Antitumor Effect of Intratumoral Administration of Bone Marrow-Derived Dendritic Cells Transduced with Wild-Type p53 Gene

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ABSTRACT

Purpose: Dendritic cells (DCs) are attractive effectors for cancer immunotherapy because of their potential to function as professional antigen-presenting cells for initiating cellular immune responses. The tumor suppressor gene p53 is pivotal in the regulation of apoptosis, and ~50% of human malignancies exhibit mutation and aberrant expression of p53. We investigated the antitumor effect of intratumoral administration of bone marrow-derived dendritic cells transduced with wild-type p53 gene.

Experimental Design: We examined whether intratumoral administration of DCs infected with recombinant adenovirus expressing murine wild-type p53 (Ad-mp53) could induce systemic antitumor responses against mutant p53-expressing tumors, highly immunogenic MethA, or weakly immunogenic MCA-207 implanted in syngeneic mice.

Results: Accumulation of wild-type p53 protein in bone marrow-derived murine DCs could be successfully achieved by Ad-mp53 infection. Treatment with intratumoral injection of Ad-mp53-transduced DCs caused a marked reduction in the in vivo growth of established MethA and MCA-207 tumors with massive cellular infiltrates. Administration of p53-expressing DCs suppressed the growth of both injected MCA-207 tumors and untreated distant MCA-207 tumors, but not unrelated Lewis lung carcinoma tumors, suggesting the augmentation of systemic immunogenicity against MCA-207 tumor cells. Moreover, intratumoral injection of p53-expressing DCs had a greater antitumor effect than did s.c. immunization.

Conclusions: Our results indicate that intratumoral administration of DCs expressing murine wild-type p53 leads to significant systemic immune responses and potent antitumor effects in mutant p53-expressing murine cancer models. These findings raise the possibility of using this strategy of intratumoral injection of p53-expressing DCs for human cancer treatment.

INTRODUCTION

Dendritic cells (DCs) are potent professional antigen-presenting cells and play a key role in initiating primary immune responses. DCs have been considered recently as cellular adjuvants in tumor immunotherapy because of their capacity to enhance antigen presentation. Several advantageous characteristics of DCs have been identified, as compared with other antigen-presenting cells: (a) DCs are capable of uptaking (endocytosis and phagocytosis) and processing an antigenic source in a state of immaturity (1); (b) DCs can present tumor-associated antigen in the context of both MHC class I and class II in association with costimulatory molecules, and thus they can stimulate both cytotoxic T cells and helper T cells (2); and (c) DCs can migrate to the draining lymph nodes and spleen, resulting in a systemic immune response (3, 4). Much attention has been directed to the problem of how and what antigens should be pulsed to DCs in their clinical application as immunotherapy. The most common approach to pulse DCs with tumor antigens is to coculture DCs with tumor-specific peptides or tumor lysate. Recent studies have reported that vaccinations with tumor-associated antigen peptide-loaded DCs elicited antitumor immunity (5–7). More recent approaches have used DCs that were transduced with antigen encoding cDNA or RNA (2, 8). Several viral vectors have been shown to transfer genes encoding antigenic protein or peptide to DCs, and DCs transduced with virus vector encoding tumor-associated antigen were shown to induce specific antitumor immunity in vitro and in vivo (9, 10).

In this respect, p53 protein is one of the most attractive candidates for antigenic protein, because it is overexpressed in ~50% of human malignancies and is expressed at very low levels in normal cells. Mutations in p53 abrogate its function as a tumor suppressor gene and are associated with a prolonged half-life, resulting in marked nuclear and cytoplasmic accumulation of p53 protein. Previous studies have reported successful induction of HLA-A2- or A24-restricted p53-specific CTLs by using p53 peptide-pulsed DCs (11, 12). Moreover, murine experimental models have demonstrated the ability to target overexpressed p53 protein as a means of achieving tumor rejection (13). These observations led us to investigate whether p53 could be used potentially for immunotherapy by using the entire p53 sequence as an antigen.

