus[12,13] that has been linked to GBM; *iv)* activation of intracellular signaling of EGFR[14] by miR-1238 and/or translocation of the cellular VEGFR2 protein[15] could lay foundation for the tumor cell adaptive response involving activation of cell proliferation and invasiveness as well as suppression of apoptosis. Although these events found mainly in primary glioblastomas are important for understanding the mechanisms of GBM drug resistance and tumor progression, the exact cause of GBM recurrence is still unclear and warrants further investigation.[16–18] The mechanisms of protein expression and intracellular accumulation determine cellular responses to therapy with alkylating agents.[19] In our studies we have identified multiple cellular proteins promoting angiogenesis, cell proliferation and division capable of promoting drug resistance and survival of glioma cells, suggesting the existence of several mechanisms for GBM adaptation to TMZ therapy.

In addition to the recent progress in our understanding of the mechanism of tumor adaptation, multiple attempts have been made to increase the efficacy of existing therapeutic approaches and develop new strategies targeting the affected tumor environment, while sparing tumor-surrounding healthy brain cells. However, combination of temozolomide (TMZ) and radiation therapy (RT) remains the standard of care for GBM treatment as the clinical trial

conducted by Stupp et al., demonstrated efficacy of TMZ + RT in gliomas that improved patient survival.[20]

Having evolved from basic research in the field of neuro-oncology and complemented by recent developments in medicinal chemistry,[21] cancer immunology,[22,23] virology[24] and nanotechnology,[25,26] current GBM therapies undergo a continuous improvement. Advancements in glioma therapy are based on the growing body of knowledge in the field of molecular mechanisms of cancer. Further improvement of the disease treatment efficacy requires new strategies involving personalized proteomic[27] and genomic[28,29] data collection and analysis. This approach holds promise for the improvement of therapeutic outcomes for GBM patients through identification of patient-specific therapeutic targets.

Various new strategies of GBM treatment utilize the principle of targeted delivery of cytotoxic modalities into the cancer cells via a specific receptor,[30,31] or a unique "signature" protein exposed selectively on the target cells' surface.[32,33] Those include targeted delivery of chemotherapy drugs,[34] and other therapeutic vehicles by means of non-viral, viral or cell-based vectors, or functionalized nanoparticles. Although various reports suggested autophagy as a potential new anticancer strategy, the overall benefits of targeting specific glioma receptors and autophagy activation for glioma treatment remain to be further investigated. In this regard, any evidence for the connection between autophagy regulation and the expression of GBM molecular markers may have clinical relevance and potential therapeutic implications.

Targeting via a glioma-specific surface receptor: the rationale and the perspectives for the experimental GBM therapy

Unfortunately, none of the existing anti-glioma therapies is capable of providing a complete cure for this cancer, primarily because the molecular mechanisms of GBM progression and recurrence are not fully understood. Recent studies have demonstrated that modulation of the expression of certain cell surface molecules plays a pivotal role in determining the degree of disease progression[35] and tumor aggressiveness.[36] Multiple receptor-mediated signaling pathways transmitting molecular signals from the cell surface could be involved in the mechanism of glioma recurrence. While some of them are universally involved in the modulation of GBM progression,[37,38] others are being activated only as a consequence of changes in the brain tumor microenvironment[39] and/or a stress response.[40,41] Those pathways involve mediators of inflammation and cytokine release, modulators of apoptosis[42] and an immune response, such as VEGF, which exerts suppressive effects on the innate immunity and pro-angiogenic function of microglia/macrophages.

The induction of glioma cell surface markers in response to stress could be partially explained by the increased production of proliferation-activating molecules,[43] limited glucose availability and hypoxia.[44] These are observed together with some tissue remodeling that leads to changes in the composition of extracellular matrix and promotes glioma cell proliferation and invasion. In the past decade, the major efforts were made towards investigating the role of molecular markers of GBM, such as fibroblast growth factor receptor (FGFR),[45,46] CD44 cell surface glycoprotein,[47] platelet-derived growth factor receptor (PDGFR)[48] and epidermal growth factor receptor (EGFR),[49] in glioma progression as well as the perspective of their utilization for nanotechnology-based applications. Those molecules were used in a variety of hybrid vector constructs, such as *i*) recombinant adenoviral vector dual-targeted to both surface EGFR and integrin receptors,[50] *ii*) a boronated dendrimer (BD)-EGF bioconjugate for boron neutron capture therapy (BNCT),[51] *iii*) hyaluronan-based lipid nanoparticles[52] and solid lipid nanoparticles (CASLNs) carrying carmustine (BCNU) grafted with anti-epithelial growth factor receptor (EGFR) antibody,[53] *iv*) iron-oxide nanoparticles conjugated with EGFRvIII antibody for targeted therapy,[54] and *v*) quantum dots (QDs) bound to the extracellular domain of EGFR protein.[55]

