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Adenoviral virotherapy for malignant brain tumors

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Abstract

Glioblastoma multiforme (GBM) is the most common form of primary brain cancer. In the past decade, virotherapy of tumors has gained credence, particularly in glioma management, as these tumors are not completely resectable and tend to micro-metastasize. Adenoviral vectors have an advantage over other viral vectors in that they are relatively non-toxic and do not integrate in the genome. However, the lack of coxsackie and adenovirus receptors (CAR) on surface of gliomas provides for inefficient transduction of wild-type adenoviral vectors in these tumors. By targeting receptors that are over-expressed in gliomas, modified adenoviral constructs have been shown to efficiently infect glioma cells. In addition, by taking advantage of tumor specific promoter (TSP) elements, oncolytic adenoviral vectors offer the promise of selective tumor-specific replication. This dual targeting strategy has enabled specificity in both laboratory and pre-clinical settings. This review looks at current trends in adenoviral virotherapy of gliomas, with an emphasis on targeting modalities and future clinical applications.

Malignant glioma

Malignant gliomas are one of the most aggressive cancers for which limited therapeutic improvement has been made over the past decade. Glioblastoma multiforme (GBM, astrocytoma grade IV) accounts for the majority of all primary brain tumors. Although new drugs like bis-chloronitrosourea (BCNU, Gliadel) and temozolomide (Temodar) have been approved by the Food and Drug Administration (FDA) in recent years, they provide only marginal benefit, with the majority of patients eventually dying from the disease.

Treatment of gliomas

Conventional treatment of GBMs includes surgical resection and radiation therapy. However, due to its location and its tendency to infiltrate the surrounding brain tissue, complete surgical removal is frequently not possible. Even after surgical debulking of the tumor, GBMs tend to micro-metastasize within the brain [1]. Standard radiation therapy is usually in the form of high dose radiation ranging from 50 Gy to 60 Gy [2] and is usually concurrent with chemotherapy. However, radiation is not a complete therapy as a fraction of glioma cells remain radioresistant and evade cellular damage and eventually re-form the tumor. This radioresistance could be due to the presence of CD133+ brain tumor stem cells [3] or other mutations like p53 [4] or the upregulation of the tumor specific protein survivin [5], the complete discussion of which is beyond the scope of this review.

The current course of chemotherapy in gliomas consists of temozolomide (TMZ) according to the Stupp protocol [6]. Compared to radiotherapy alone, combination of TMZ and radiation has prolonged median survival to 14 months [7]. Other drugs that are currently FDA approved and in use include Gliadel [8,9]. Gliadel, in combination with radiation and TMZ, has also

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been used in phase I/II clinical trials and this combination has improved median survival to 21.4 months compared with 12.4 months with radiation and Gliadel alone [7].

Virus as a therapeutic tool

The advent of recombinant DNA technology has enabled pathogenic virus to be harnessed for human good. It has been possible for some time now to create viruses that retain infectivity while attenuating their pathogenicity. This strategy has been utilized to target tumors and express a gene of interest inside the tumor. Alternatively, viruses that are conditionally replicative (CRAds) have been used to multiply inside tumor cells and eventually kill these cells, a method termed virotherapy. In this section, we summarize the different strategies and the different types of viruses used to date.

Treatment of brain tumors using virotherapy has a long history. The earliest publication of virus-glioma interaction was in 1961 when rabies virus was studied in glial tumors [10]. Subsequently, growth of herpes simplex virus and measles virus was also examined in gliomas and other cells of neural origin although the oncolytic effects were not clearly established [11-15]. Although these studies were intended to study growth of viruses more than advance the cause of glioma therapy, they provided valuable resources for subsequent research that looked into virotherapy of gliomas. The first research exclusively focusing on virotherapy of glioma was conducted in Japan in 1982 using mumps virus, a member of the paramyxoviridae family of viruses [16].

