Intravenous administration of adenoviruses targeting transforming growth factor beta signaling inhibits established bone metastases in 4T1 mouse mammary tumor model in an immunocompetent syngeneic host

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We have examined the effect of adenoviruses expressing soluble transforming growth factor receptor II-Fc (sTGFβRIIFc) in a 4T1 mouse mammary tumor bone metastasis model using syngeneic BALB/c mice. Infection of 4T1 cells with a non-replicating adenovirus, Ad(E1−).sTβRIIFc, or with two oncolytic adenoviruses, Ad.sTβRIIFc and TAd.sTβRIIFc, expressing sTGFβRIIFc (the human TERT promoter drives viral replication in TAd.sTβRIIFc) produced sTGFβRIIFc protein. Oncolytic adenoviruses produced viral replication and induced cytotoxicity in 4T1 cells. 4T1 cells were resistant to the cytotoxic effects of TGFβ-1 (up to 10 ng/ml). However, TGFβ-1 induced the phosphorylation of SMAD2 and SMAD3, which were inhibited by co-incubation with sTGFβRIIFc protein. TGFβ-1 also induced interleukin-11, a well-known osteolytic factor. Intracardiac injection of 4T1-luc2 cells produced bone metastases by day 4. Intravenous injection of Ad.sTβRIIFc (on days 5 and 7) followed by bioluminescence imaging (BLI) of mice on days 7, 11 and 14 in tumor-bearing mice indicated inhibition of bone metastasis progression (P<0.05). X-ray radiography of mice on day 14 showed a significant reduction of the lesion size by Ad.sTβRIIFc (P<0.01) and TAd.sTβRIIFc (P<0.05). Replication-deficient virus Ad(E1−).sTβRIIFc expressing sTGFβRIIFc showed some inhibition of bone metastasis, whereas Ad(E1−).Null was not effective in inhibiting bone metastases. Thus, systemic administration of Ad.sTβRIIFc and TAd.sTβRIIFc can inhibit bone metastasis in the 4T1 mouse mammary tumor model, and can be developed as potential anti-tumor agents for breast cancer.

Keywords: breast cancer; mouse model; oncolytic adenovirus; systemic delivery; TGFβ

INTRODUCTION

In the United States alone, of the nearly 209,000 women diagnosed with breast cancer each year, about 43,000 die.1 A majority of the women develop bone metastases, tumor-induced bone destruction, hypercalcemia and spinal cord compression during the advanced stages of breast cancer, thus seriously compromising the lifestyle of the affected patients.2 Currently, there are only limited therapies for bone metastases. Although the two types of drugs—bisphosphonates and denosumab, an anti-body against RANKL (receptor activator of nuclear factor kappa-B ligand)—can inhibit bone resorption, their ability to cure bone metastases remains to be established.3 Thus, development of novel therapies to treat breast cancer bone metastasis is a major unmet need in medicine.

In the recent years, oncolytic adenoviruses have shown some potential in the treatment of cancer.4−12 In an attempt to develop novel therapeutic approaches for bone metastases, our laboratory has developed oncolytic adenoviruses that would kill the cancer cells and simultaneously express a soluble form of transforming growth factor beta (TGFβ) receptor II-Fc (sTGFβRIIFc) that can target TGFβ-induced signaling pathways.10−12 We chose to target the TGFβ pathway because high levels of circulating TGFβ protein is a poor prognostic marker in breast cancer patients.13,14 Furthermore, aberrant TGFβ signaling at the bone metastasis site has been postulated to be a key factor in the progression of breast cancer bone metastases.14−19 Therefore, there is a growing interest in developing inhibitors of TGFβ signaling for the treatment of various cancer metastases.20−24 Using an MDA-MB-231 human breast cancer bone metastasis model in immunodeficient mice, we have recently shown that intravenous delivery of oncolytic adenoviruses, Ad.sTβRIIFc and TAd.sTβRIIFc, in tumor-bearing mice are effective in inhibiting the established bone metastases.11,12 However, before initiating a clinical trial in breast cancer patients, it is important to examine the efficacy of these oncolytic adenoviruses in an immunocompetent animal model because they have the ability to limit adenoviral replication and thus its efficacy. Keeping that in mind, we have now conducted in vitro and in vivo studies using a mouse mammary 4T1 tumor cell model. We report here that infection of 4T1 cells with recombinant adenoviruses produced transgene expression, and 4T1 cells

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supported adenoviral replication and were killed by oncolytic adenoviruses. Although 4T1 cells were resistant to TGFβ-1-induced cytotoxicity, TGFβ1 was able to activate signaling. More importantly, intracardiac inoculation of 4T1 cells in BALB/c mice produced bone metastases and osteolytic lesions, and thus is an appropriate pre-clinical model for our purpose. We report here that intravenous injections of Ad.sT[RIIFc and TAd.sT[RIIFc inhibited the progression of skeletal metastases in BALB/c mice. Based on our findings, we believe that oncolytic adenoviruses targeting TGFβ1 pathways can be developed for treating breast cancer bone metastases.