Intratumoral injection has been reported to be an attractive approach for delivering DCs directly to the tumor site. In addi-
Immunotherapy with temic immune responses against tumors with high p53 expressed mutated p53. Furthermore, this strategy can enhance systemic immune responses against tumors with high p53 expression.

MATERIALS AND METHODS

Animals. BALB/c and C57BL/6 (5–8-week-old female) mice were purchased from Charles River Japan (Yokohama, Kanagawa, Japan) and housed at the Animal Experiment Facility of Okayama University School of Medicine for a minimum of 1 week before the experiments. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Okayama University School of Medicine.

Cell Lines. The MethA tumor is a transplantable 3-methylcholanthrene-induced sarcoma of BALB/c origin passed as an ascitic tumor (14). MethA p53 is known to have three missense point mutations in codons 132, 168, and 234 (15). The MCA-207 tumor, which is a weakly immunogenic, MCA-induced fibrosarcoma of C57BL/6 origin (16), was kindly provided by Dr. Yutaka Kawakami (Keio University School of Medicine, Tokyo, Japan). MCA-207 cells expressed a mutant form of p53 (17). The Lewis lung carcinoma (3LL) was provided by Cell Resource Center for Biomedical Research (Institute of Development, Aging and Cancer, Tohoku University, Tohoku, Japan). These cell lines were maintained in RPMI 1640 supplemented with 10% FCS, 100 units/ml penicillin, and 100 mg/ml streptomycin, glutamine, and 50 mm 2-mercaptoethanol, referred to as complete medium. The transformed embryonic kidney cell line 293 was grown in DMEM with high glucose (4.5 g/liter), supplemented with 10% FCS, 100 units/ml penicillin, and 100 mg/ml streptomycin. The 293 cells were used for the production of adenovirus vectors.

Adenovirus Vectors. The recombinant, replication-deficient adenovirus vector, Ad-mp53, encodes full-length murine wt-p53 cDNA. The E1A-deleted adenovirus vector lacking cDNA insert (dl312) was used as a control vector. These viruses were obtained by lysing infected 293 cells. Titers of the viral stocks were determined with a plaque-forming assay using 293 cells.

Antibodies. Supernatants containing monoclonal antibodies produced by the following hybridomas, which were obtained from American Type Culture Collection (Rockville, MD), were used to deplete lymphocytes and granulocytes, antitumor CD4 (TIB207), antitumor CD8 (TIB105), and antimouse B220/CD45R (TIB146).

Generation of BM-DCs. BM-DCs were generated as described previously (18, 19). Briefly, BM cells were flushed out from the femurs and tibias. The erythrocytes were lysed by RBC lysis buffer containing ammonium chloride, and the lymphocytes were immunodepleted by treatment with anti-B220/CD45R, anti-CD4, and anti-CD8 monoclonal antibodies, and rabbit complement (Cedarlane, Ontario, Quebec, Canada). The remaining cells were incubated overnight in complete medium, and the nonadherent cells (DC precursor cells) were harvested to be incubated with the complete medium supplemented with recombinant murine-granulocyte macrophage colony-stimulating factor (1000 IU/ml; Genzyme Technne) and recombinant murine-interleukin-4 (1000 IU/ml; Genzyme Technne) for an additional 7 days. On day 3 of culture, 50% of the medium was replaced, and on day 7, nonadherent cells (DCs) were harvested.

Adenovirus-Mediated Gene Transduction into DCs. Gene transfer to DCs was performed using an adenovirus vector with centrifugal force method, as described previously (20). Briefly, 1 × 10⁶ DCs were suspended in 500 μl of PBS containing 1% FCS and then mixed with adenoviral vector that was suspended in 500 μl of PBS containing 1% FCS. The mixtures of DCs and virus vectors were centrifuged (2000 × g at 37°C) for 2 h. Centrifuged DCs were washed twice in PBS and used for subsequent experiments.

Flow Cytometry. The cell surface expression of DC markers was assessed by flow cytometric analysis. DCs were washed once and stained for 30 min at 4°C with the primary antibody followed by a FITC-conjugated rabbit antitumor IgG. After being washed twice, the cells were analyzed by the FACScan with the window set to exclude dead cells and debris, using CELL Quest software. Ten thousand cells were examined for each determination. Anti-CD11c, -CD80, and -Ia-Dº (MHC class II; PharMingen, San Diego, CA) were used as the primary antibodies.