The most commonly studied virus in glioma virotherapy has been the herpes simplex virus (HSV). The first of these studies involved a mutation in the thymidine kinase (tk) gene, *dls*ptk that resulted in attenuated neurovirulence. This vector killed glioma cells *in vitro* and controlled tumor growth *in vivo* in an intracranial model of glioma [17]. Further studies showed that mutation in the herpes viral gene $\gamma_1 34.5$ reduced neurotoxicity and its capacity to inhibit tumor growth but most importantly retained sensitivity to common anti-virals used for herpes infections [18]. Most studies with oncolytic HSV have been carried out using this mutation as a backbone and have been elegantly reviewed by Markert [19]. Other major studies on virotherapy of gliomas have been conducted using retrovirus [20-22] and have been reviewed in Rainov and Ren [23]. Measles virus [24], reovirus [25,26], adeno-associated virus [27], Newcastle disease virus (NDV) [28,29], Semliki Forest virus [30], vaccinia virus [31-33] and poliovirus [34,35] have also been used. However, the discussion of these viruses as an effective oncolytic strategy for gliomas is beyond the scope of this review.

Adenovirus as a therapeutic tool

Recombinant adenovirus has been explored as an alternative to conventional chemotherapy and radiotherapy and their combination for almost a decade. Adenovirus has a double stranded DNA genome of 36kbp. Adenovirus serotype-5 (Ad5), the most common of the various subtypes, belongs to subgroup C and infects human cells using the coxsackie and adenovirus receptor (CAR), a 46kDa cell-surface receptor [36-38]. This binding, along with a secondary interaction between the virus penton base proteins and host surface integrins ($\alpha_v\beta_3$ and $\alpha_v\beta_5$), is responsible for adenoviral internalization into cells [39]. The entry of an adenovirus into tumor cells is, however, complicated by the reduction or total absence of CAR in tumor cells in general and GBMs in particular [40]. Targeting the adenovirus to human tumors therefore remains a challenge.

An elegant yet simplistic approach taken to overcome resistance to infection by Ad5 to refractory cells, like tumor cells, is the modification of the Ad5 knob. Since the CAR levels in gliomas are low, the knob is a redundant element of the Ad5 viral structure for the purpose of cancer therapy and therefore can be modified or replaced without loss of infectivity. Strategies

to enhance Ad infection in tumor cells involve modification of this CAR binding domain in the knob region of Ad5. A wide variety of approaches have been taken so far to modify Ad5 knob. One of these approaches involves incorporating the Arg-Gly-Asp (RGD) motif into the Ad knob in order to bind $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins expressed on tumor cells [41]. By modifying the HI loop of Ad5 to incorporate this RGD motif, it is possible to markedly improve Ad tropism to tumor cells in a CAR-independent mechanism. The CRAd, AdΔ24 was also enhanced by the addition of the RGD moiety and tested in panel of glioma cell lines and in an *in vivo* xenograft model [42]. In 10 primary glioma samples tested, cellular toxicity increased by at least 10% to a maximum of 70% in AdΔ24 RGD compared to AdΔ24 alone. *In vivo*, the tumor burden decreased dramatically over a period of 120 days compared to no virus injection as control [42].

Another modification of the knob that enhances tropism of Ad5 is the poly-lysine modification (pK7), whereby addition of multiple lysine residues enhances the electrostatic attraction between the knob and the anionic cell surface receptors like heparan sulfate proteoglycans [39,43]. A vector which incorporates the pK7 modification (AdZ.FpK7) infected U87MG and GL261 cell lines at significantly higher levels than AdZ.F control *in vitro* and in an *in vivo* subcutaneous model [44]. A hybrid fiber-knob domain comprising of Ad5 fiber shaft and Ad3 knob has been used to enhance binding to gliomas. The Ad3 knob has an affinity for binding to CD46, CD80 and CD86 receptors, resulting in more efficient binding and replication compared to that of the wild-type Ad5 virus. First tested in head and neck cancers, Ad5/3 displayed higher infectivity by several hundred-fold over Ad5 vectors [45]. Ad5/3 was also tested in a panel of cell lines including, the U118MG glioma line, and showed an increase in viral tropism vs. Ad5[46]. The authors also generated a mosaic virus by recombining Ad5 and Ad 5/3. These adenoviruses had different fractions of Ad5 and Ad3 fiber on the same viral particle that resulted in significantly higher infectivity in a panel of cell lines, including U118MG. The Ad5/3 chimera was also tested in a panel of glioma cell lines and primary GBM samples for infectivity [47]. The cell lines tested all showed high CD46 levels, making them a good target for this vector. Later studies also showed that CD80/86 levels were high in these cells, an often discussed potential target for the Ad5/3 chimeras [48]. Although *in vitro* results were not significantly higher than Ad5 alone, *ex vivo* analysis of GBM samples showed a 10-fold increase in infectivity compared to the Ad5 vector. Comparison of Ad pK7, AdRGD, and Ad5/3 revealed that while different modifications to the knob work better in some cell lines, there is an increase in viral infectivity in all the modified vectors compared to the Ad5 knob alone [47].

