Gene Transfer into Brain Parenchyma Elicits Antitumor Effects

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Abstract

Gene therapy strategies for cancer currently aim at targeting gene delivery to the malignant cell. In a mouse model of intracerebral Lewis lung carcinoma (3LL), adenoviral vectors transduce not only 3LL cells but also brain parenchymal cells including endothelial cells, neurons, microglia, and astrocytes in vivo. Furthermore, transgene expression persists longer in brain than in tumor. Transfer of IFN-γ into brain parenchymal cells rather than tumor is both necessary and sufficient to generate antitumor therapeutic benefits. Therefore, parenchymal cells represent an effective and necessary target for delivery of genes that render the brain uninhabitable by the tumor.

Introduction

Current experimental gene therapy strategies for malignant brain tumors aim at targeting delivery of the transgene to the tumor cell. The genes transferred include those that induce a suicide effect, modulate the immune response, arrest the cell cycle, induce apoptosis, or inhibit neovascularization. Historically, delivering the suicide gene herpes simplex thymidine kinase into brain tumors was the first gene therapy strategy for brain cancer to show efficacy in animal models, and this therapy has been tested in humans (2, 11). Because of the inability of retroviral vectors to infect quiescent cells, Culver et al. (2) hypothesized that recombinant retroviruses can target suicide gene delivery into rapidly multiplying tumor cells while sparing the backdrop of nondividing neural tissue. Data from this laboratory showed that treatment of mice harboring a poorly immunogenic carcinoma (3LL) implanted in the brain with an adenoviral vector to deliver IFN-γ (AdIFN) at the same coordinates as the tumor elicits significant prolongation of survival times and tumor rejection. In this mouse brain tumor model, the antitumor effects of AdIFN are independent of adaptive cellular immune mechanisms and appear to be mediated by antiangiogenesis (12). Evidence against a cellular immune-mediated response include: (a) absence of a memory immune response on challenge; (b) lack of antitumor effects at sites distal to inoculation of AdIFN; and (c) preservation of the therapeutic benefits in scid, beige, iNOS C57/B6 knockout mice and mice treated with N’-nitro-l-arginine-methyl ester. High concentrations of IFN-γ do not inhibit tumor growth in vitro, making it unlikely that the antitumor effect of this treatment acts directly on the growth of the tumor cell. However, gene transfer of IFN-γ inhibits neovascularization of the tumor in the avascular s.c. space in a Matrigel assay in vivo, and AdIFN induces apoptosis of endothelial cells in vivo, thus supporting the idea that AdIFN represses tumor growth by inhibiting angiogenesis (12).

Results and Discussion

To identify the cell types transduced with AdIFN in vivo, groups of mice that received intracerebrally 3LL implants were injected 10 days later with AdIFN at the same coordinates as the tumor, and their brains were sectioned 4 days later. Immunofluorescence staining for astrocytes (green) and IFN-γ (red) reveals IFN-γ reactivity predominantly in the brain, outside and surrounding the area of the tumor (Fig. 1). Furthermore, double immunofluorescence staining demonstrates that astrocytes (Fig. 2, a–c), microglia (Fig. 2, d–f), neurons (Fig. 2, g–i), and endothelial cells (Fig. 2, j–l) are all transduced with AdIFN in vivo.

To examine whether selective gene transfer into brain parenchymal cells is sufficient to generate the antitumor effects of AdIFN, mice first received implants of either AdIFN or AdBGAL and were then reinjected 4 days later with wt3LL at the same coordinates and followed for survival. The therapeutic benefits of AdIFN in these experiments were identical to the effects of AdIFN in the treatment of mice with 3LL brain tumors (Fig. 3, a and b); AdIFN generated statistically significant prolongation of survival times as well as tumor rejection in 4 of 10 mice (Fig. 3b). The latter survived for >85 days, and histological analysis of their brains showed cavity formation (data not shown). To study the biological effects of selective gene transfer of IFN-γ into tumor cells, 3LL cells transduced in vitro to secrete 1.125 μg IFN-γ/10⁶ cells/24 h were implanted in mice, and the mice were followed for survival. The survival benefits in these experiments were modest but statistically significant; however, none of these mice survived longer than 36 days (Fig. 3c).

