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Plasmacytoid Dendritic Cells in the Tumor Microenvironment: Immune Targets for Glioma Therapeutics[1,2]

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Abstract

Adenovirus-mediated delivery of the immune-stimulatory cytokine Flt3L and the conditionally cytotoxic thymidine kinase (TK) induces tumor regression and long-term survival in preclinical glioma (glioblastoma multiforme [GBM]) models. Flt3L induces expansion and recruitment of plasmacytoid dendritic cells (pDCs) into the brain. Although pDCs can present antigen and produce powerful inflammatory cytokines, that is, interferon α (IFN-α), their role in tumor immunology remains debated. Thus, we studied the role of pDCs and IFN-α in Ad.TK/GCV + Ad.Flt3L–mediated anti-GBM therapeutic efficacy. Our data indicate that the combined gene therapy induced recruitment of plasmacytoid DCs (pDCs) into the tumor mass; which were capable of *in vivo* phagocytosis, IFN-α release, and T-cell priming. Thus, we next used either pDCs or an Ad vector encoding IFN-α delivered within the tumor microenvironment. When rats were treated with Ad.TK/GCV in combination with pDCs or Ad-IFN-α, they exhibited 35% and 50% survival, respectively. However, whereas intracranial administration of Ad.TK/GCV + Ad.Flt3L exhibited a high safety profile, Ad-IFN-α led to severe local inflammation, with neurologic and systemic adverse effects. To elucidate whether the efficacy of the immunotherapy was dependent on IFN-α–secreting pDCs, we administered an Ad vector encoding B18R, an IFN-α antagonist, which abrogated the antitumoral effect of Ad.TK/GCV + Ad.Flt3L. Our data suggest that IFN-α release by activated pDCs plays a critical role in the antitumor effect mediated by Ad.TK/GCV + Ad.Flt3L. In summary, taken together, our results demonstrate that pDCs mediate anti-GBM therapeutic efficacy through the production of IFN-α, thus manipulation of pDCs constitutes an attractive new therapeutic target for the treatment of GBM.

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Abbreviations: Ad, adenoviral vector; APC, antigen-presenting cell; DC, dendritic cell; dLN, draining lymph node; Flt3L, *fms*-like tyrosine kinase 3; GBM, glioblastoma multiforme; IFN-α, interferon α; MHC, major histocompatibility complex; pDC, plasmacytoid dendritic cell; TK, thymidine kinase; TLR, Toll-like receptor

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[2]This article refers to supplementary materials, which are designated by Figures W1 to W5 and are available online at www.neoplasia.com.

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Introduction

Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor. Its invasiveness in the surrounding nonneoplastic brain and its intrinsic resistance to traditional therapeutic approaches make this disease a therapeutic challenge [1–4]. Several therapeutic approaches have attempted to stimulate the immune system to target and kill GBM cells. To this end, interferon α (IFN-α) has recently been proposed for the treatment of GBM owing to its effects on antitumor immunity, angiogenesis, and tumor cell proliferation and death [5–8]. IFN-α has shown a direct antitumor effect by reducing angiogenesis in GBM growing in the rat brain [7], inhibiting the proliferation of GBM cells [9] and sensitizing them to proapoptotic agents [10]. IFN-α has also shown indirect antitumor activity in several types of cancers through the stimulation of the host immune system: it can activate dendritic cells (DCs), upregulate major histocompatibility complex (MHC) types I and II expression, increase the recruitment of immune cells into the tumor microenvironment, and enhance cellular and humoral antitumor immunity [6]. Although IFN-α was the first cytokine to be approved for cancer treatment, its considerable toxicity has limited its use [11,12].

Our efforts toward developing an immunotherapeutic approach for the treatment of GBM that would be highly effective and nontoxic to the surrounding brain parenchyma consist of the delivery of a conditional cytotoxic molecule and a cytokine using adenoviral vectors (Ads) [13–16]. One Ad encodes Herpes simplex virus 1 thymidine kinase (Ad.TK), which kills proliferating cells in the presence of ganciclovir, and the second Ad encodes *fms*-like tyrosine kinase 3 ligand (Ad.Flt3L), which recruits antigen-presenting cells (APCs) to the brain tumor microenvironment [17]. This combined gene therapy eradicates established intracranial tumors in several rat and mouse preclinical GBM models [18]. We have previously shown that this treatment induces an antitumor immune response that is dependent on APCs, $CD4^+$, and $CD8^+$ T cells [14,19,20].