Using the σ1 protein, the receptor-binding molecule of serotype 3 bearing reovirus, in fusion with Ad5 fiber, a recombinant Ad was created that binds to sialic acid and JAM1 molecules in human cell lines including glioma [49]. This fusion Ad 5-Sigma vector was also found to have increased infectivity in a panel of glioma cells compared to wild type Ad5 [47].

Another approach to increase the tropism of adenovirus to glioma cells is the use of “xenotype” knobs. In this method, the knob from a different species of adenovirus is linked to the wild-type human Ad5 fiber. Using the canine, porcine, murine and ovine adenovirus knobs has enabled greater transduction efficiency of these modified viruses [50,51]. The canine adenovirus type I knob, CAV1, was fused to Ad5 to generate Ad5-CAV1 and tested by Zheng et al., [47] for increased tropism to good effect. The canine and porcine knobs were both found to enhance infectivity along with Ad5/3 compared to Ad5 alone in four glioma cell lines tested as well as in primary GBM tissues [50].

An undesired advantage in cancer cells is their upregulation of several receptors that play a role in aberrant growth and migration. This is also true for gliomas and several such receptors, like EGFR, PDGFR, FGFR, IGFR and VEGFR have been found to be highly expressed in

primary gliomas [52]. Modifying Ads that target these receptors bypasses CAR dependency in adenoviral transduction. One of these approaches uses the EGFR receptor that was found to be over-expressed in gliomas [53]. In CAR deficient but EGFR expressing cell lines, the potency of this adenoviral vector is about 1000-fold higher than the control vector that has no EGFR biding domain in the fiber. Fibroblast growth factor (FGF) is another such receptor and has been used to modify the Ad knob for better targeting [54]. The FGF receptor 1 (FGFR1) was shown to be over-expressed in four glioma cell lines as well as in 7 GBM samples tested. By chemically conjugating the FGF2-Fab' antibody to wild-type Ad expressing GFP, the modified virus FGF2-AdGFP was created. The number of GFP positive cells using this modified vector was higher than AdGFP infected cells by at least 5% (U87MG) to as high as 80% in a primary GBM culture. In an intracranial model of mouse glioma, using U118MG cells, the number of GFP positive cells increased dramatically using the recombinant vector than the wild-type GFP expressing vector or PBS controls [54]. More recently, a mutant variation of EGFR, EGFRvIII, has been found to be upregulated in up to 50% of gliomas. This presents a new and attractive target for knob modifications [55,56]. Although these approaches bypass the need for CAR and have met with varying successes both *in vitro* and *in vivo*, tumor biologists have not stopped looking for more innovative solutions.