However, recent reports have suggested that glioma resists therapies based on targeting the cell surface EGFR due to accumulation of the cell population with exclusively nuclear localization of this receptor.[56] Moreover, the intrinsic mechanism of accumulation of truncated EGFR variants in glioma cells with different genetic and treatment resistance profiles should be taken into account by the ongoing GBM clinical trials utilizing EGFR-targeting through EGF ligand fusing/binding approaches as a therapeutic strategy.[57]

Cell surface markers overexpressed on both glioma stem cells and brain endothelial cells (implicated in transfer across BBB), such as transferrin receptor (TfR), are of particular interest for glioma targeting due to their lack or lower abundance on noncancerous cells.[58] For example, an efficient uptake of nanoparticles conjugated with transferrin by glioma cells offers an effective strategy for glioma-specific surface receptor delivery of quantum dots,[59] liposomes,[60] magnetic,[61] gold[62] and PLGA nanoparticles,[62] or even encapsulated drugs, such as resveratrol,[63] or zoledronic acid[64] via the ligand-specific route of internalization.

Several investigators proposed to use $\alpha v\beta 3$ integrin or PDGFR as alternative platforms for targeted cancer therapy and bio-imaging.[65] For example, targeting glioblastoma cells with hybrid cRGD peptide-conjugated nanoparticles loaded with doxorubicin made it possible to accumulate and retain the nanocarriers in tumors and achieve strong anti-proliferative effects in U87 glioblastoma cells in culture.[66]

Nonetheless, despite the efforts in developing new efficient therapies utilizing $\alpha v\beta 3$ integrins, LRP1 or PDGFR receptors as molecular targets, a long-term survival of mice bearing glioma xenografts has not yet been achieved. Thus, specificity of glioma targeting requires further improvement.

The IL13 alpha 2: opportunities for binding and targeting

Recent advancements in understanding the structure of interleukins IL4 and IL13, the functional mechanism of signaling through their cognate receptor IL13Rα1 and its role in GBM progression have made IL13R an attractive molecular target for intracellular delivery of various anti-glioma therapeutics. This review focuses on the use of

surface molecules such as IL13R alpha 2 (IL13R domain) for experimental therapy and discusses the possible mechanisms and prospects for GBM therapy based on this strategy. Intensive studies of the molecular mechanisms of the IL13-IL13R interaction,[67,68] including the recent X-ray diffraction analysis of the IL13/IL13R complex[69] (modelled on Fig. 1), have revealed a remarkably high affinity of IL13 to the alpha 2 subunit of the IL13R. Subsequently, Wykosky et al.[70] showed that the main subunit of IL13 receptor IL13R alpha 2 (IL13α2R) is differentially expressed in glioma cells and its expression levels correlate with the disease progression.

Glioma specificity of the cell surface receptor IL13α2R is a critical finding for the development of IL13Rα2-targeting applications. A recent study by Brown et al. demonstrated the presence of IL13α2R also on the surface of tumor infiltrating macrophages (CD11b high/Gr-1 intermediate),[71] indicating a new venue for targeting the glioma environment. Besides, the expression of IL13α2R in a subset of glioma initiating stem cell-like population could become a crucial factor for the future success of GBM targeted therapy. Aberrations in the post-transcriptional splicing of IL13α2R mRNA that lead to various forms of a soluble cytoplasmic IL13α2R protein represent the most intriguing observation.[51–53] Although many of those splice variants lead to non-functional IL13α2R molecules, several studies documented their presence in human tissues. IL13α2R splice mutants lacking the IL13 ligand-binding site appear to be unsuitable for targeting applications using nano- or T-cell-based technologies. According to the findings of several studies, splice variants of IL13α2R are lacking the transmembrane motif and accumulate only in the soluble form.[51,53] Thus, it remains unclear whether the splice variants are even exposed on the surface of human glioma cells. As reported by Bhardwaj et al., two AP-1 transcription factors (c-Jun and Fra-1) become overexpressed at mRNA and protein levels in IL13α2R positive U251 and A172 glioma cell lines in response to treatment with IL-13.[72] Activation of Fos/Jun transcriptional factors in tumor cells leads to their resistance to anticancer drugs.[73–75]