Flt3L is a growth factor that induces the expansion of DCs [21]. Our previous results have shown that intracranial administration of an Ad encoding Flt3L (Ad.Flt3L) induces the expansion and recruitment of plasmacytoid DCs (pDCs), which are a subset of IFN-α–producing DCs, into the rat brain parenchyma [22]. The role of pDCs in tumor immunology remains debated because it has been reported that pDCs can induce immunologic tolerance or antitumor immunity [23]. In patients with head and neck squamous cell carcinoma, tumor-infiltrating pDCs were found to have a decreased response to Toll-like receptor 9 (TLR9) activation [24]. pDCs detected in ovarian cancer were involved in the generation of an immunosuppressive tumor microenvironment [25] and the promotion of tumor angiogenesis [26].

pDCs can mature and express costimulatory molecules and inflammatory cytokines and migrate to the draining lymph nodes (dLNs) to present tumor antigens to naive $CD4^+$ T cells [23]. Although there is evidence that pDCs have the ability to present antigens, their main role seems to be as IFN-α–producing cells [27]. pDCs are considered the professional IFN-α–producing cells, being able to release between 100 to 1000 times more type I IFN than other immune cells after activation [27]. Here we aimed to elucidate the role of pDCs in the antitumor immune response mediated by Ad.TK + Ad.Flt3L. Thus, we assessed the ability of intratumoral pDCs to uptake and transport tumor cell remnants. We also purified tumor-infiltrating pDCs and characterized the expression of costimulatory molecules and their ability to prime T cells and produce inflammatory cytokines.

Because here we demonstrate that treatment with Ad.TK + Ad.Flt3L induces the recruitment of IFN-α–producing pDCs into the brain tumor mass, we hypothesized that this cytokine could play a critical role in mediating the antitumor therapeutic efficacy elicited by this treatment. Our data suggest that IFN-α release by activated pDCs mediates antitumor effects elicited by Ad.TK/GCV + Ad.Flt3L gene therapy. Therefore, manipulation of pDCs constitutes an attractive novel target for the treatment of GBM.

Materials and Methods

Adenoviral Vectors

The vectors used in this study were first-generation, replication-deficient, recombinant adenovirus type 5 vectors (Ad), with deletion in the E1 and E3 regions; the expression cassette containing the appropriate transgene is inserted within the E1 region [28]. Five Ad vectors were used: Ad.TK (encodes HSV1-thymidine kinase under the control of the human CMV promoter [14,19]), Ad.Flt3L (encodes human soluble *fms*-like tyrosine kinase ligand under the control of the human CMV promoter [14,17,19,22,29]), Ad.IFN-α (kindly donated by Dr Kazunori Aoki, National Cancer Center Research Institute, Tokyo, Japan; it encodes rat IFN-α under the control of the CAG promoter, which combines the human cytomegalovirus immediate-early enhancer and a modified chicken β-actin promoter [30]), Ad.B18R (encodes B18R an IFN-α decoy receptor under the control of the murine CMV promoter [31]), and, as a control, we used an Ad without transgene (Ad.0 [14]). For the generation of Ad.B18R, the *B18R* gene was polymerase chain reaction amplified from purified Vaccinia viral DNA (kind gift from Dr R Mark L. Buller, Saint Luis University, MO [31]) using the following primers: B18R-for ccgctcgaggatatcgtcgacATGAGTCGTCGTCTGATT (5′ overhangs containing *Xho*I, *Eco*RV, and *Sal*I restriction sites) and B18R-rev ccgctcgaggactagtgtcgacCTATACTTTGGTAGGTGG (3′ overhangs containing *Xho*I, *Spe*I, and *Sal*I restriction sites). Thirty thermal cycles (94°C denature, 55°C anneal, 72°C extension) were performed using an Applied Biosystems thermocycler (Foster City, CA). The polymerase chain reaction product was purified (Qiagen, Hilden, Germany) and cloned into pGEM-T-easy vector (Promega, Madison, WI). The *B18R* gene was excised with *Xho*I and cloned into the *Sal*I site of pAL120, an adenoviral shuttle plasmid containing the powerful murine CMV promoter in the E1 region of adenovirus [32] to generate pAL120-B18R. 293 cells were cotransfected with pAL120-B18R and pJM17 (Microbix, Toronto, Canada) using TransIT-293 Transfection Reagent (Mirus Bio, Madison, WI) to rescue the viral vector. Ad-mCMV-B18R was amplified and purified as described by us previously [28]. All viral preparations were tested free of replication-competent adenovirus and lipopolysaccharide contamination using methods previously described [28].

Rat GBM Model

Rats were housed in a pathogen-free environment and humidity and temperature–controlled vivarium on a 12:12-hour light/dark cycle (lights on 7:00 A.M.) with free access to food and water. All animal experiments were performed after prior approval by the Institutional Animal Care and Use Committee at Cedars-Sinai Medical Center and conformed to the policies and procedures of the Comparative Medicine Department. After administration of anesthesia, animals were placed in a stereotactic apparatus and injected unilaterally into the right striatum. Rats were injected using a 10-μl Hamilton syringe (coordinates: 1 mm forward from bregma and 3.1 mm lateral). Animals were allowed to recover, and their health status was closely monitored. CNS-1 cells (4500) were injected as described before [33]. CNS-1 cells were grown in Dulbecco modified Eagle culture medium