Successful entry of Ad5 or its derivatives is just one of many steps for Ad virotherapy to be an effective tool in glioma therapy. A second and equally important step is its replication once inside the cells. Efficient replication with subsequent viral progeny release is essential to cell killing and effective glioma therapy. Consequently, a number of innovative approaches have been taken in this regard. Initially, replication incompetent viruses were used for glioma therapy. These viruses were genetically modified with a mutation in the early promoter E1A or both E1A and E1B [57,58]. A replication incompetent adenovirus for example, carrying the cytochrome P450 gene into glioma cells, increased the response of these cells to cyclophosphamide [59]. Several other approaches were used for replication incompetent adenovirus [60-65]. Most notable among them was the approach taken by Zhang et al. [66] in which the entire E1 region of Ad5 was deleted and replaced by the p53 gene driven by the cytomegalovirus (CMV) promoter. This construct, Ad-p53 (also known as Advexin), was tested in a variety of cancers including gliomas. *In vitro*,Ad-p53 induced apoptosis in glioma cells and inhibited the growth of glioma xenografts *in vivo* [67,68]. A phase I clinical trial of 15 patients was conducted showing minimal toxicity and a median recurrence time of 7 months with one patient tumor free after 30 months. The biggest drawback of the study was the lack of diffusion of the viral bolus a few millimeters beyond the injection site [68,69].

In order to overcome the limitations of spread, several groups have focused on the importance of connective tissue and extracellular matrix (ECM). For example, a study on the role of ECM in inhibiting viral spread was done by Kuriyama[70]. The authors observed that addition of proteases such as trypsin and a mixture of collagenase-dispase aided the viral diffusion process. In a separate study, an ECM degrading enzyme, relaxin was used in a replication incompetent (dl-lacZ-RLX) and competent (Ad-ΔE1B-RLX) virus. Increased transduction and viral spread throughout the tumor mass was observed in these subcutaneous models of glioma, showing the importance of the ECM in preventing viral spread [71]. Increased expression of MMP-1 and MMP-8 has also been correlated with increased viral distribution by modulating the ECM [72].

Trask [73] conducted a phase I study in which a replication deficient Ad bearing the HSV-tk gene driven by the Rous Sarcoma Virus (RSV) promoter (Adv.RSVtk) was injected intratumorally followed by ganciclovir (GCV) treatment. The virus was safely tolerated even at the highest dose but failed to achieve significant tumor cell death. A similar study with HSV-tk gene, this time with the CMV promoter, was conducted by Sandmair [74]. There was an increase in frequency of epileptic attacks and a 'tendency' for improved survival was reported

[74]. Another trial was conducted by controlling the HSV-tk gene using the Ad major late promoter (MLP) [64]. The study concluded that patients tolerated the virus and the subsequent GCV treatment, but did not proceed further. Similar studies by Germano et al.,[75] and Immonen et al.,[76] using the Ad vector used by Sandmair, showed little to moderate improvement in survival rates and moderate tolerability. A replication competent version of this Ad was tried in lung cancer and melanoma [77] and in gliomas [78]. In the latter case, it showed increased cytotoxicity, reduced glioma volume, and increased survival in a mouse xenograft model.

These trials with non-replicating adenoviruses failed to significantly change prognosis and the problem of glioma recurrence due to its disseminating nature was not addressed. This underscored the need for replication competent viruses that would infect cells, lyse them, and release viral progeny that can further infect cells in the vicinity and thereby cause the virus to spread. This replicative cycle can potentially prevent recurrences due to micrometastatic lesions. Another huge disadvantage with these viruses was that while they were able to target normal brain and low-grade tumors, they were unable to infect highly aggressive gliomas [58,79]. In light of these limitations, CRAds have entered the clinical scene.

Conditionally replicative adenoviruses (CRAds)

CRAds replicate under certain conditions that are usually found in cancer cells and absent in normal healthy cells. These viruses may have either (1) inessential regions of their genomes deleted, or (2) transduce only cancer cells and spare normal cells because of the over-expression of receptors on the cell surface of gliomas, or (3) tumor specific promoter driving an essential gene in the Ad genome (for example E1A early promoter) [42,80-88]. Earlier in this review we have discussed the second of the three points mentioned here and therefore the remainder of this review will be dedicated to remaining points.