Signaling through IL13R alpha 2 could be enhanced by its cooperation with other signal transduction pathways such as the MMP8-related cell signaling pathway. Metalloproteinases are involved in the direct cleavage of IL13α2R to release the extracellular domain from the membrane surface-associated receptor, thereby generating a soluble form.[76] MMP8-related pathways implicated in the digestion of the extracellular matrix have been shown to become upregulated during the normal brain development. Most recently, Han et al., analyzed mRNA expression profiles in GBM patients' samples submitted to TCGA database and found that, although there was no correlation between IL13α2R levels and GBM drug resistance, the receptor expression contributed to the induction of immunosuppression genes such as *CCL2, FMOD* and *OSM*.[67] On the other hand, the expression of cathepsin O and ATG4A proteins showed a significant Spearman correlation with that of IL13α2R mRNA (TCGA, Agilent 4502a dataset) suggesting a possible link between the IL13 signaling and the autophagy-mediated cell survival pathways. This indicates that autophagy activation could play an important role of a common effector explaining the observed synergy between the IL13α2R-targeting and drug-based therapies. Nonetheless, it is still unclear to what degree these pathways may affect the IL13α2R signaling and thus could become the focus of future studies, like the ones carried out with titanium oxide nanoparticles.[77]

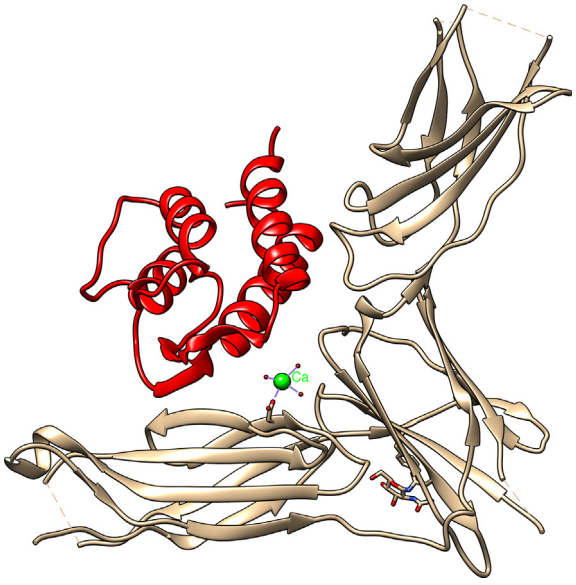

Figure 1 A structure of the high affinity complex between IL13 and IL13R alpha 2. The 44.25 kDa IL13R alpha 2 subunit (*golden*) binds as a monomer to its cognate 14.33 kDa IL13 ligand (*red*) in the presence of calcium ion (*asterisk*). PDB 3LB6 (protein data bank, #3LB6, structure of IL13 in complex with IL13α2R subunit (https://www.rcsb.org/structure/3lb6)).

IL13R alpha 2 and immunotherapeutic strategies

The recent emergence of therapeutic antibodies opened wide perspectives for the development of new promising GBM treatment strategies. The contemporary protein engineering technology offers a new opportunity for targeting any tumor-specific antigen on the cell surface. A monoclonal antibody to any tumor-associated marker can be engineered to deliver therapeutic payloads that cannot be specifically and efficiently delivered by the conventional methods. With regard to the IL13R alpha 2 receptor, several studies have confirmed this antigen presentation by glioblastoma multiforme tumors.[72,78]

The development of a hybridoma cell line secreting high-affinity antibody specific for the tumor-associated antigen IL13α2R offered a new therapeutic strategy for the treatment of glioblastoma both *in vitro* and *in vivo*.[79] Remarkably, the engineered antibody against the IL13α2R receptor is capable of completely blocking the interaction between the soluble form of IL13 ligand and its cognate receptor, consistently with the high binding specificity and affinity observed for this antibody *in vitro*. The availability of high-affinity antibodies against IL13α2R led to the emergence of immunotherapeutic approaches such as dendritic cell-based therapy.[80] The latter is based on