Reverse Transcription-PCR. Molecular analysis of the expression of transduced murine p53 was performed by standard reverse transcription-PCR methods. Total RNA was isolated from untreated DCs or DCs infected with Ad-mp53 or dl312 using RNAzol (Cinna/Biotec, Friendwood, TX) in a one-step phenol extraction method. Reverse transcription was performed using 0.5 μg of total/cyttoplasmic RNA reaction to ensure that the amount of amplified DNA was proportional to that of the specific mRNA in the original sample. PCR with specific primers was performed for 20–30 cycles in a 50-μg volume according to the manufacturer’s protocol (PCR kit; Perkin-Elmer/Cetus, Norwalk, CT) using a Perkin-Elmer thermal cycler (Foster City, CA). Specific primers were used for the promoter/p53 region of Ad-mp53 (sense, 5’-CAC GTA CTC TCC TCC CCT CA-3’; and antisense, 5’-CTC GGG TGG CTC ATA AGG TA-3’) and β-actin (sense, 5’-GCC ATC CTG CGT GAC TTG-3’; and antisense, 5’-CAC TGG CTC TGG ACG ATG GAG-3’), which served as a control. The amplification reactions involved denaturation at 96°C for 1 min, annealing at 59°C for 1 min, and extension at 72°C for 3 min. The PCR products were resolved on 1% agarose gels and visualized by ethidium bromide staining. The density of products was quantified using an image analyzer with β-actin as the internal control.

Western Blot Analysis. DCs were collected by trypsinization and washed twice in cold PBS. Cells were then dissolved in lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.5% Triton X-100, and protease inhibitors [0.2 mM phenylmethylsulfonyl fluoride, 0.2 mM 4-(2-aminoethyl) benzenesulfonylfluoride, 10 μg/ml leupeptin, 10 μg/ml pepstatin, and 1 μg/ml aprotinin]. Lysis was performed at 4°C for 30 min, and the lysate was centrifuged at 15,000 rpm. The protein
concentration of the supernatant was determined using the Bio-Rad protein determination method (Bio-Rad, Hercules, CA). Equal amounts (15 μg) of proteins were electrophoresed under reducing conditions on 12% (w/v) polyacrylamide gels. Proteins were electrophoretically transferred to a Hybond-polyvinylidene difluoride transfer membranes (Amersham, Arlington Heights, IL) and incubated with primary antibodies against murine p53 followed by peroxidase-linked secondary antibody. An Amersham enhanced chemiluminescent Western system (Amersham, Tokyo, Japan) was used to detect secondary probes.

**In Vivo Experiments.** MethA or MCA-207 tumor cells (1 × 10⁶ cells/100 μl) were inoculated s.c. into the right and/or left flanks of 6–12-week-old BALB/c or C57BL/6 mice, respectively. The 3LL tumor cells (1 × 10⁶ cells/100 μl) were also inoculated s.c. into C57BL/6 mice, and when average tumor diameters reached ~5 mm, mice were treated with intratumoral or s.c. injection of HBSS (100 μl), untreated DCs (1 × 10⁶ cells/100 μl), dl312-transduced DCs (1 × 10⁶ cells/100 μl), or p53-transduced DCs (1 × 10⁶ cells/100 μl). The s.c. injections were performed >2.5 cm distant from the tumors. The s.c. MethA, MCA-207, or 3LL tumors were measured with calipers for perpendicular diameters, and tumor volume was calculated using the following formula: tumor volume (mm³) = a × b² × 0.5, where a is the longest diameter, b is the shortest diameter, and 0.5 is a constant to calculate the volume of an ellipsoid.

**Statistical Analysis.** Data are expressed as mean ± SE. Differences in the tumor volumes between the treatment groups were compared using the Student’s t test. Statistical significance was defined as P < 0.05.