The human adenovirus E1B 55kDa protein interacts with the tumor suppressor p53 and blocks its transcriptional activity. Deletion of this region in the Ad5 genome prevents this interaction and this principle has been used to create a CRAd that replicates in glioma cells that lack p53 protein. Deletion of this 55kDa protein in Ad5 led to the creation of the adenovirus dl1520, also known as ONYX-015. This virus replicated efficiently in gliomas that are attenuated for p53 but not in p53 wild-type tumors or normal human cells that have normal levels of p53 [89]. Initial studies with ONYX-015 showed significant tumor cell killing and reduction in tumor mass in preclinical experiments both *in vivo* and *in vitro* [90]. In clinical studies, ONYX-015 has been used in a phase I clinical trial [91]. Safety and efficacy being the objective of this study, they were met satisfactorily in all 24 patients enrolled, 17 of which were grade IV glioma (GBM) patients. The virus was well tolerated even at its highest dose of 10^{10} pfu. Ten out of 24 patients had some adverse effects but were eventually determined to be not related to the virus treatment. However, only about 50% gliomas are p53 negative, making this virus ineffective in the remaining 50% that are p53 positive, reducing the scope of its application.

Another more successful conditionally replicative approach was undertaken by Fueyo and collaborators. In this approach, a 24-bp deletion in the Ad 5 E1A CR2 domain inhibited the binding of E1A protein to the retinoblastoma protein (pRb). Since the pRb protein negatively regulates cell growth by releasing E2F, a failure to release this inhibition stops cell growth [81]. This E1A mutant Ad, known as Ad5-Δ24 replicates only in tumor cells and not in normal brain cells [81]. Both *in vitro* and *in vivo* studies with this tumor have shown potent cytolytic activities, particularly *in vivo* where a single low dose local injection inhibited tumor grown in a mouse graft model of glioma [81]. Building on the success of these preclinical studies, a modified virus Ad5-Δ24RGD was created that has dual transductional targeting. Addition of this modification increased the cytotoxic effect of the virus in glioma cell lines and *in vivo*

experiments showed 9 out of 10 animals showing complete tumor regression [42]. A still further modification of this virus was the ICOVIR series: ICOVIR-2 and ICOVIR-5. In addition to the modifications in Ad5-Δ24RGD, ICOVIR-5 has an E2F responsive element in lieu of the E1A promoter that further confines the replication of this Ad in tumor cells [92]. *In vitro*, ICOVIR-5 showed an increased anti-glioma effect and increased replication in glioma cell lines; however *in vivo*, there was no significant increase over the second generation Ad5-Δ24RGD virus in controlling tumor growth and long term survival was lower for ICOVIR-5 than Ad5-Δ24RGD (46.5d vs. 71.0d) in a mouse xenograft model of glioma [92]. A summary of adenoviral vectors in clinical trials is listed in Table 1.

Brain tumor specific promoters (TSP), like nestin and GFAP, have been previously used in non-replicating adenoviruses to test the hypothesis of tumor specificity and glioma targeting [94]. These TSPs can be used to drive the E1A gene of Ad5, which would then replicate selectively in glioma cells only where the levels of these proteins are high and not in normal brain tissues. The midkine promoter is one such protein over-expressed in malignant gliomas and a CRAd driven by this promoter showed strong virolytic effects in glioma cells but not in midkine negative normal brain cells. In animal experiments, Ad-MK ablated tumor xenografts in midkine expressing cells [85]. The loss of pRb in tumor cells potentially leads to an excess of free E2F in tumor cells. This hypothesis was elegantly tested by Parr et al., [86] in a rat glioma model. Using a βgal reporter system, an Ad.E2F1.βgal adenoviral vector specifically targeted tumor cells in an *in vivo* model while sparing normal brain tissues when compared to a similar construct driven by the CMV promoter or wild type Ad5. The human telomerase RNA (hTR) and the human telomerase reverse transcriptase (hTERT) is active in a vast majority of cancer cells including gliomas, however, their use in CRAds for glioma specific treatment has been proposed but not yet achieved [95,96].