MATERIALS AND METHODS

Cell culture

4T1 (ATCC, Manassas, VA) mouse mammary tumor cells, 4T1-luc2 (Caliper life sciences, Hopkinton, MA), MV1Lu (ATCC) mink epithelial cells, and HEK 293 (ATCC, Manassas, VA) human embryonic kidney cells were grown in Dulbecco’s modified Eagle’s medium containing 10% bovine calf serum (Invitrogen, Grand Island, NY). 4T1 cells were plated in 6-well dishes (4 x 10^3 cells/well). The following day, cells were infected with viral vectors (5 x 10^4 VPs/cell) for 7 days before sulforhodamine B staining.

Protocol was used except that 4T1 cells were incubated with various doses to have 100% survival. To examine viral-induced cytotoxicity, the same cells were infected with Ad(E1-).Null, an E1 minus replication-deficient adenovirus containing no foreign gene; Ad(E1-).sTGF, a replication-deficient adenovirus expressing EGFP protein; Ad(E1-).sT[RIIFc, a replication-deficient adenovirus expressing sTGF[RIIFc gene; Ad.sT[RIIFc, an oncolytic adenovirus expressing sTGF[RIIFc gene (constructed using dl01/07 mutant of Ad5, containing two deletions in E1A region as previously described) and TAd.sT[RIIFc, an oncolytic adenovirus expressing sTGF[RIIFc gene with the human TERT promoter driving the adenoviral replication as published. Adenoviral vectors were grown in HEK 293 cells and purified by double CsCl gradient as described. Viral particle (VP) numbers were determined by measuring OD_260 (optical density 260) of the sodium dodecyl sulfate-treated adenoviral solutions.

Adenoviral replication assay

4T1 cells were plated in 24-well dishes (2 x 10^4 cells/well). The following day, cells were infected with viral vectors (5 x 10^4 VPs/cell) and the incubations were continued for 3 days. Cells were then subjected to immunohistochemistry for adenoviral hexon staining using an Adenoviral Titer commercial kit (Clontech, Mountain view, CA) as described earlier. Positive hexon expressing brown cells were photographed, and counted under the microscope to quantify viral replication.

Cytotoxicity assays

To measure TGFβ-1-induced cytotoxicity, cells were plated in 96-well plates 10^3 cells/well. The following day, cells were infected with various concentrations of TGFβ-1 (0.001–10 ng/ml) (Sigma, St Louis, MO), and the incubations were continued for 7 days. Cells were washed, fixed and stained with sulforhodamine B (Sigma), and the A_564 (absorbance at 564 nm) measured as previously described. Untreated control cells were considered to have 100% survival. To examine viral-induced cytotoxicity, the same protocol was used except that 4T1 cells were incubated with various doses of adenoviral vectors for 7 days before sulforhodamine B staining.

GFP expression

4T1 cells were plated in 6-well dishes (4 x 10^5 cells/well). The following day, cells were infected with Ad(E1-).sTGF (2.5 x 10^4 VPs/cell) and incubated for 48 h. Cells were photographed using a fluorescent microscope (x 200).

sTGF[RIIFc expression

To examine adenoviral vector-mediated sTGF[RIIFc expression, 4T1 cells were plated in 6-well dishes (4 x 10^5 cells/well). The following day, cells were infected with various viral vectors (2.5 x 10^4 VPs/cell). After 24 h, media was changed to serum-free media, and the incubations continued for another 24 h. sTGF[RIIFc expression in the media and the cell lysates were examined by western blot analyses as previously described.

sTGF[RIIFc protein amounts in the media were measured by enzyme-linked immunosorbent assay using antibodies against the human IgG Fc fragment (Jackson ImmunoResearch, West Grove, PA, USA) as previously described.