pulsing dendritic cells with peptides derived from IL13α2R, EGFRvIII or gp100 antigens for enhancement of tumor antigen presentation and augmentation of anti-tumoral immune responses. This approach has been tested both in *in vitro*[81] and clinical trials,[82] where GBM patients not only developed strong immune response, but also exhibited an extended progression-free survival (PFS). On the other hand, genetic manipulation with T cells allows overcoming the suppression of anti-tumoral immune response, known to develop within the tumor environment, and represents a promising strategy for GBM therapy. Redirecting chimeric antigen receptor (CAR)-engineered T-cells to neoantigens fused to ligands or antibodies, triggers activation of T-cell response against tumor cells expressing IL13α2R.[83] Activated T-cells in turn begin to secrete cytokines like TNF alpha, IFN-gamma and GM-CSF, which is followed by cytotoxic response (lysis) of the IL13α2R-positive cells.[80]

The successful use of IL13α2R targeting platform for nanotechnology-based therapeutic applications[84] that includes utilization of adenovirus-[85] or herpesvirus-based[86] vectors, titanium oxide-based nanoparticles,[87] magnetic-vortex disks[88] (Fig. 2) and modified T-cells[89] underscores its potential benefit for the human clinical applications (Fig. 3). Whether the ablation of IL13α2R is a better therapeutic strategy than the one based on internalization through this receptor requires future investigation. Likewise, the potential utility of the IL13α2R-targeting for TMZ/XRT anti-glioma therapy is yet to be evaluated.

IL13Rα2-targeted nanocarriers: a twist in tumor targeting

It is commonly accepted that GBM drug resistance is the main reason for clinical inefficiency of the standard anti-glioma therapies. Various drugs showed low efficiency for glioma due to acquired resistance arising from the heterogeneity of glioma tumor cells and, particularly, from their genetic diversity, allowing the resistant cell populations to evade therapies and selectively proliferate, leading to post-treatment tumor recurrence.[90] However, it is not the only hurdle in drug-based glioma therapies. Although the natural blood—brain barrier (BBB) leakage during glioma progression has been noted,[91] pre-clinical and clinical applications of advanced drug delivery based on BBB permeabilization techniques, such as convection-enhanced delivery (CED), play an essential role in improving survival in GBM animal models and/or human patients. Thus, selection of a delivery method for a multidrug combinational therapy utilizing different cell signaling pathways could offer a significant step forward in the improvement of glioma treatment.

The primary mechanism for glioma drug resistance involves internal and external factors regulated by the stress response and the tumor microenvironment.[92,93] Several mechanisms proposed for GBM chemoresistance link it to the following molecular events and factors: TMZ/XRT sensitivity, induction of artificial autophagy[94] and apoptosis,[95] expression of mismatch- and MGMT-regulated genes,[96] unfolded protein response,[97] long non-coding RNA (lncRNA) and miRNA expression,[98] chromatin remodeling proteins,[99] membrane transporters[100] or DNA double-strand break repair.[101] Various factors and therapeutic approaches show synergy with TMZ/XRT therapy and can be utilized as potential adjuvants to help overcome the therapeutic resistance and prolong the survival of GBM patients (Fig. 3). The tentative mechanisms of this synergistic effect are likely to involve dysregulation of glioma signaling and activation of such pathways, as those of necrotic cell death,[102] autophagy,[103] apoptosis[104,105] or transition of the adaptive stress response to cell death, such as ferroptosis.[106] Earlier, our laboratory showed that conditionally replicative adenoviral vectors work synergistically with TMZ and XRT at the level of autophagy and apoptosis activation.[107] In 2010 we proposed a conceptually novel approach utilizing microfabricated ferromagnetic-vortex disks (MDs) conjugated to IL13α2-specific antibodies (MD—anti-IL13α2R), as illustrated in Figure 2. In these experiments N10[88] and A172[108] glioma cells that selectively overexpress IL13α2R receptor on their surface were targeted by the disks carrying anti-IL13α2R Ab. Owing to their anisotropic shape, the magnetic particles respond to externally-applied magnetic field with a mechanical torque, similar to how a compass needle aligns with the earth's geomagnetic field. Therefore, MDs function as mediators or vectors for delivery of external source energy (the magnetic field) to the cell membrane via a magneto-mechanical coupling.

Application of an AC field energy of as low as 90 Oe and frequency of 10—20 Hz for only 10 min result in drastic cell morphology and biochemistry changes.[88] Tunnel immunofluorescence staining, calcium re-localization microscopy, and studies including caspase-3 inhibitor evidenced the disk-mediated activation of apoptosis in target cells, leading to DNA damage and glioma cell destruction. Considering that TMZ promotes apoptosis in glioma cells,[109] a combination of MDs with TMZ and/or ionizing radiation could potentially augment the suppression of glioblastoma growth.