Survivin is a tumor specific promoter that has recently come into prominence [5,97-99]. It has been used in *in vitro* and *in vivo* studies in glioma models and has shown promise in targeting gliomas [100-102]. Specifically, a CRAd with its E1A driven by the survivin promoter and its knob modified with a pK7 moiety (CRAd-S-pK7) has been shown to have increased targeting to glioma cell lines and to improve survival of animals with intracranial tumors [102,103].

A very ingenious system was developed by Wohlfahrt et al., [104] in which an Ad5 vector was deleted for all E1A and E1B genes. By use of inverted repeats, this virus replicated only in tumor cells and produced E1A, which in turn synergistically allowed more viral replication. By placing tumor necrosis factor related apoptosis-inducing ligand (TRAIL) under the control of this E1A promoter, the replicating cells apoptosed and released TRAIL to the surrounding cells [104]. This virus also used the chimeric Ad 5/Ad 35 knob: a dual modification that promotes more specificity. Such a therapeutic approach was also used along with an Ad5 vector expressing Bax and Caspase-8. Shinoura et al. [87] showed increased cell killing by apoptosis in glioma cell lines using this virus.

Delivery systems using CRAds are an exciting approach and therapeutic genes such as p53 and TRAIL have been tested in gliomas [105]. More recently, a combination of CRAd and TMZ, the drug of choice in gliomas, was tested both *in vitro* and *in vivo* in a mouse xenograft model [106]. The authors observed an increase in toxicity in the combination treatment group as compared to CRAD-S-pK7 or TMZ treatment alone. There was also an increase in long-term survival in a mouse model of glioma after treatment with the combination therapy. This approach should be further tested in a pre-clinical setting.

Limitations of virotherapy

Despite the large body of research cited here on adenoviral therapy of malignant gliomas, the challenge remains unabated. All GBM tissues do not uniformly over-express the same

receptors; therefore they cannot be transduced uniformly by a single fiber modification. Similarly, even after viral entry, efficient adenoviral replication remains a challenge. All glioma cells do not express the same TSPs, nor are they uniformly high. Furthermore, because GBMs tend to disseminate into the brain parenchyma, tumor cells remain out of reach and newer delivery methods have to be devised to overcome this problem [107,108].

In order to overcome the limitations associated with deliver of oncolytic vectors, mesenchymal stem cells (MSC) were used as a carrier of CRAds. These cells have an inherent tropism towards gliomas using growth factors as chemo-attractants. Since MSCs can be easily isolated in an autologous manner and they possess the potential to migrate to tumor-bearing regions of the brain inaccessible by surgical intervention, they are an attractive candidate as a delivery vehicle. MSCs were therefore tested in a mouse model of intracranial glioma [107]. At a viral concentration of 100vp/MSC, a significant increase in viral delivery to distal regions of the brain was achieved compared to a similar concentration of virus alone. This concentration of virus was also found optimal as it reduced cytotoxicity to the host MSCs long enough for them to be able to migrate to these distally located glioma cells in the mouse brain.

The second strategy involves using neural stem cells (NSC) instead of MSCs. These cells have also been shown to have a preference for migration towards gliomas *in vivo* in response to the cytokines released by the tumor [109]. In the study by Tyler et al. [108], NSCs were shown to be able to efficiently migrate towards gliomas and target them by releasing their viral load via a suicide mechanism common for all adenoviral infections. This study also showed that CRAd-loaded NSCs target tumors that are located away from the site of injection of the CRAd-bearing NSCs. Therefore, both of these approaches could be extremely beneficial in targeting and killing glioma cells that are embedded in the brain away from surgical and drug injection sites and need to be evaluated in greater detail.