**RESULTS**

Adenovirally Transduced Murine WT-p53 Gene Expression in Murine BM-DCs. BM-DCs generated *in vitro* with granulocyte macrophage colony-stimulating factor plus interleukin-4 were infected with a replication-deficient adenovirus vector carrying a murine WT-p53 gene under the control of the cytomegalovirus promoter (Ad-*mp53*) at a multiplicity of infection of 100, 200, or 300. A semiquantitative reverse transcription-PCR assay demonstrated enhanced *p53* mRNA expression as early as 24 h after infection (Fig. 1A). Nishimura *et al.* (20) reported that adenovirus-mediated gene transduction into DCs was enhanced by centrifugation. Centrifugally transduced DCs showed a marked increase in exogenous *p53* expression (Fig. 1B). In addition, to confirm the functional *p53* gene transduction, we conducted Western blot analysis using an antibody against murine *p53* protein. Western blotting showed that readily detectable murine *p53* protein expression was achieved in BM-DCs 36 h after Ad-*mp53* infection by the centrifugal method but not in uninfected and dl312-infected DCs (Fig. 1C). These results suggest that the adenovirus-mediated gene transfer with centrifugal manipulation is highly efficient in murine BM-DCs. There was no apparent toxicity of adenovirus infection up to 300 multiplicity of infection in DCs (data not shown).
Influence of Ad-mp53 Infection on Differentiation and Activation of BM-DCs. Recombinant adenovirus infection has been reported to induce maturation and activate human DCs (21, 22). To determine whether Ad-mp53 infection affected DC activity, we analyzed the cell surface phenotype of DCs infected with either Ad-mp53 or control dl312 by flow cytometric analysis. As shown in Fig. 2, the intensity of expression of MHC class II, CD11c, and CD80 on Ad-mp53-infected DCs did not differ from that on uninfected or dl312-infected DCs. These results suggest that recombinant adenovirus infection as well as exogenous wt-p53 gene expression had no apparent effect on DC differentiation and activation.

Eradication of Established MethA Tumors by Intratumoral Administration of BM-DCs. To examine the antitumor effect of intratumoral injection of BM-DCs, we established s.c. tumors in BALB/c mice with an injection of $2 \times 10^6$ MethA sarcoma cells that carry three mutations at codons 132, 168, and 234 in the p53 coding sequence. Mice with palpable s.c. MethA tumors received 3-day courses of intratumoral injections of uninfected, dl312-infected, or Ad-mp53-infected DCs or PBS (mock) on days 7–9, and tumor size was measured. As shown in Fig. 3A, intratumoral administration of DCs transduced with murine wt-p53 gene resulted in substantial MethA tumor growth inhibition; 62.5% of the treated mice showed complete tumor regression. Unexpectedly, 50% of mice that received uninfected or dl312-infected DCs also exhibited apparent tumor regression. All of the control mice died within 80 days of tumor inoculation (7 of 7), whereas 4 of 8 (50%) mice injected with untreated or dl312-infected DCs and 5 of 8 (62.5%) mice treated with Ad-mp53-infected DCs remained tumor-free over an observed period of 120 days (Fig. 3B).

The experiment was repeated with $5 \times 10^5$ MethA sarcoma cells to evaluate the systemic immunity against MethA tumor cells. Intratumoral injections of uninfected, dl312-infected, or Ad-mp53-infected DCs cured most of the mice within 30 days after tumor inoculation. All of the animals cured of established MethA tumors were protected against subsequent s.c. rechallenge with the same dose of MethA tumor cells 70 days after treatment (Fig. 4). In contrast, MethA tumors grew rapidly in naive mice. These results suggest that local DC administration alone might be sufficient to induce substantial inhibition of the growth of MethA tumors as well as rechallenge resistance, probably because of their highly immunogenic properties.

Treatment of Established MCA-207 Tumors by Intratumoral Injection of DCs Transduced with Murine WT-p53 Gene. To assess the ability of Ad-mp53-infected DCs to induce a specific immunological response against p53-expressing tumor cells, we used MCA-207 tumors expressing mutant p53, which are weakly immunogenic, methylcholanthrene-induced fibrosarcomas of C57BL/6 origin. In a representative experiment, MCA-207 tumors that were injected intratumorally with Ad-mp53-infected DCs for 3 consecutive days demonstrated a significant reduction in tumor volume, as compared with PBS (mock; P < 0.05). In contrast, no statistically significant difference in mean tumor volumes was observed between the mock group and the group treated with uninfected or dl312-infected DCs (Fig. 5A).