SMAD phosphorylation

4T1 cells were plated in 6-well plates (4 x 10^5 cells/well). The following day, cells were serum starved for 6 h, and then treated with TGFβ-1 (1 ng/ml) in the absence or presence of sTGF[RIIFc (250 ng/ml) for 1 h. Cells were analyzed for p-SMAD2, p-SMAD3 and for total SMAD2/3 using western blots as previously described. The blots were visualized by enhanced chemiluminescence substrate (Amersham Biosciences, Piscataway, NJ).

Interleukin (IL)-11 assays

4T1 cells were plated in 6-well plates (2 x 10^5 cells per well). The following day, cells were serum starved over night, and then exposed to TGFβ-1 (0.1, 104 VPs/cell). Cell lysates and media were analyzed for sTGFβRIIFc protein expression.

Figure 1. Adenoviral-mediated transgene expression in 4T1 cells. (a) 4T1 cells were infected with Ad(E1−).sTGF (2.5 x 10^4 VPs/cell) for 24 h. Cells were photographed (x 200) using a fluorescent microscope. Same viewing fields were used to take phase contrast (left) or fluorescent (right) images. (b) 4T1 cells were infected with various adenoviral vectors (2.5 x 10^4 VPs/cell). Cell lysates and media were analyzed by western blots for sTGFβRIIFc protein expression. (c) Extracellular media were used to examine sTGFβRIIFc levels by enzyme-linked immunosorbent assay method.
1 or 5 ng ml\(^{-1}\)) for 48 h. Media were analyzed for IL-11 levels by enzyme-linked immunosorbent assay using the previously described method.\(^{28}\)

Animal model
All animal experiments were conducted using the animal protocols approved by the IACUC committee of the NorthShore University HealthSystem. To establish bone metastases, 4T1-luc2 cells were injected in the left heart ventricle (day 0) of 4-week-old BALB/c mice (Charles River laboratories, Wilmington, MA). On day 4, the mice were subjected to bioluminescence imaging (BLI) in dorsal and ventral positions using Xenogen IVIS Spectrum imaging equipment (Caliper life sciences, Hopkinton, MA). Photon signals were quantified using living image software 3.0 (Caliper life sciences, Hopkinton, MA) as previously described.\(^{12}\) Mice were divided into various groups, with statistically indistinguishable BLI signals among each group. Various viral vectors were administered via tail vein on days 5 and 7 (5 \(\times\) 10\(^{10}\) VPs per injection/mouse, each injection in a 0.1-ml volume). The control group of mice was administered the buffer alone.

BLI
Mice were imaged in dorsal and ventral positions on days 7, 11 and 14 using the IVIS Spectrum imaging system (Caliper Life Sciences). Whole-body BLI signals were used to quantify the metastasis as previously described.\(^{12}\) Signals in the hind limbs were separately quantified to measure the skeletal metastases as described.\(^{12}\)

X-ray radiography
On day 14 following tumor cell injections, mice were also subjected to X-ray radiography in prone position using Faxitron (Faxitron X-ray Corporation, Wheeling, IL). Skeletal lesion sizes were measured in the femur and tibia of both the hind limbs using Image J software as described earlier.\(^{11,12}\)

Statistical evaluation
All statistical analyses were performed using GraphPad Prizm 5 (GraphPad software, San Diego, CA). Data are presented as mean \(\pm\) s.e.m. To analyze BLI signal progression, a two-way repeated-measure analysis of variance followed by Bonferroni post-tests was used. For multiple groups, statistical significance was analyzed using one-way analysis of variance followed by Bonferroni post-tests. \(P<0.05\) was considered a statistically significant difference.

RESULTS
4T1 cells can be infected with human adenoviral vectors
Experiments were conducted to examine the infectability of 4T1 cells with replication-deficient and replication-competent adenoviral vectors. 4T1 cells were infected with Ad(E1\(\rightarrow\)).GFP, a
non-replicating adenovirus, for 48 h, and the cells were visualized under a fluorescent microscope. The vast majority of cells produced a strong GFP signal (Figure 1a). In another experiment, cells were infected with Ad(E1−)–sTGFRIIFc, a replication-deficient adenovirus, and two oncolytic adenoviruses—Ad.sTGFRIIFc and TAD.sTGFRIIFc. Cell lysates and the extracellular media were subjected to western blot analyses for sTGF|RIIFc expression. Infection of 4T1 cells with Ad(E1−)–sTGFRIIFc, Ad.sTGFRIIFc and TAD.sTGFRIIFc resulted in sTGF|RIIFc protein production, which could be detected in the cell lysates as well as the extracellular media (Figure 1b). The amounts of sTGF|RIIFc were quantified in the media using enzyme-linked immunosorbent assay, and were found to be in the range of 6.21–15.48 ng/ml of media (Figure 1c). These results indicate that 4T1 cells can be infected with human adenoviruses and that infection with Ad(E1−)–sTGFRIIFc, Ad.sTGFRIIFc or TAD.sTGFRIIFc leads to the production of sTGF|RIIFc protein, which is secreted into the media.

