Dominant-Negative Fibroblast Growth Factor Receptor Expression Enhances Antitumoral Potency of Oncolytic Herpes Simplex Virus in Neural Tumors

Ta-Chiang Liu, Tingguo Zhang, Hiroshi Fukuhara, Toshikiko Kuroda, Tomoki Todo, Xavier Canron, Andreas Bikfalvi, Robert L. Martuza, Andreas Kurtz, and Samuel D. Rabkin

Abstract

Purpose: Oncolytic herpes simplex viruses (HSV) appear to be a promising platform for cancer therapy. However, efficacy as single agents has thus far been unsatisfactory. Fibroblast growth factor (FGF) signaling is important for the growth and migration of endothelial and tumor cells. Here, we examine the strategy of arming oncolytic HSV with a dominant-negative FGF receptor (dnFGFR) that targets the FGF signaling pathway.

Experimental Design: A mouse Nf1:p53 malignant peripheral nerve sheath tumor (MPNST) cell line expressing dnFGFR was generated by transfection. The effects of dnFGFR expression on cell growth and migration in vitro and tumor formation in vivo were determined. The dnFGFR transgene was then inserted onto oncolytic HSV G47Δ using a bacterial artificial chromosome construction system. Antitumoral and angiogenic properties of bG47Δ-dnFGFR were examined.

Results: MPNST 61E4 cells expressing dnFGFR grew less well than parental control cells. bG47Δ-dnFGFR showed enhanced killing of both tumor (human U87 glioma and F5 malignant meningioma cells and murine PMPN 61E4 and 37-3-18-4 cells) and proliferating endothelial cells (human umbilical vascular endothelial cell and Py-4-1) in vitro compared with the control vector bG47Δ-empty without inhibiting viral replication. In vivo, bG47Δ-dnFGFR was more efficacious than its nonexpressing parent bG47Δ-empty at inhibiting tumor growth and angiogenesis in both human U87 glioma and mouse 37-3-18-4 MPNST tumors in nude mice.

Conclusions: By using multiple therapeutic mechanisms, including destruction of both tumor cells and tumor endothelial cells, an oncolytic HSV encoding dnFGFR enhances antitumor efficacy. This strategy can be applied to other oncolytic viruses and for clinical translation.

Replication-selective oncolytic herpes simplex virus (HSV) vectors have emerged as a new platform for cancer therapy (1). Most of the engineered HSV mutants currently being used as oncolytic agents have deletions in the neurovirulence gene γ34.5 and/or ICP6, the large subunit of HSV ribonucleotide reductase (RR). Consequently, these HSV mutants replicate selectively in and kill dividing tumor cells, possibly due to the fact that dividing cells express mammalian RR and growth arrest DNA damage 34 (GADD34). Although not fully characterized, these gene products regulate the cell cycle and complement the mutations of these viruses (2). In particular, mammalian RR generates deoxyribonucleotides in place of the HSV RR, and the COOH terminus of GADD34 substitutes for the homologous region of mammalian RR. Consequently, these HSV mutants replicate selectively in and kill dividing tumor cells, possibly due to the fact that dividing cells express mammalian RR and growth arrest DNA damage 34 (GADD34). Although not fully characterized, these gene products regulate the cell cycle and complement the mutations of these viruses (2). In particular, mammalian RR generates deoxyribonucleotides in place of the HSV RR, and the COOH terminus of GADD34 substitutes for the homologous region of γ34.5.

Several oncolytic HSV mutants (e.g., 1716, G207, NV1020, and OncoVexGM-CSF) have entered phase I to II clinical trials with various solid tumors (3–7). However, despite the significant efficacy in preclinical models and safety in humans, therapeutic benefits have been limited (4, 8). It is therefore prudent to incorporate mechanisms in addition to direct oncolysis for tumor cell destruction. To this end, we have previously shown that HSV mutant G47Δ (9), in addition to enhanced viral replication, also possesses an immunoregulatory function in which MHC class I presentation was increased compared with its parent, G207, while maintaining the safety profile of G207. This provides for the possibility of an enhanced cytotoxic lymphocyte response toward tumor cells and increased efficacy of the virus.