Although clinical trials in GBM patients using the above approach have not yet been carried out, preclinical evaluation using *in vivo* intracranial glioma models is being carried out in numerous research groups around the world.[110] In our earlier study,[87] we utilized the IL13α2R receptor targeting strategy in conjunction with 5 nm colloid titanium dioxide (anatase) nanoparticles (NPs), as shown in Figure 2. Since TiO_2 is a semiconductor catalyst with a relatively wide band gap of 3.2 eV that restrains its activation to UV-region of the electromagnetic spectrum ($\lambda \leq 387.5$ nm), we modified the surface of the NPs with catecholate linker molecules (e.g., dopamine) to adapt TiO_2 NPs for visible-light activation as well as conjugation with the IL13α2R antibody. Specific targeting of the nanoconjugate to a single A172 GBM cell was then directly demonstrated by using a synchrotron-based X-ray fluorescence microscopy imaging at the sub-micrometer scale.[87] The nanobiohybrid at concentrations ranging from 6 to 600 ng/ml showed an efficient photodynamic therapy when illuminated with white light for a few minutes, which led to destruction of more than 80% of A172 glioma cells (with high IL13α2R overexpression levels), while cytotoxicity for U87 cells with lower levels of the receptor expression achieved a plateau at about 50% and, furthermore, no cytotoxicity was observed under the same conditions in

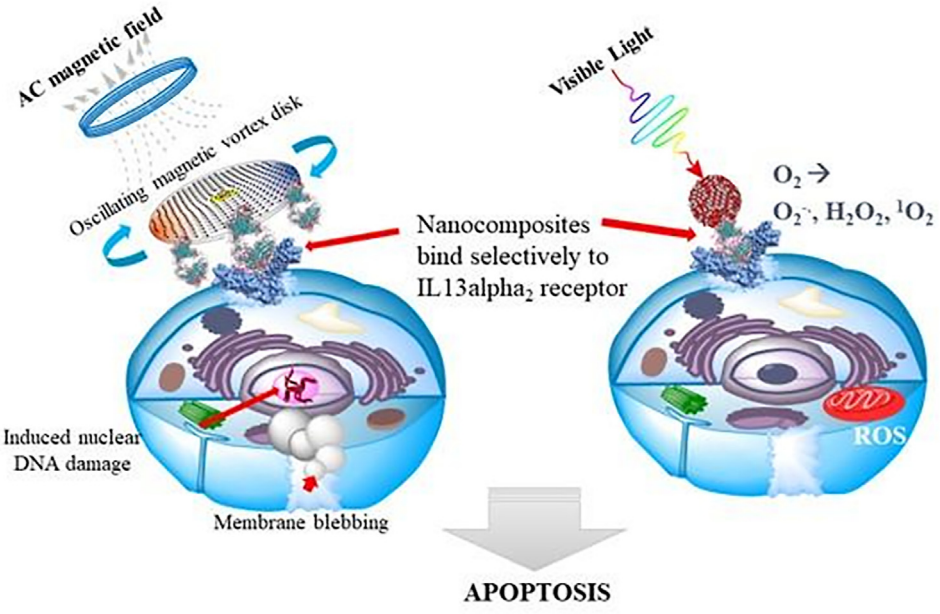

Figure 2 A schematic representation of the mechanism of apoptosis in human glioblastoma cells elicited by innovative nanotechnology-based approaches employing a selective targeting to IL13α2R. The magnetic-vortex disks (*Left*) and TiO_2 anatase nanoparticles (*Right*) are biofunctionalized with anti-human-IL13α2R antibody. When an AC magnetic field or visible light are applied, the disks or the nanoparticles trigger a programmed cell death (apoptosis) via a magneto-mechanical cell destruction mechanisms or by releasing reactive oxygen species (ROS), respectively.