Oncolytic adenoviruses replicate and induce cytotoxicity in 4T1 cells

Next, we examined the replication potential of adenoviral vectors in 4T1 cells. Cells were incubated with various adenoviral vectors (5 × 10^3 VPs/cell) at 37 °C for 72 h, and viral titer was determined by hexon staining. Figure 2a shows typical hexon staining of 4T1 cells exposed to various viral vectors. There were very few hexon expressing brown cells in Ad(E1−)–Null- or Ad(E1−)–sTGFRIIFc-treated samples (Figure 2a). However, a large number of 4T1 cells infected with Ad.sTGFRIIFc or TAD.sTGFRIIFc were hexon positive (Figure 2a). Quantification of hexon-positive cells indicated that Ad.sTGFRIIFc produced viral titers (Figures 2b and c), which were about 257-times higher than the non-replicating adenovirus Ad(E1−)–Null (P < 0.001). TAD.sTGFRIIFc produced 175-times higher viral titer than Ad(E1−)–Null (P < 0.01) (Figures 2b and c). However, viral titers in Ad(E1−)–sTGFRIIFc-infected cells were similar to those in Ad(E1−)–Null-treated cells (Figures 2b and c). From these results, we conclude that continuous incubation of 4T1 cells with oncolytic adenoviruses can produce viral replication.

To examine whether viral replication can result in cytotoxicity, 4T1 cells were incubated with various adenoviral vectors for 7 days, and the cytotoxicity assays were performed. Both the oncolytic adenoviruses, Ad.sTGFRIIFc and TAD.sTGFRIIFc, produced a dose-dependent cytotoxicity in 4T1 cells (Figure 2d). Based on the IC50 values, Ad.sTGFRIIFc and TAD.sTGFRIIFc were about 34.2-fold and 24.0-fold, respectively, more toxic than the non-replicating virus Ad(E1−)–Null (Figure 2e). By contrast, a non-replicating virus Ad(E1−)–sTGFRIIFc produced toxicity that was comparable with Ad(E1−)–Null (Figures 2d and e).

4T1 cells are resistant to killing by TGFβ, but retain TGFβ-mediated signaling pathways

Next, we investigated the killing effect of TGFβ in 4T1 cells. 4T1 cells were exposed to various concentrations of TGFβ-1, and 7 days later cytotoxicity was measured. As a positive control, another rodent cell-type MV1Lu, known to be sensitive to TGFβ, was used. As shown in Figure 3a, there was little to no cytotoxic effect of TGFβ-1 even at the highest concentration used (10 ng/ml) in 4T1 cells. However, MV1Lu cells were killed even by a very low concentration of TGFβ-1, with an IC50 of <0.1 ng/ml.

To examine whether TGFβ could induce signaling in 4T1 cells, we exposed 4T1 cells to TGFβ-1 and analyzed the cell lysates for SMAD2 and SMAD3 phosphorylation. Figure 3b shows that TGFβ-1 induced SMAD2 and SMAD3 phosphorylation in 4T1 cells. Co-incubation of sTGFβRIIFc with TGFβ-1 inhibited TGFβ-1-dependent SMAD2 and SMAD3 phosphorylation (Figure 3b). We also examined the effect of TGFβ-1 on IL-11 production, a known osteolytic factor in human breast cancer cells.28,29 TGFβ-1 induced IL-11 protein production in a dose-dependent manner (Figure 3c).

These results indicate that 4T1 cells respond to TGFβ-1 and undergo activation of signaling pathways that are known to favor bone metastases in human breast cancer cells.18,28,30 Importantly, sTGFβRIIFc is able to abolish the TGFβ signaling.