Authors’ Affiliations: 1Molecular Neurosurgery Laboratory, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts and 2Institut National de la Santé et de la Recherche Médicale E0113, Molecular Mechanisms of Angiogenesis, University Bordeaux 1, Talence, France.

Requests for reprints: Samuel D. Rabkin, Molecular Neurosurgery Laboratory, Massachusetts General Hospital, CPZN-3800 Simchess Research Building, 185 Cambridge Street, Boston, MA 02114. Phone: 617-726-6817; Fax: 617-643-3422; E-mail: rabkin@helix.mgh.harvard.edu.

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Note: A. Kurtz and S.D. Rabkin contributed equally to this work. Current address for T. Zhang: Department of Pathology, Shandong University Hospital, Jinan, China; H. Fukuhara: Department of Urology, University of Tokyo, Tokyo, Japan; T. Todo: Department of Neurosurgery, University of Tokyo, Tokyo, Japan; A. Kurtz: Robert Koch-Institute, Berlin, Germany.

Requests for reprints: Samuel D. Rabkin, Molecular Neurosurgery Laboratory, Massachusetts General Hospital, CPZN-3800 Simchess Research Building, 185 Cambridge Street, Boston, MA 02114. Phone: 617-726-6817; Fax: 617-643-3422; E-mail: rabkin@helix.mgh.harvard.edu.
To improve efficacy, oncolytic viruses have been armed with therapeutic transgenes. Armed oncolytic HSV expressing cytokines (e.g., granulocyte macrophage colony-stimulating factor, interleukin-12, and interleukin-18), produg-activating enzymes (e.g., thymidine kinase and cytosine deaminase), and other therapeutic transgene products have been shown to enhance the antitumoral effect (10–14). However, we propose that the targets of armed oncolytic viruses should be expanded to include tumor stromal cells and vasculature to maximize the therapeutic effect, using fibroblast growth factor (FGF) as an example.

The FGF family contains 22 members, which interact with four receptors (FGFR1–FGFR4; ref. 15). Prototypical FGF1 (acidic FGF) and FGF2 (basic FGF) both interact with FGFR1. FGFs play critical roles in tumorigenesis (15–17). The binding of FGF to FGFR activates a variety of signal transduction cascades through its intrinsic tyrosine kinase, including Ras/mitogen-activated protein kinase, phosphatidylinositol 3-kinase/Akt, and phospholipase C-γ, which in turn regulate cell proliferation, survival, differentiation, and migration (17, 18). FGF therefore acts as a mitogenic factor, angiogenic factor, and antiapoptotic factor. Overexpression of FGF, by either tumor cells or surrounding stromal cells, can result in autocrine and/or paracrine effects and is found to occur in tumors, such as gliomas (19). On the other hand, overexpression or activating mutations of FGFR have been shown in brain, breast, prostate, thyroid, and melanoma tumors (15, 20). Thus, dysregulated FGF signaling is present in most human cancers. Of note, two nervous system tumors [i.e., gliomas and malignant peripheral nerve sheath tumors (MPNST)] are well known to be vascular and aggressive, characteristics that have been attributed to angiogenesis and dysregulated FGF signaling pathways (21, 22). In particular, FGFR1 is generally elevated in gliomas and correlates with the degree of malignancy (19, 23).

Blocking FGF signaling for cancer treatment therefore seems appealing due to its ability to target not only tumor cells but also surrounding stromal cells and tumor vasculature. Indeed, inhibition of FGF/FGFR signaling with dominant-negative FGFR (dnFGFR), antisense, soluble FGFR, etc., as well as downstream pathways or partially autocrine feedback pathways, can result in dysregulated FGF signaling. In vivo tumor cells or surrounding stromal cells, or both, cell as well as tumor cells that would be targeted by this approach. The concept was tested by engineering an oncolytic HSV with a dnFGFR1, a truncated receptor that can dimerize, forming heterodimers with endogenous receptors, but not signal (28). This vector (bG47Δ-dnFGFR) shows enhanced antitumoral effects both in vitro and in vivo via direct antitumoral and antiangiogenic mechanisms.