The experiments in this study were designed to determine: (a) the identity of the brain parenchymal cells transduced with AdIFN; and (b) whether the antitumor effects are generated by delivery of IFN-γ into the tumor cell versus brain parenchyma.

Materials and Methods

Animals and Vectors. C57/BL6 mice were purchased from the Jackson Laboratories (Bar Harbor, ME). Animal care was in accordance with institutional guidelines. 3LL (Lewis lung carcinoma; C57/BL6) is a generous gift from Dr. I. Fidler (M. D. Anderson Cancer Center, Houston, TX). The generation of adenoviral vectors AdIFN and AdBGAL was described elsewhere (12). The titers of AdIFN and AdBGAL of viral particle:plaque-forming unit ratios are 43 and 81, respectively. Mice were anesthetized and injected intracerebrally as described previously (12). Statistical calculations were performed by the JMP software (SAS Institute, Cary, NC).

Tissue Staining. Antibodies against mouse IFN-γ (American Type Culture Collection, Manassas, VA) and tomato lectin (Sigma Chemical Co., St. Louis, MO), anti-GFAP (DAKO, Carpenteria, CA), anti-NeuN (Chemicon, Temecula, CA), and anti-factor VIII (DAKO) were used. Biotinylation reactions were performed following the manufacturer’s specifications (Vector Laboratories, Burlingame, CA). Immunofluorescence and immunohistochemistry were performed as described previously (12).
Fig. 1. AdIFN induces IFN-γ reactivity predominantly in brain parenchyma. Mice received intracerebral implants of 3LL, followed 10 days later by AdIFN (10 μl; 24 × 10⁷ viral particles) at the same coordinates, sectioned 4 days later and reacted sequentially with biotinylated anti-IFN-γ and anti-GFAP and then with Cyan3-coupled streptavidin and FITC-coupled antirat IgG (a). The area of the tumor, surrounded by white arrows (a) and shown as a hypercellular area by H&E staining (b), is delineated by paucity of astrocytes (green). IFN-γ reactivity (red) is located predominantly in brain parenchyma surrounding the tumor bed. Double staining (yellow) identifies IFN-γ-producing astrocytes. o.m. is ×400 and ×40 for a and b, respectively.

To determine the persistence of transgene expression in brain and tumor, groups of mice (n = 6 each) received implants of: (a) 3LL cells transduced with AdBGAL in vitro; or (b) AdBGAL followed 4 days later with wt 3LL cells at the same coordinates. AdBGAL-transduced tumor cells instead of AdIFN transduced tumor cells were used because IFN-γ secreted by the tumor induces microglia to secrete endogenous IFN-γ, thus obscuring the localization of the virus. The brains were sectioned 10 days after the first injection. Although all 3LL cells transduced with AdBGAL in vitro contained β-gal activity at day 0 (Fig. 4a), none of the brains implanted with these tumors stained for β-gal at day 10 (Fig. 4, b and c). Nonetheless, mice first injected with AdBGAL showed prominent β-gal expression in brain parenchyma surrounding the tumor (Fig. 4, d and e).

The results show that AdIFN transduces not only tumor cells but also brain parenchymal cells including endothelial cells, microglia, neurons, and astrocytes in vivo. Although implantation of 3LL cells transduced in vitro into the brain is associated with modest survival benefits (Fig. 3c), it fails to duplicate the therapeutic effects of AdIFN in treating 3LL brain tumors (Fig. 3a), making it unlikely that the antitumor effects of AdIFN in the latter model are mediated solely by transduction of the tumor cell. Thus, the data argue that gene transfer into brain parenchyma is necessary to optimize the therapeutic benefits. Furthermore, selective transfer of IFN-γ into brain parenchyma reproduces the survival benefits of AdIFN in treating 3LL brain tumors (Fig. 3b), suggesting that brain transduction alone is sufficient to generate the therapeutic response (Fig. 3a). The gains of gene transfer into brain parenchymal cells may stem from the fact that unlike rapidly multiplying tumor cells, they are less likely to lose the transgene and are more likely to produce sustained high amounts of the gene product for longer periods of time (Fig. 4).