normal human astrocytes (NHAs) that do not express IL13α2R. The other approach that could potentially improve TMZ/XRT-based glioma therapy is based on the induction of autophagy. Autophagy activation involves signaling pathways prerequisite for cytotoxicity induction by TMZ that may have evolved as a response to cell damage by reactive oxygen species (ROS) and Ca^{2+} mobilization.[111] ROS production enhances the TMZ-induced anti-glioma therapeutic effect *in vitro* and *in vivo*. In line with the recent publications,[112,113] the titanium dioxide (TiO_2)-induced stress also promotes the ROS-mediated stress,[114] which is sufficient to suppress the PI3K/AKT pathway and elicit the TMZ-mediated anti-glioma effect. Of note, ionizing radiation has also been shown to produce ROS[115] necessary for ionizing radiation-induced cytotoxicity effect. Hence, modulation of autophagy offers another innovative solution for overcoming glioma chemoresistance, which remains a serious obstacle for glioma therapy.[94] As a consequence of such modulation, the TiO_2-induced autophagy surpasses the autophagy suppression that occurs in glioma cells and can sensitize the tumor cells to TMZ/XRT therapy. In addition, numerous studies reported Ca^{2+} exchange upon treatment with titanium dioxide. This suggests that modulation of Ca^{2+} levels can provide a basis for synergy with current anti-glioma therapeutic modalities. It is also important to emphasize that different research laboratories/teams work with TiO_2 particles that differ with regard to their crystalline phase, size, porosity and surface structure. Differences in physical characteristics could affect therapeutic effectiveness of TiO_2 -NPs upon their light activation. Most recently, a study by Valentini et al. demonstrated a possibility of intracellular Ca^{2+} release in neuronal cells in response to their exposure to TiO_2 -NPs.[116] The latter have been shown in various cancer models to exert their genotoxic effect through modulation of intracellular Ca^{2+} levels and sensitization of the exposed cells to alkylating agents.[117] By studying the protein expression profile of temozolomide-treated glioma cells in the presence of various inhibitors suppressing the caspase-dependent apoptosis, necroptosis, and cathepsins, Buccarelli et al.[106] found that autophagy proteins could be used as important targets in glioma therapy. One of such potential targets is a cysteine protease cathepsin B, a member of the papain family implicated in glioma radioresistance and self-renewal of glioma-initiating cells.[118] The mechanism of cell damage induced by TiO_2 NPs operates through the induction of cathepsin B expression,[119] leading to lysosome leakage into the cytoplasm and initiation of the caspase-mediated cleavage cascade.[120] This may provide a foundation for the synergy with alkylating agents. In line with this, Kruthika et al. demonstrated that expression of a large variety of cathepsins is associated with recurrence of GBM tumors.[104] Finally, a molecular profiling of GBM tumors suggests that, in addition to activation of autophagy signaling, GBM also responds to TiO_2 treatment by upregulation of modulatory proteins, such as STAT3,[121] ELK1,[122] and NF-κb.[123] Therefore, augmentation of therapeutic effects of TMZ/XRT therapy can be achieved by a combination treatment with nanosized TiO_2-NPs or MDs as potential adjuvants.

Preclinical studies using IL13α2R targeting: variety, efficacy and specificity

In order to target glioblastoma cells via IL13α2R, several therapeutic strategies have been proposed. A number of IL13α2R-targeting moieties, including IL13R ligands and