Attempts to modulate the immune system in order to both augment the anti-tumor immune response while at the same time decrease the anti-viral immune response are an important and ongoing area of research. Using an adenovirus expressing IL-12, GBM cells have been shown to elicit an anti-tumor immune response [110]. Adenoviral expression of the human decorin gene, a known TGF-β antagonist, has also been found to induce an anti-tumor immune response [111]. Pretreatment of gliomas using cyclophosphamide (CPA) resulted in increase in infection of tumor cells by HSV in both athymic and syngeneic models of brain tumor. This increase was found due to immunosupression and down-regulation of IFNs α, β, γ, TNFα, IL-15 and IL-18 genes by CPA [112]. In an adenovirus model, injection of CPA decreased the rate of viral depletion as evidenced by luciferase expression. This effect was found in both replication-deficient and replication-competent adenoviruses AdΔ24CMV-Luc and Ad.CMV-Luc, respectively in athymic mouse model [113]. Another immunotherapy approach consists in the injection of an adenovirus expressing IFN-β to the tumor bed. In a phase I study, there was an increase in apoptotic cells with an associated increase in tumor necrosis [114]. However, detailed studies on the anti-tumor immune response mechanism in glioma are still lacking.

Previous studies have demonstrated that MSCs inhibit mitogen-and alloantigen-induced T cell proliferation [115-118] and NK cell proliferation [119]. Our laboratory has sought to exploit the immunosuppressive capabilities of MSC to enhance the persistence of oncolytic adenoviral vectors *in vivo*. Using the permissive cotton rat model, we have confirmed that MSC loaded with oncolytic adenoviral vectors retain the ability to suppress T cell activation, proliferation, and IFN-γ production in response to mitogenic stimulation *in vitro* (unpublished observation). Studies are currently underway to assess the immunosuppressive ability of MSC *in vivo*.

The future of adenovirus therapy for gliomas (Expert Opinion)

The history of adenovirus as a therapy for gliomas is a short one; however it is one that holds promise. Clinical trials with ONYX-015 have established that adenoviruses are safe for use in humans and that they have minimal toxic side-effects [91]. The key to success in virotherapy in gliomas is delivery and specific targeting of tumor cells. The fact that after surgical resection these tumors have a tendency to micrometastasize and disseminate into healthy tissues in the brain makes it especially so. However, a huge 'advantage' in targeting gliomas is that the surrounding healthy brain tissue is quiescent as opposed to the actively replicating tumor cells. Replicative adenovirus therapy is therefore the key to this strategy. As mentioned earlier, due to the non-uniform expression of cell surface receptors and tumor specific promoters, a single CRAd treatment is unattainable. All options are to be analyzed for a particular patient with GBM and individualized molecular-profiled treatment is therefore the correct approach. Although a lot of progress has been made in the laboratory settings, clinical trials with CRAds need to go forward. Researchers also cannot be complacent with their successes in non-clinical setting, as clinical prognosis has remained grim. Alternate transductional and transcriptional targets needs to be identified. Another aspect that needs special attention is the delivery of these viruses so that they reach the lesion targeted. Recently, stem cells have become an attractive delivery vehicle and this line of research needs to be pursued. Adenovirotherapy for gliomas holds promise in the near future and should be actively pursued on all fronts.