Targeting gene transfer into brain parenchymal cells located in proximity of the tumor is an attractive adenoviral-mediated strategy for delivering molecules that are either antiangiogenic, exert immunomodulatory effects on both the tumor and immune cells, inhibit tumor cell growth directly, or induce selective tumor cell apoptosis. Tumor cells constitute an unstable source for transgene production because they multiply rapidly, thus diluting out the episomal transgene (Fig. 4). Furthermore, by killing a large number of malignant cells, a potentially successful therapeutic strategy may fail because of prematurely reducing or eliminating transgene production by the tumor. Because brain parenchymal cells are nondividing or replicate slowly, they make up a reliable and potentially controllable factory for producing a gene product cloned under an inducible promoter (Fig. 4). Furthermore, such a strategy is particularly attractive for gliomas because they tend to recur within a few centimeters of the original tumor site. In summary, the results present a proof of principle that the tumor bed, in this instance, brain parenchymal cells, is an effective and necessary target for delivering genes that render the brain uninhabitable by the tumor.

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Fig. 3. Gene transfer into brain parenchymal cells but not tumor cell reproduces the therapeutic effects of AdIFN on 3LL brain tumors. Mice received intracerebral implants of 1500 3LL cells in 3 μl. Two days later, mice were treated with either AdIFN (10 μl; 24 × 10^6 viral particles; n = 8) or AdBGAL (10 μl; 35 × 10^6 viral particles; n = 6) at the same coordinates (a). Survival times were examined by Kaplan-Meier analysis. Mean survival times were 29.7 days and >57.1 days for AdBGAL- and AdIFN-treated mice, respectively (log-rank P < 0.0003). Animals were also injected with either AdIFN or AdBGAL (10 μl; 24 × 10^6 viral particles; n = 10 each) first, followed 4 days later by 3LL at the same coordinates (b). Mean survival times were 31.6 days and >54.6 days for AdBGAL- and AdIFN-treated mice, respectively (log-rank P < 0.0093). c shows the survival times of animals that received intracerebral implants of 3LL cells transduced in vitro with either AdIFN or AdBGAL (n = 10 each). One million 3LL cells were cultured in the presence of AdIFN or AdBGAL (45 × 10^6 viral particles each) in vitro. Two days later, the cells were washed extensively with PBS, and 1500 cells were injected intracerebrally. Mean survival times were 24.1 and 31.2 days for AdBGAL- and AdIFN-treated mice, respectively (log-rank P = 0.0002).

Fig. 4. Transgene expression persists longer in brain than tumor. One million 3LL cells were transduced with AdBGAL in vitro (45 × 10^6 viral particles); 2 days later, they were stained for β-gal (a) or washed extensively, and 1500 cells were injected intracerebrally into naïve mice (n = 6). Ten days after injection, consecutive frozen sections of the brain were stained for β-gal activity and reacted with anti-GFAP antibodies (b) or stained with H&E (C). Arrows point to the tumor site, showing a paucity of astrocytes (b and c). Whereas all transduced 3LL cells expressed β-gal before injection into the brain (a), intracerebral tumors examined 10 days after implantation do not show β-gal activity (b and c). C57/BL6 mice (n = 6) were implanted with AdBGAL followed 4 days later with 1500 wt 3LL cells (d and e). Ten days after the viral injections, consecutive frozen sections of the brain were stained for β-gal activity and reacted with biotinylated anti-GFAP antibodies (d) or stained with H&E (e). β-Gal expression is prominent in brain parenchyma surrounding the area of the tumor showing a paucity of astrocytes (d and e), arrows point to β-gal staining. o.m. is ×200 for a–e.

References
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