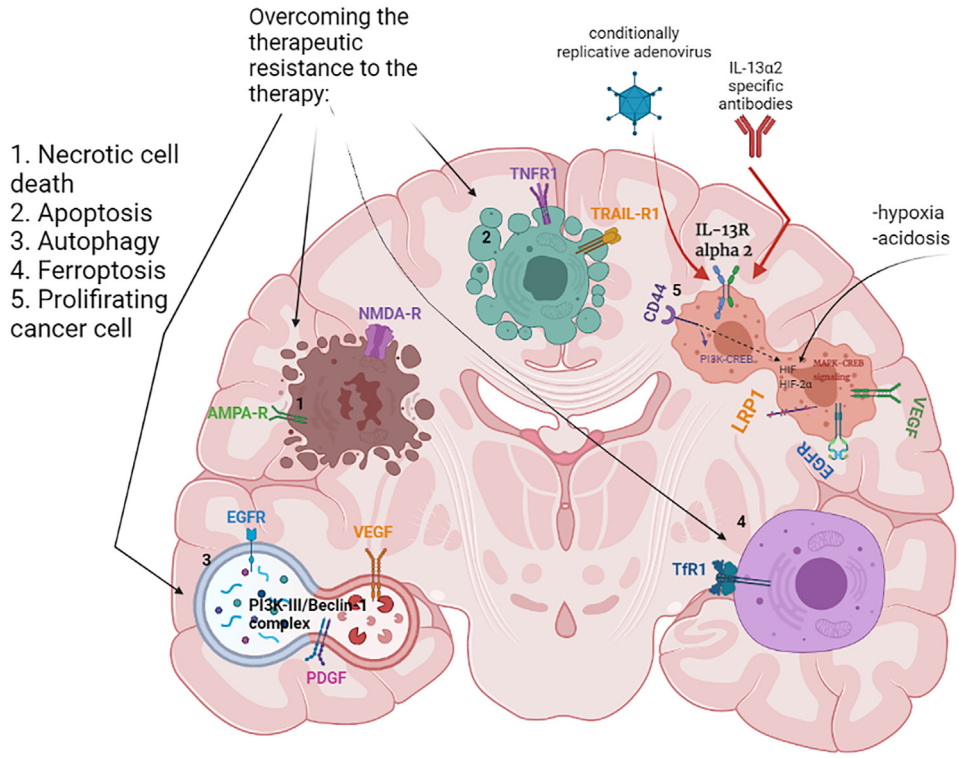

Figure 3 Factors promoting the proliferative activity of human glioblastoma cells. A schematic representation of factors promoting the proliferative activity of the human glioblastoma cell. Those include microenvironmental factors, such as hypoxia and acidosis and cell surface receptors initiating cancer cell proliferation and tumor growth. Targeting receptors with the chemotherapy agents (TMZ/XRT), conditionally replicative adenovirus or magnetic-vortex microdiscs (MDs) or "rotating disks" conjugated to the IL-13R alpha 2 ligand (IL-13) or IL-13α2 specific antibodies may help overcome the therapeutic resistance of glioblastomas through interfering with signaling pathways regulating apoptosis, autophagy and necrotic cell death. The main canonical death receptors of the cell surface responsible for apoptosis are represented by the superfamily of tumor necrosis factor (TNF) receptors,[141] the ones involved in necrosis including AMPA-R and NMDA-R, and the ones regulating ferroptosis including TfR1.[142] The main autophagy regulators are the 5′-AMP-activated protein kinase (AMPK) and the serine/threonine protein-kinase (mTOR) activated mainly through VEGF, PDGF and EGFR.[143]

anti-IL13R single chain antibodies, have been developed for use in the context of targeting applications of adenoviral vectors,[85] liposomal formulation of doxorubicin,[124] and *Pseudomonas aeruginosa* exotoxin A conjugates.[125,126] Owing to their low immunogenicity,[127] high biocompatibility and high affinity to peptide ligands, liposomes offer superior retargeting benefits relative to antibodies and, therefore, targeted delivery of anti-glioma agents to the cancer tissues via the IL13α2R ligands currently represents the most promising anticancer strategy. The IL13α2R ligands[128] have proved useful for targeted delivery of viral vectors,[129] nanoparticles,[114] and magnetic disks (MDs)[88] to glioblastoma cells. Recently, significant efforts have been made in improving identification of GBM tumors and defining their physical margins. The first evidence for overexpression of IL13α2R chain (subunit) in high-grade gliomas, was published in 1998.[130] In this regard, Joshi et al.[131] demonstrated that healthy brain sections contain significant levels of IL13α1R and IL-4R, but only marginal levels of the GBM-restricted IL13α2R form, which led to a clinical trial based on the IL13α2R targeting strategy. This discovery was also highlighted by Debinski et al. in their review.[132] Most recently, Sattiraju et al.[133] suggested utilizing a radiolabeled Pep-1 ligand (Pep-1L) capable of high-affinity binding to IL13α2R subunit as a GBM-targeting platform for selective delivery of anti-glioma agents.

Safety issues related to nanotechnology applications

Glioma progression is a process whereby individual cancer cells with certain properties promote tumor growth and dissemination by proliferation and invasion into the brain parenchyma. It has been shown that glioblastoma-derived extracellular vesicles[134] and exosomes[135] are capable of inducing physiological changes in normal cells of the surrounding brain environment. Pathophysiology of glioma involves its ability to modulate the tumor microenvironment for achieving the most favorable conditions for tumor progression. Those include overcoming contact inhibition of normal cells via disruption of intracellular contacts and modification of surrounding non-cancer cells to suppress their barrier role in preventing tumor development and dissemination. Thus, new experimental approaches to GBM

targeting should include high degree of selectivity for neoplastic cells and produce no or minimal toxicity to normal cells. Most recently, a study by Peng et al.[136] suggested that nanoparticles used in experimental cancer therapies could elicit permeability of endothelial cells, thereby stimulating intravasation of cancer cells, favoring the development of metastatic foci. Specifically, TiO_2-, silica- and gold-basic NPs can exert a strong protumorigenic effect on breast cancer circulating tumor cells (CTCs). In this regard, serious precautions should be taken for biomedical application of nanoparticles, and the prevalence of titanium-oxide based products in cosmetic industry should warrant strong biosafety measures.