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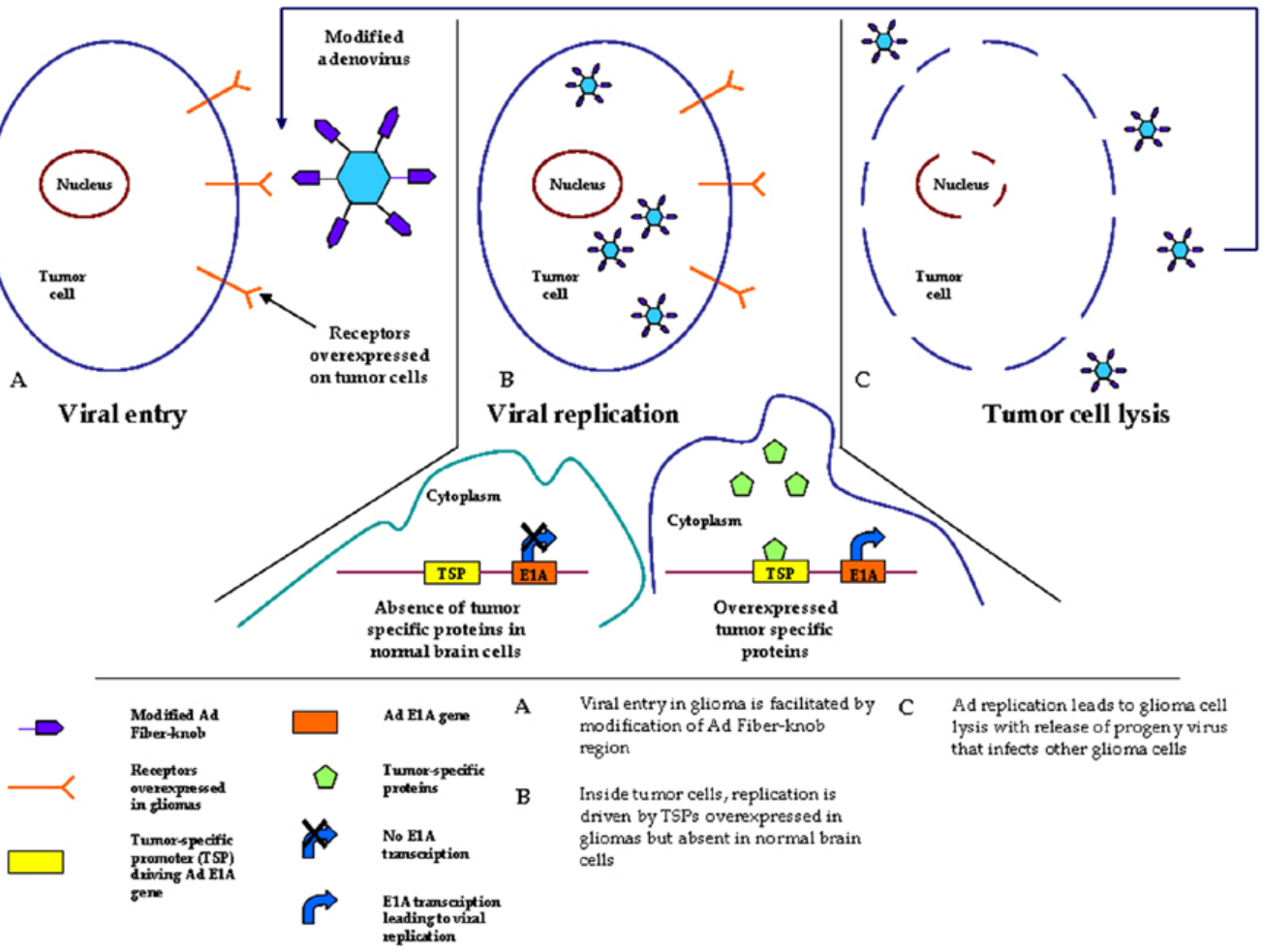

Figure 1.
Schematic overview of CRAds in glioma.

Table 1
Adenovirus in clinical trials for glioma.

Gene/*Prodrug*	Clinical Trial	Reference
Replication Defective Adenovirus		
Adv.RSVtk/*Gancyclovir*	Phase I, dose escalation	Trask et al, 2000 [73].
Ad-p53 (INGN 201; Advexin)	Phase I, dose escalation	Lang et al, 2003 [69].
IG.Ad.MLPI.TK/*Gancyclovir*	Phase I, dose escalation	Smitt et al, 2003 [64].
ADV/HSV-tk/*Gancyclovir*	Phase I, dose escalation	Germano et al, 2003 [75].
HSV-tk/*Gancyclovir* (Cerepro)	Phase III (European Medicines Agency)	Immonen et al, 2004 [76].
AdV-tk/*Valacyclovir* (GliAtak)	Phase I, dose escalation Phase Ib, currently ongoing	New et al, 2008 [93]. Protocol ID: NCT00751270
AdV-tk /*Valacyclovir*/Radiation (GliAtak)	Phase II, currently ongoing	Protocol ID: NCT00589875
Replication Competent Adenovirus		
Delta-24-RGD	Phase I, currently ongoing	Protocol ID: NCT00805376
ONYX-015	Phase I, open label, dose escalation	Chiocca et al, 2004 [91].