### Materials and Methods

**Cells.** Human glioblastoma cell lines U87 and T98 and African monkey kidney cell line Vero were obtained from the American Type Culture Collection (Manassas, VA) and grown in DMEM plus 10% calf serum. Human umbilical vascular endothelial cells (HUVEC) and its culture medium EGM-2 were obtained from Cambrex (Walkersville, MD) and maintained as described by the vendor. Human malignant meningioma cell line F5 (29) was provided by Dr. Anil Menon (University of Cincinnati, Cincinnati, OH). Murine MPNST cell lines 61E4 and 37-3-18-4, isolated from spontaneously arising tumors in Nij/Nij : p53+/− mice (30), were provided by Dr. Luis Parada (University of Texas, Southwestmern Medical Center, Dallas, TX) and cultured in DMEM plus 10% calf serum. Murine endothelial cell line Py-4-1, isolated from hemangiosomas of polyomavirus transgenic mice (31), was provided by Dr. Victoria Bautch (University of North Carolina, Chapel Hill, NC) and maintained in DMEM plus 10% calf serum.

**Transfection of cells.** For stable constitutive expression of dnFGFR, 61E4 cells were transfected with mouse dnFGFR1 (tyrosine kinase domain deleted, two immunoglobulin-like loop form, IIc splice variant) cDNA (provided by Dr. Lewis Williams, University of California at San Francisco, San Francisco, CA; ref. 28) subcloned into pRC/CMV (Invitrogen, Carlsbad, CA) using LipofectAMINE (Life Technologies, Carlsbad, CA). Control cells (61E4-Zeo) were transfected with pRC/CMV alone. Clones were selected with geneticin (Life Technologies).

**In vitro wound-healing assay.** 61E4-Zeo and 61E4-dnFGFR cells were seeded into six-well plates, and an artificial wound was created with a scalpel when the cells were confluent. Images were captured before and 12 hours after scratching.

**Chicken chorioallantoic membrane assay.** On embryonic day 10, a plastic ring was placed on the chicken chorioallantoic membrane as described before (32), and 5 million 61E4-Zeo or 61E4-dnFGFR tumor cells in 20 μl of medium were deposited after gentle laceration of surface. Digital photos were taken under a stereomicroscope (Nikon SMZ800, Tokyo, Japan). One week after deposition, tumor volume was determined by the formula V = 4/3πr³, with r = 1/2((A1 × A2), and compared as described before (33).

**Virus construction.** The backbone for the constructed viruses is G4ΔA (9), which was cloned into a bacterial artificial chromosome (BAC) vector for propagation in Escherichia coli. The details of G4ΔA-BAC plasmid and virus constructions have been described (14). For the generation of the vectors described here, the Hind III blunt-ended/BamHI dnFGFR cDNA fragment from p5W2 was ligated into Stul/BamHI-digested pVec91 (containing Lacz, IoxP, and FRT sites, a cytomegalovirus promoter, and multiple cloning site) to generate pVec91-dnFGFR. pVec91-dnFGFR or pVec91 was inserted into the IPC6 region of pG4ΔA-BAC using Cre recombinase to generate pG4ΔA-BAC-dnFGFR and pG4ΔA-BAC-empty, respectively. Cotransfection of pG4ΔA-BAC-dnFGFR or pG4ΔA-BAC-empty and a FLPe recombinase expression plasmid (pCAGGSFLpIRES, provided by Dr. Pedro Lowenstein, Cedars-Sinai Medical Center, University of California at Los Angeles, Los Angeles, CA; ref. 34) into Vero cells resulted in the removal of FRT-flanked BAC sequences and generation of bG4ΔA-dnFGFR or bG4ΔA-empty viruses.