The survival curves for all of the four groups were shown in Fig. 5B. All of the control mice without DC treatments died within 62 days of tumor inoculation. Only 1 of 4 mice treated with uninfected DCs or dl312-infected DCs survived for 80 days after tumor inoculation, whereas 2 of 5 mice treated with Ad-mp53-infected DCs survived. These results suggest that local administration of murine p53-transduced DCs exhibits therapeutic efficacy against mutant p53-expressing, weakly immuno-
genic MCA-207 tumors, although no treated mice underwent complete tumor regression.

To investigate the mechanisms for the antitumor effect, we histopathologically analyzed MCA-207 tumors injected with Ad-mp53-infected DCs for the distribution of inflammatory and/or immune cells. Histological analysis at 7 days after the first injection of Ad-mp53-infected DCs revealed massive cellular infiltrates at the central portions of the tumors where DCs were injected (Fig. 5C). In contrast, tumors treated with uninfected DCs showed less intensive cellular infiltrates. Intratumoral injection of PBS as a control induced no cellular infiltrates. These results indicate that the presence of inflammatory infiltrates might be because of transduced p53 expression in DCs rather than DC injection.

Inhibition of MCA-207 Tumor Growth at Distant Sites After Intratumoral Injection of DCs Transduced with Murine WT-p53 Gene. We also tested whether intratumoral injection of Ad-mp53-transduced DCs could mediate a therapeutic benefit on distant, un.injected MCA-207 tumors in a dual tumor model. MCA-207 tumors were established in both the left and right flanks of C57BL/6 mice, and the growth of tumors in the left flank was assessed after intratumoral inoculation of uninfected, dl312-infected, or Ad-mp53-infected DCs or PBS (mock) into the right flank. As shown in Fig. 6A, Ad-mp53-infected DCs, but not untreated and dl312-infected DCs, mediated substantial, significant inhibition of the growth of distant MCA-207 tumors compared with that of treated mice with complete regression; 3 weeks after intratumoral injection of Ad-mp53-transduced DCs into established MCA-207 tumors with s.c. injection of Ad-mp53-transduced DCs induced systemic antitumor immunity against injected tumors, but not unrelated tumors, even with mutant p53 overexpression.

Therapeutic Advantage of Intratumoral Administration of DCs on MCA-207 Tumors. To additionally define the impact of intratumoral injection of DCs, we compared the antitumor effect of intratumoral injection of Ad-mp53-infected DCs on MCA-207 tumors with that of s.c. immunization. Mice received three intratumoral injections of Ad-mp53-infected DCs on days 7–9. Another cohort of mice was immunized daily for 3 consecutive days by s.c. administration of DCs transduced with Ad-mp53. Although s.c. immunization with murine p53-transduced DCs resulted in a slower tumor growth rate compared with control groups, this antitumor effect could be significantly enhanced further by intratumoral injections (P < 0.05; Fig. 7A). Treatment of MCA-207 tumors with s.c. injection of Ad-mp53-infected DCs was associated with inhibition of tumor growth but not with complete regression; 3 weeks after intratumoral injection of Ad-mp53-infected DCs, however, tumors had completely regressed in 3 of 5 mice (Fig. 7B). These results suggest that intratumoral immunization with murine p53-expressing DCs have efficient antitumor activity against mutant p53-expressing syngeneic tumors.
DISCUSSION

Previous studies have suggested that CTL-mediated immunotherapy targeting p53 would be primarily applicable to mutant p53-overexpressing tumors (23); however, the most effective strategy for inducing p53-specific CTLs in vivo remains unclear. DCs are highly efficient antigen-presenting cells and can generate antigen-specific CTLs in a MHC class I- and II-restricted manner. Increased numbers of intratumoral DCs have been reported to be associated with better outcomes in patients with a variety of carcinomas (24–26), indicating that intratumoral DCs play an important role in antitumor immune responses. Here, we have shown that intratumoral administration of DCs transduced with murine wt-p53 using an adenovirus vector induced a systemic antitumor effect against mutant p53-expressing murine tumors in vivo and that this effect was greater than that associated with s.c. immunization.