Oncolytic adenoviral-mediated inhibition of 4T1-induced metastases: BLI analyses

Next, we examined the effect of systemic administration of adenoviral vectors expressing sTGFβRIIFc in a 4T1 bone metastasis model. 4T1-luc2 cells were inoculated into the left heart ventricles of BALB/c mice. After 4 days, mice were subjected to whole-body BLI in both dorsal and ventral positions. Mice were split into multiple groups, with nearly equal BLI signal within each group. Two doses of adenoviral vectors were given via the tail vein—the
first dose on day 5 (5 x 10^{10} VPs/mouse) and a second dose on
day 7 (5 x 10^{10} VPs/mouse). Mice were subjected to BLI on days 7,
11 and 14 following tumor-cell injection. A representative mouse
showing BLI signal from each treatment group is shown
in Figure 4a. Whole-body BLI signals were quantified and are shown
in Figure 4b. There was a time-dependent increase in the whole-
body BLI signal to 0.88 x 10^{10} photons sec^{-1} in the control
group of mice that received buffer alone (Figure 4b). There was no
significant inhibition of BLI signal in the Ad(E1-)-Null, Ad(E1-)-sTJRFc
or TAd.sTJRFc treatment groups (Figure 4b). However, Ad.sTJRFc
induced a significant inhibition (P < 0.05) of the whole-body BLI. As 4T1
cells also established bone metastasis in the hind limbs (Figure 4a), the effect of viral vectors on the BLI
signal in the hind limbs was quantified. In the control group of
mice, the BLI signal in the hind limbs reached to 0.86 x 10^{10}
photons sec^{-1}, and there was a significant inhibition of BLI signal accumulation in the hind limbs in the Ad.sTJRFc-treated group
(P < 0.05). However, Ad(E1-)-Null, Ad(E1-)-sTJRFc and TAd.sTJRFc
treatments had no significant effect on the BLI signal intensity in
the hind limbs (Figure 4c).

Oncolytic adenoviral vectors’-mediated inhibition of 4T1-induced metastases: X-ray analyses
To further examine the effects of vectors’ administration on bone metastases, mice were subjected to X-ray radiography on day 14.

A representative example of X-ray radiographs from each group is shown in Figure 5a.

Figure 4. Effect of systemic delivery of viral vectors on 4T1 bone metastases: BLI analysis. 4T1-luc2 cells were injected in BALB/c mice
(5 x 10^6 cells/mouse) on day 0. Initial BLI was performed on day 4; mice with positive tumors were administered viral vectors or buffer (via tail vein)
on days 7 and 14. The numbers of mice in each treatment group were: buffer (n = 9), Ad(E1-)-Null (n = 9), Ad(E1-)-sTJRFc (n = 9), Ad.sTJRFc (n = 11) and TAd.sTJRFc (n = 11). Representative mice of each treatment group are shown. (b) BLI signal in the whole body of mice in various treatment groups were quantified and are shown. (c) To measure bone metastases, BLI signals in the hind limbs (shown by red circles) were quantified in each treatment group and are shown. *P < 0.05.
Adenoviruses targeting TGFβ for bone metastasis

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**Conflict of interest**

The authors declare no conflict of interest.

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Figure 5. Effect of systemic delivery of viral vectors on 4T1 bone metastases: X-ray radiography. (a) Mice from the above experiment described in Figure 4, were subjected to X-ray radiography on day 14. (b) Lesion sizes in each mouse were calculated using Image J software. Results shown are the average lesion sizes in the hind limbs in each of the treatment groups. The numbers of mice in each treatment group were: buffer (n = 9), Ad(E1–)Null (n = 9), Ad(E1–)sT[RFC] (n = 9), Ad.sT[RFC] (n = 11) and TAd.sT[RFC] (n = 11). *P < 0.05, **P < 0.01.

Ad(E1–)sT[RFC], Ad.sT[RFC] and TAd.sT[RFC] treatment groups, respectively. Thus, although the intravenous injection of Ad(E1–)sT[RFC], Ad.sT[RFC] and TAd.sT[RFC] resulted in sTGFβRIIFc production, it appears that the replicating viruses expressing sTGFβRIIFc were the most effective in inhibiting bone metastases.

**Discussion**

The key finding here is that intravenous delivery of oncolytic virus Ad.sT[RFC] expressing sTGFβRIIFc can inhibit bone metastasis in the 4T1 mouse mammary tumor bone metastasis model in a syngeneic host as revealed by BLI studies. X-ray radiographic analyses showed inhibition of tumor growth by Ad.sT[RFC] and TAd.sT[RFC], though Ad.sT[RFC] was superior to TAd.sT[RFC]. A non-replicating virus, Ad(E1–)sT[RFC], expressing sTGFβRIIFc showed some inhibition of bone metastasis in X-ray analyses; Ad(E1–)Null was not effective in either BLI or X-ray analyses.