**Western blotting.** Vero cells were mock infected or infected with viruses at 1 plaque-forming unit (pfu)/cell for 20 hours and harvested. Proteins (30 μg) were subjected to SDS-PAGE, transferred to polyvinylidene difluoride Plus membrane (MSE Micron Separations, Westborough, MA), and blotted overnight with FGF primary antibody McAb6 (diluted 1:250; provided by Dr. Pamela Maher, The Scripps Research Institute, La Jolla, CA; ref. 35), anti-ICP4 (diluted 1:6,000; U.S. Biological, Swampscott, MA), or anti-actin (diluted 1:500; Sigma, St. Louis, MO). The membrane was then washed, blotted with either anti-mouse secondary antibody (horseradish peroxidase conjugated; diluted 1:10,000; Amersham, Piscataway, NJ) or anti-rabbit secondary antibody (horseradish peroxidase conjugated; diluted 1:10,000; Amersham), washed, exposed to ECL Plus, and developed.

For FGF signaling, U87, 61E4, and 37-3-18-4 cells were seeded into six-well plates (3 × 10⁴ per well). The cells were cultured in serum-free DMEM (U87) or DMEM plus 0.3% heat-inactivated FCS (61E4 and 37-3-18-4) for 48 hours before treatment. Cells were then mock
infected or infected with bG47-empty or bG47-dnFGFR (5 pfu/cell). Sixteen hours after infection, cells were treated with (61E4 and 37-3-18-4) or without (U87) acidic FGF (1 ng/mL; PeproTech, Rocky Hill, NJ) for 5 minutes. Acidic FGF was not added to U87 cells because these cells endogenously secrete FGF. Cells were then harvested, subjected to SDS-PAGE, transferred to polyvinylidene difluoride Plus membrane, and blotted overnight with antibody to total extracellular signal-regulated kinase (ERK) or phosphorylated ERK (diluted 1:1,000; Cell Signaling, Danvers, MA). The membrane was then washed, blotted with anti-rabbit secondary antibody (diluted 1:10,000; horseradish peroxidase conjugated), washed, exposed to ECL Plus, and developed. The same membrane blotted for phosphorylated ERK was stripped and blotted for total ERK.

**Cell survival assay.** Cells were seeded into 96-well plates at 5,000 to 7,500 per well. After 24 hours, cells were infected with the indicated viruses at various concentrations (3-fold dilution from 30 to 0.001 pfu/cell) and incubation was continued for a further 72 hours when a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Sigma) was done and dose-response curves were obtained. Experiments were repeated at least four times with each condition in quadruplicate.

**Virus replication assays.** Cells were seeded into 12-well plates (1 × 10⁶ per well) and infected at 70% to 80% confluency with different viruses, and the inoculum was removed after 2 hours and replaced with medium (DMEM/1% inactivated FCS). Cells and medium were harvested at the indicated times after infection, processed with three
Directions per injection) of virus (1/10 nL/C2 givenevery3days; for U87 tumors, two intratumoral injectionsofvirus per group): PBS, bG47Δ-empty, or bG47Δ-dnFGFR. For 37-3-18-4 tumors, four intratumoral injections (three directions per injection) of virus (1 × 10⁷ pfu/injection) or PBS were given every 3 days; for U87 tumors, two intratumoral injections of virus (1 × 10⁵ pfu/injection) or PBS were given. Tumor volumes were monitored two to three times weekly.

**Intratumoral biological end point studies.** Mice with tumors (50-100 mm³) implanted as above were randomized into three groups (n = 10 per group): PBS, bG47Δ-empty, or bG47Δ-dnFGFR. Two intratumoral injections with 1 × 10⁷ pfu/injection (37-3-18-4 tumors) or 5 × 10⁵ pfu/injection (U87 tumors) were given on days 1 and 4. Animals were sacrificed at indicated time points, and tumors were harvested, snap frozen, and processed for immunohistochemistry.