Viral vector-mediated genetic modification of DCs to express tumor antigens or immunomodulatory proteins has been successfully achieved in preclinical animal models of tumor treatment (27, 28). However, if transgenes have proapoptotic or cell cycle-regulatory functions, it may be difficult to obtain constitutive transgene expression in DCs. Although overexpression of the wt-p53 gene has been known to induce apoptosis in many types of human cancer cells (29, 30), we were able to generate murine BM-DCs expressing high levels of the wt-p53 gene by adenovirus-mediated transduction combined with centrifugation (Fig. 1). In addition, recombinant adenovirus infection reportedly induces maturation of DCs via a nuclear factor κB-dependent pathway (22). We found, however, that the adenoviral vector had no detrimental effect on the DC phenotype in our experimental systems (Fig. 2). Nishimura et al. (20) reported also that infection of adenovirus encoding enhanced green fluorescent protein had little effect on expression of surface markers of DCs, indicating that the effect of recombinant adenovirus infection on DC maturation is controversial.

Intratumoral injection of adenovirus expressing the wt-p53 gene had substantial therapeutic activity in animal models as well as in clinical trials (31, 32); the effects, however, seem to be limited within the local areas. Nikitina et al. (33) reported that MethA tumor growth was significantly slowed by immunization of mice with Ad-mp53-transduced DCs (4 × 10^5 and 2 × 10^5 cells were injected i.v. and s.c., respectively). The fact that increased DC numbers within solid tumor masses are associated with improved prognosis (34) suggests the possible application of direct tumoral administration of DCs. We hypothesized that intratumoral administration of Ad-mp53-infected
Fig. 5  A, effect of intratumoral injection of bone marrow-derived dendritic cells (DCs) transduced with Ad-mp53 on the in vivo growth of established s.c. MCA-207 tumors. C57BL/6 mice received $2 \times 10^6$ MCA-207 tumor cells s.c. on day 0, and then were intratumorally injected on days 7–9 with $1 \times 10^6$ cells of uninfected DCs, dl312-infected DCs, or Ad-mp53-infected DCs. Ten mice were used in each group. The tumor growth was expressed by the tumor mean volume; bars, ±SE. B, survival of mice bearing s.c. established MCA-207 tumors treated with intratumoral injection of bone marrow-derived DCs transduced with Ad-mp53. Survival was monitored over time after tumor injection and plotted as a Kaplan-Meier plot. C, histological examination of MCA-207 tumors s.c. implanted into C57BL/6 mice. Mice were treated either with uninfected DCs or Ad-mp53-infected DCs as described above. Tumors were dissected 7 days after the first DC injection, and paraffin sections were stained with H&E. C, panel 1, mock; C, panel 2, uninfected DCs; and C, panel 3, Ad-mp53-infected DCs. Original magnification, ×200.
DCs could enhance antitumor effects compared with other approaches. We first used MethA tumor cells as the target because Noguchi et al. (14) demonstrated that a peptide containing the codon 234 mutation that was identified in MethA p53-induced CD8+ CTLs. Our results, however, were unexpected: DCs delivered intratumorally markedly suppressed the growth of MethA tumors and prolonged the survival of mice, even without Ad-mp53 transduction (Fig. 3). All of the animals cured of established MethA tumors were protected against subsequent s.c. rechallenge with the same dose of MethA tumor cells (Fig. 4). One possible explanation for these unexpected results is the strong immunogenicity of MethA tumor cells. DCs could readily acquire antigens by uptake of apoptotic immunogenic tumor cells. Indeed, it has been reported that intratumoral injection of syngeneic DCs resulted in inhibition of murine tumor growth in vivo (17). Moreover, in the clinical pilot study, DCs mediated biological activity in patients with metastatic tumors after intratumoral injection (35).