Most of the previously published studies using oncolytic adenoviruses have been conducted in human xenografts established in immunodeficient nude mice, mainly because mouse tumor cells are not considered good targets for the human adenoviruses. However, it is critical that we continue to explore the animal models in which oncolytic adenoviruses can be examined in immunocompetent syngeneic hosts as described here. It is quite interesting that 4T1 cells can be infected with human adenoviruses resulting in high levels of transgene expression, indicating the presence of adenoviral receptors even in mouse 4T1 cells. Moreover, continuous exposure of 4T1 cells to oncolytic adenoviral vectors can produce a viral titer. This indicates that human adenoviruses can result in virus entry and replication, clearly demonstrating that human adenoviruses can indeed infect and replicate in 4T1 mouse tumor cells, which is consistent with a previous report.31 This infection could be via the previously known adenoviral receptor, by an unknown adenoviral receptor or even by other pathways, including clathrin-independent mechanisms such as macropinocytosis, phagocytosis or trans-endocytosis.32 Once the VPs are internalized by the cells, however, viral replication proceeds as in human breast cancer cells. Although the exact relationship of the Ad.sT[RFC] and TAd.sT[RFC]-induced replication resulting in the cytopathic effect of the 4T1 tumor model remains to be examined, it is tempting to speculate that the viral replication resulting in the cytopathic effects of the adenoviral vectors in the mouse tumor cells could have a role in mediating the in vivo anti-tumor responses reported here.

Another important observation is the inability of TGFβ to kill 4T1 cells and yet induce the TGFβ signaling pathway (SMAD-phosphorylation), and the production of IL-11 (a well-known osteolytic factor in human breast cancer bone metastasis). Thus, in this regard, 4T1 is an appropriate tumor model for examining the role of TGFβ signaling in bone metastases. In the radiographic analyses, a non-replicating adenovirus expressing sTGFβRIIFc showed some inhibition of bone metastasis, albeit weaker than oncolytic adenovirus Ad.sT[RFC]. Again, these studies suggest that the expression of sTGFβRIIFc, coupled with viral replication and cytopathic effects, is potentially having a role in mediating the inhibition of bone metastases.

**Intravenous delivery of adenoviruses will result in their uptake mainly in the liver, and in smaller amounts in other tissues and the skeletal tumors.**3,10,11,12,27,33 We believe that the infection of tumor cells in vivo will result in the viral replication in the tumor cells causing cell killing and partial tumor destruction. Infection of tumor cells and other mouse organs will result in the production of sTGFβRIIFc that will be released in the blood. The sTGFβRIIFc production resulting in the inhibition of TGFβ signaling at the tumor/bone site will also contribute towards the inhibition of bone metastases. Among the three vectors expressing sTGFβRIIFc, the most effective vector is the Ad.sT[RFC]; TAd.sT[RFC] is slightly weaker than Ad.sT[RFC], and the least effective is the Ad(E1–)sT[RFC]. As all the three vectors produce nearly equal amounts of sTGFβRIIFc, Ad.sT[RFC] is the most effective, which is perhaps due to its higher replication potential in the tumor cells. TAd.sT[RFC] can also replicate in the tumor cells, but its replication potential is slightly lower than Ad.sT[RFC]; and Ad(E1–)sT[RFC] is replication-deficient. Based on these results, we believe that both viral replication and the sTGFβRIIFc expression have an important role in the inhibition of bone metastases.

In addition to understanding the role of viral replication and the inhibition of TGFβ signaling at the tumor–bone microenvironment, the future availability of a 4T1 bone metastasis model will also allow us to further explore the role of the adenoviral vector-induced innate and humoral immune responses,34–36 the role of TGFβ in suppressing the immune system3,10,15,37 and how that can be reversed by the oncolytic adenoviruses expressing sTGFβRIIFc. These questions can be addressed only in fully immunocompetent animal models.

In conclusion, our work described here shows that oncolytic adenoviruses targeting the TGFβ pathway can inhibit breast cancer bone metastases in a mouse mammary tumor model established in a syngeneic immunocompetent host and represents an important step in developing oncolytic adenoviruses for the treatment of breast cancer bone metastases. This animal model will now allow us to investigate the underlying molecular mechanism of action of the oncolytic adenoviruses, which may help in refining this method of treatment.
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