**Immunohistochemistry.** Cryosections (5-7 μm) were obtained from snap-frozen tissues, fixed in cold methanol for 10 minutes, blocked with serum-free blocking agent (DAKO, Carpinteria, CA) for 10 minutes, quenched for endogenous hydroxide (DAKO) for 5 minutes, and incubated with primary antibody (rabbit anti-mouse von Willebrand factor (VWF); 1:100; DAKO) overnight. Sections were then washed, incubated with secondary antibody (donkey anti-rabbit; 1:500; Amersham), developed with 3,3′-diaminobenzidine substrate-chromagen system (DAKO), and counterstained with hematoxylin. VWF-positive structures were analyzed under light microscopy.

**Microvessel counting.** Areas of vascularization after VWF staining were randomly chosen for microvessel counting at a low optical power (40×). Microvessel counting was done on 200× fields (area of a 200 × field, 0.724 mm²). The final microvessel density was the mean value of five individual, nonoverlapping fields in the area of vascularization.

**Statistical analysis.** Student’s t test or one-way ANOVA was used for statistical analysis. Kaplan-Meier curves for survival were compared by log-rank tests.

**Results**

dnFGFR expression reduced tumor cell proliferation and migration. To investigate the effect of blocking FGF/FGFR signaling in tumor cells, murine MPNST 61E4 cells were stably transfected with dnFGFR. dnFGFR-expressing cells (61E4-dnFGFR) showed slower proliferation in vitro compared with the parental line transfected with a control plasmid Zeo (61E4-Zeo; P = 0.033; Fig. 1A). In an in vitro wound-healing assay, dnFGFR expression decreased migration of cells into a scratch in a cell monolayer (Fig. 1B). The growth of tumor cells 61E4-Zeo and 61E4-dnFGFR was compared in vivo with two different models. We first tested tumor growth in a novel model on chicken chorioallantoic membrane that we recently described, which assays tumor growth and invasion in addition to angiogenesis (33). Control cells formed a homogenous well-organized tumor mass with marked angiogenesis, whereas dnFGFR-expressing cells formed small tumors with reduced angiogenesis (Fig. 1C). One week after implanting the tumor cells onto chorioallantoic membrane, 61E4-dnFGFR tumors had significantly smaller volumes than the 61E4-Zeo tumors (P = 0.042; Fig. 1C and D). Furthermore, angiogenesis is reduced in 61E4-dnFGFR tumors by ~30% (vessel density,
Expression of dnFGFR inhibits migration of endothelial cells. We therefore tested whether expression of dnFGFR by oncolytic HSV could block activation of FGFR signaling using phosphorylation of ERK as a readout. Indeed, bG47Δ-dnFGFR infection strongly inhibited ERK phosphorylation in all three cell lines tested (U87, 61E4, and 37-3-18-4), whereas bG47Δ-empty infection did not alter ERK phosphorylation compared with PBS treatment (Fig. 3B). Therefore, the expression of dnFGFR after bG47Δ-dnFGFR infection inhibits FGFR signaling.

Expression of dnFGFR did not inhibit viral replication. To investigate whether the expression of dnFGFR inhibits viral replication, cells were infected with 1 pfu/cell, and viral yield was determined at 24 and 48 hours after infection. As seen in Fig. 4A, bG47Δ-dnFGFR showed a comparable viral burst with bG47Δ-empty at both time points in all tumor and endothelial cell lines tested (P > 0.05). Of note, replication of both bG47Δ-empty and bG47Δ-dnFGFR was significantly lower in quiescent HUVEC compared with proliferating HUVEC (P = 0.005–0.017; Fig. 4A), suggesting that these viruses would not cause significant damage to normal vasculature as opposed to tumor vasculature. Virus replication and spread was also determined at a low multiplicity of infection (0.01 pfu/cell), which allows for multiple replication cycles. bG47Δ-dnFGFR showed comparable viral growth kinetics with bG47Δ-empty in both human U87 glioma and HUVEC (P > 0.05; Fig. 4B). Therefore, dnFGFR expression did not inhibit bG47Δ viral replication.