Conclusions

The clinical statistics provides an evidence for increasing incidence of GBM disease across the world in the past few years.[137] Although most of the GBM cases have a favorable prognosis, about two-thirds of them become eventually resistant to conventional therapeutic modalities. Therefore, a clear understanding of the GBM therapeutic resistance mechanisms could aid in the development of a better therapeutic approach based on selective targeting of drug-resistant GBM cell populations within glioblastoma tumor mass. A number of such targeting strategies have been proposed for GBM. Features of GBM pathobiology, such as glioma-specific surface receptors FGFR, EGFR, PDGFR or $\alpha v\beta 3$ integrin, can be implemented for designing targeting approaches for GBM therapy. However, the results of preclinical and clinical testing have been so far quite contradictory. In this regard, a combination of different therapeutic approaches could offer a better way of improving survival of GBM patients. Based on preclinical data, IL13α2R receptor is upregulated in most GBM cancer stem cells and in other types of brain cancer stem cells, including breast,[138] ovarian and colon cancer metastases.[139] The evidence of correlation between IL13α2R expression and regulation of autophagy-mediated cell survival[140] makes IL13α2R a suitable target for anti-glioma therapy. It is known that multiple receptors are frequently upregulated via an autocrine mechanism, thereby promoting stem cell-like features, tumor progression, and resistance to cancer therapies. In light of these considerations, the future anti-GBM strategies should be aimed at blocking activation of signaling pathways that may promote glioma invasion and drug resistance. The emergence of anti-IL13R high affinity single chain antibodies and the possibility of genetic manipulations with T-cells enable implementation of immunotherapeutic strategies. Besides, IL13R ligands due to their low immunogenicity and high biocompatibility represent a promising tool for glioma-specific targeted therapy. In the context of delivery methods, the most appropriate are IL13α2R-targeted nanocarriers, such as magnetic discs and titanium dioxide nanoparticles, which are capable of inducing cathepsin B leading to caspase-mediated cleavage cascade. Moreover, inactivation of cellular cathepsins implicated in the regulation of glioma cell stemness and EMT programs can be considered as a new promising anti-glioma approach. Based on this concept, it is reasonable to expect that inhibition of cathepsin axis and its interaction with autophagy signaling can be considered beneficial for the prevention of glioblastoma progression and could greatly improve efficacy of TMZ/XRT-based therapies.

Conflict of Interests

The authors declare no conflict of interests.

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Abbreviations

Akt	protein kinase B
ALDH1A1	aldehyde dehydrogenase 1 family member A
BBB	blood brain barrier
BHLHE40	basic helix-loop-helix family member E40
CEBPB	CCAAT/enhancer-binding protein beta
CD44 and CD133	cluster of differentiation type 44 and 133 cell surface receptors
CED	convection-enhanced delivery
CREB	cAMP response element-binding protein
DNA	deoxyribonucleic acid
EMT	epithelial-to-mesenchymal transition
ENPP2	ectonucleotide pyrophosphatase/ phosphodiesterase 2
ELK1	ETS like-1 protein
FUS	focused ultrasound
GBM	glioblastoma
GSEA	gene set enrichment analysis
HIF1 alpha and HIF 2 alpha	hypoxia-inducible factor 1 alpha or 2 alpha
IAP	inhibitor of apoptosis protein
KLK6	kallikrein related peptidase 6
MAPK	mitogen-activated protein kinase

MGMT	DNA repair enzyme O-(6)-methylguanine DNA methyltransferase
MMP8	metalloproteinase type 8
NP	nanoparticle
PBK	PDZ-binding kinase
PT	peritumoral brain zone
ROS	reactive oxygen species
STAT3	signal transducer and activator of transcription 3
STAT3	signal transducer and activator of transcription 3
SH3GL3	SH3 domain containing GRB2 like 3 protein
SCIN	Scinderin
TC	tumor core
TMZ	temozolomide
VEGF	vascular endothelial growth factor
XRT	ionizing radiation

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