Treatment of mice with intratumoral injections of Ad-mp53-transduced DCs mediated more profound antitumor effects with massive cellular infiltrates than either DCs alone or dl312-transduced DCs when weakly immunogenic MCA-207 tumors were used (Fig. 5). The possibility that intratumoral injection of Ad-mp53-infected DCs had direct antitumor activity was not excluded. However, we also presented evidence of antitumor effects even in distant established tumors (Fig. 6A). Furthermore, we speculated that the effector cells responsible for the antitumor activity in our murine models might be p53-specific CTLs. However, we detected a very low p53-specific CTL response against MCA-207 cells after 7-day in vitro stimulation of splenocytes obtained from treated mice (data not shown). The finding that mice treated intratumorally with Ad-mp53-infected DCs demonstrated a therapeutic benefit not only on injected MCA-207 tumors but also on distant, uninjected MCA-207 tumors, but not other unrelated 3LL tumors (Fig. 6), indicates that the systemic immune response against MCA-207 cells could be induced in vivo. One explanation for the failure of p53-specific lysis in vitro is the lack of acquisition of additional antigens in the process of in vitro stimulation. Although the poor immunogenicity of 3LL tumor cells could not be ruled out, the recognition of other tumor-associated antigens in addition to p53 might be required for the induction of systemic immunity against MCA-207 tumor cells; DCs delivered locally at the site of tumors could phagocytose adjacent tumor cells and present additional tumor antigens in vivo, which might be facilitated by p53 protein overexpression, although additional investigations will be required to confirm this hypothesis.

Because p53 is broadly expressed at low levels in virtually every nucleated cell, whether or not p53 can serve as targets for cancer immunotherapy has remained an open question. In some cases, successful rejection of established tumors by self-tumor antigens could only be achieved in the presence of severe autoimmune responses; in our experimental models, however, none of the mice treated with Ad-mp53-infected DCs showed signs of autoimmune distress (ruffled fur, weight loss, lethargy, or agitation) or histopathological changes in organs at autopsy (data not shown). In conclusion, we have demonstrated that intratumoral administration of BM-DCs transduced with the wt-p53 gene could elicit a systemic immune response capable of inhibiting the growth of tumor cells harboring mutant p53. Of addi-
transduced bone marrow-derived DCs daily for 3 consecutive days and treated with either intratumoral or s.c. administration of Ad-mp53

MCA-207 tumors were injected s.c. with 2*10^6 dendritic cells (DCs) on the growth of established s.c. MCA-207 tumors. Data are mean; bars, ±SE. □, mock; ■, s.c. injection; ▲, intratumoral (i.t.) injection. B, macroscopic appearance of MCA-207 tumors on C57BL/6 mice. B, panel 1, untreated mice (Mock); B, panel 2, mice treated with s.c. injection of Ad-mp53-infected DCs; and B, panel 3, mice treated with intratumoral injection of Ad-mp53-infected DCs.

Fig. 7  Effect of intratumoral versus s.c. injection of Ad-mp53-infected dendritic cells (DCs) on the growth of established s.c. MCA-207 tumors. C57BL/6 mice were injected s.c. with 2*10^6 MCA-207 tumor cells and treated with either intratumoral or s.c. administration of Ad-mp53-transduced bone marrow-derived DCs daily for 3 consecutive days beginning 7 days after tumor inoculation. A, tumors were measured and sizes presented as tumor volume. Data are mean; bars, ±SE. □, mock; ■, s.c. injection; ▲, intratumoral (i.t.) injection. B, macroscopic appearance of MCA-207 tumors on C57BL/6 mice. B, panel 1, untreated mice (Mock); B, panel 2, mice treated with s.c. injection of Ad-mp53-infected DCs; and B, panel 3, mice treated with intratumoral injection of Ad-mp53-infected DCs.

tional importance, the local delivery of p53-expressing DCs at the site of tumors was apparently more efficient at enhancing the therapeutic efficacy than s.c. administration. It re-
mains to be determined whether or not maturation of DCs after the p53 gene transfer can result in additional therapeutic benefits. Our data, however, provide a preclinical rationale for p53-expressing DC-based therapy, which may be of clinical utility.

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