Antitumoral efficacy of bG47Δ-dnFGFR. To examine the effects of dnFGFR expression on neural tumor growth in vivo, human U87 or murine 37-3-18-4 tumor cells were implanted into the flanks of nude mice, and intratumoral treatments were started when tumors grew to 50 to 100 mm³. In both tumor models, the bG47Δ-empty treatment group showed a significant delay in tumor growth compared with that of the PBS treatment group (P < 0.001; Fig. 5), as expected. However, the potency of bG47Δ in a variety of neural tumor and endothelial cells in vitro.

Expression of dnFGFR blocks FGFR signaling. Molecular pathways by which FGF signaling promotes angiogenesis have been studied (15, 35). Among those downstream targets, ERK in the mitogen-activated protein kinase pathway has been shown to play a critical role in migration and proliferation of endothelial cells (36). We therefore tested whether expression of dnFGFR by oncolytic HSV could block activation of FGFR signaling using phosphorylation of ERK as a readout. Indeed, bG47Δ-dnFGFR infection strongly inhibited ERK phosphorylation in all three cell lines tested (U87, 61E4, and 37-3-18-4), whereas bG47Δ-empty infection did not alter ERK phosphorylation compared with PBS treatment (Fig. 3B). Therefore, the expression of dnFGFR after bG47Δ-dnFGFR infection inhibits FGFR signaling.
tumor growth in the bG47Δ-dnFGFR treatment group was further inhibited \( (P < 0.001, \text{compared with bG47Δ-empty; Fig. 5}) \). Therefore, dnFGFR expression significantly enhanced the efficacy of G47Δ in both a human glioma and murine MPNST tumor model.

In vivo biological end points. To explore the antiangiogenic effects of bG47Δ-empty and bG47Δ-dnFGFR vectors in vivo, tumor tissues were harvested 8 days after treatment and tumor vasculature was assessed by VWF staining (Fig. 6A). Similar to the in vitro results, bG47Δ-empty treatment significantly reduced the number of VWF-positive structures compared with PBS treatment \( (P = 0.013; \text{Fig. 6B}) \). Importantly, dnFGFR expression further reduced the number \( (P < 0.001) \) so that there were >4-fold fewer vascular structures than in the mock-treated tumors (Fig. 6B). This large decrease in tumor vasculature likely plays an important role in the inhibition of tumor growth.

Discussion

Herein, we describe the effect of incorporating a therapeutic transgene, dnFGFR, which inhibits the FGF signaling pathway, into an oncolytic HSV vector. We first transfected dnFGFR into mouse MPNST cell line 61E4 and showed that dnFGFR-transfected tumor cells had decreased proliferation and invasion in vitro and reduced growth in vivo in two tumor models. An oncolytic HSV vector expressing dnFGFR was then constructed. dnFGFR expression from G47Δ greatly enhanced potency in both neural tumor and proliferating endothelial cells in vitro. In addition to direct oncolysis, the expression of dnFGFR blocks the FGF autocrine/paracrine loop, thereby enhancing the antitumoral effect. The autocrine effects were apparent in human glioma and malignant meningioma cells, murine MPNST cells isolated from spontaneously arising tumors in Nf1+/-;p53+/- mice, and human and murine proliferating endothelial cells. Paracrine effects were shown by the ability of conditioned medium from bG47Δ-dnFGFR-infected U87 cells to inhibit the migration of HUVECs. In vivo, bG47Δ-dnFGFR treatment significantly inhibited the growth and angiogenesis of human glioma (U87) and murine MPNST (37-3-18-4) tumors in nude mice compared with bG47Δ-empty.

The selectivity of the currently tested oncolytic HSV mutants is based in part on defective IFN signaling and mutant complementation with cellular GADD34 and/or RR. Although this provides for sufficient selectivity and safety, the genetic manipulations also reduce the potency of the virus. To counteract the reduced growth properties of \( \gamma 34.5 \) mutants, a suppressor mutation can be introduced that places the late Us11 gene under the control of the immediate-early \( \alpha 47 \) promoter, as in G47Δ (9), or \( \gamma 34.5 \) can be expressed under the control of a tumor-specific promoter, as in Myb34.5 (37), or tumor cells can be treated to induce GADD34 or RR expression, as occurs after temezolomide treatment (38). Another approach is to insert therapeutic transgene(s) that will enhance antitumoral potency independent of the viral mutants.
Our data indicated that inhibition of FGF activity by dnFGFR can disrupt both angiogenesis-dependent and angiogenesis-independent pathways required for tumor growth and invasion, consistent with previous studies (24). Furthermore, as the FGF signaling pathway is critical to tumor growth and angiogenesis, the enhanced potency should be seen in both FGF-secreting and FGF-nonsecreting tumors, thus broadening the application of the vector. Of note, our data support previous publications that oncolytic HSV mutants with deletions in γ34.5 and/or ICP6 have antiangiogenic activities by themselves, inhibiting capillary tube formation in vitro and in vivo and replicating in tumor endothelial cells but not in normal vasculature in vivo (41, 42). We showed that G47Δ is a potent antiangiogenic virus; it significantly reduced endothelial cell proliferation by direct cell killing as well as HUVEC migration in a Transwell coculture system, presumably due to a reduction in angiogenic factors secreted by infected tumor cells. Expression of dnFGFR further enhanced this effect, showing that different mechanisms work cooperatively in inhibiting angiogenesis.

The FGF signaling pathway is complex and involves multiple molecules and interactions with other signaling pathways (18). The approach taken in this study is to block FGF signaling, whether because of mutant FGFR or elevated FGF, at the receptor level. It will be interesting to see whether blocking the pathway at other checkpoints will result in a similar effect. In addition, apart from the FGF/FGFR pathway, other signaling pathways are implicated in tumorigenesis and/or angiogenesis (vascular endothelial growth factor pathway, epidermal growth factor, insulin-like growth factor signaling pathways, etc.). The approach taken in this study can also be applied to these pathways. Of note, it has been shown that tumors are able to use different angiogenic pathways and may switch, for example, from the vascular endothelial growth factor pathway to FGF (43). The switch from vascular endothelial growth factor to FGF after vascular endothelial growth factor inhibition has been experimentally shown but never the opposite. Although tumors often use multiple redundant angiogenic pathways and treatment might select for FGF-independent tumor cells, the endothelial cells are less likely to loose responsiveness to FGF. Because oncolytic HSV vectors can accommodate large and/or multiple transgenes, construction of vectors that express transgenes targeting multiple pathways is possible. As tumors evolve, they secrete a variety of angiogenic factors (44, 45); targeting these pathways should enhance the therapeutic efficacy of oncolytic HSV and also benefit late-stage cancer patients (46).

These studies indicate that dnFGFR is an excellent therapeutic transgene for oncolytic vectors, affecting not only infected tumor and endothelial cells but also the surrounding environment. Therefore, even tumors that do not support robust oncolytic virus replication, such as the mouse MPNST cells, can be effectively treated. This strategy should be applicable to other oncolytic viruses and considered for clinical translation.

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**Fig. 5.** bG47Δ-dnFGFR has enhanced antitumoral efficacy in athymic mice. A, U87 s.c. tumors were inoculated with PBS or virus (1 × 10^6 pfu) on days 1 and 4. B, 37-3-18-4 s.c. tumors were inoculated with PBS or virus (1 × 10^7 pfu) on days 1, 4, 7, and 10. More virus was inoculated into the 37-3-18-4 tumors because these cells are less permissive to G47Δ replication than U87 (see Fig. 2B). *., P < 0.05 between PBS and bG47Δ-empty treatment groups; ▲, bG47Δ-dnFGFR treatment groups; c, PBS treatment groups; ○, bG47Δ-empty treatment groups; ▲, bG47Δ-dnFGFR treatment groups. *.*, P < 0.01 between PBS and bG47Δ-empty treatment groups (n = 10).
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