Antitumor Effect of in Vivo Somatostatin Receptor Subtype 2 Gene Transfer in Primary and Metastatic Pancreatic Cancer Models

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INTRODUCTION

Pancreatic ductal carcinoma is, at present, the fifth leading cause of cancer-related deaths in Western countries. Because of late diagnosis due to a long silent clinical phase during tumor development and the absence of early markers of the disease, the prognosis of this cancer is very poor (1). Up to now, the only curative treatment for pancreatic cancer is surgical resection. Unfortunately, surgery for curative purposes is possible in only 10–15% of cases, and among patients undergoing surgery, only 5% have a 5-year survival. The overall 5-year survival rate for pancreatic carcinoma is no more than 3%. At the time of diagnosis, 15–30% of patients with pancreatic adenocarcinoma also observed (16). Furthermore, we recently conducted studies in a

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The abbreviations used are: sst2, cloned somatostatin receptor subtype 2; PEI, polyethylenimine; PCNA, proliferating cell nuclear antigen; PARP, poly(ADP-ribose) polymerase; CMV, cytomegalovirus; GPP, green fluorescent protein; TGP, tumor growth progression; RT-PCR, reverse transcription-PCR; pfu, plaque-forming unit(s); X-Gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling.
transplantable model of pancreatic carcinoma in hamsters demonstrating that reexpression of \( \text{sst2} \) gene caused inhibition of primary tumor growth and metastatic progression (17). All these results strongly suggest that the \( \text{sst2} \) gene acts as a tumor suppressor in pancreatic cancer cells. Its transfer may represent a novel therapeutic approach to this cancer. Our previous studies were based on \textit{ex vivo} approaches using cellular clones transfected with \( \text{sst2} \) or control genes (16, 17). A rational preclinical approach to gene therapy for pancreatic cancer requires \textit{in vivo} gene transfer experiments, and we tested in this way both viral and synthetic vectors to transduce \( \text{sst2} \) gene in either primary or metastatic tumors.

The transplantable model of pancreatic carcinoma established in hamsters (17) and a new transplantable model of hepatic metastasis developed by us were used in the present study. We observed that \textit{in vivo} \( \text{sst2} \) gene transfer resulted in significant inhibition of growth progression for both pancreatic primary tumor and hepatic metastases. This therapeutic effect required an antitumor bystander effect, whose molecular mechanisms were investigated here.

**MATERIALS AND METHODS**

**Reagents**

ExGen 500/PEI in \textit{vivo} was from Euromedex (Souffelweyersheim, France). Oligonucleotides, RNAbler, and SuperScript II reverse transcriptase were from Eurobio (Les Ulis, France) and Invitrogen, respectively. RNAsin was from Promega (Charbonnières, France). Taq polymerase and DNase I were from Boehringer Mannheim France (Meylan, France).

**Plasmids**

The murine \( \text{sst2} \) cDNA, subcloned into pCMV6c vector, was kindly provided by G. I. Bell (Howard Hughes Medical Institute, The University of Chicago, Chicago, IL). Control plasmid pGLCMV-\beta-GAL was a gift from H. Paris (INSEMe U388, Toulouse, France). All plasmids were purified using the Endoffree system (Qiagen, Courtaboeuf, France).

**Construction and Preparation of Recombinant Adenoviruses**

The Ad.RSV.\textit{nsLacZ} adenoviral vector used was a recombinant human serotype 5 replication-deficient adenovirus containing nuclear-targeted \textit{E. coli} \( \beta \)-galactosidase (\textit{LacZ}) reporter gene driven by the Rous sarcoma virus long terminal repeat promoter.

Replication-deficient (\textit{ΔE1,E3}) adenoviral vector expressing murine \( \text{sst2} \) cDNA under the transcriptional control of the human CMV promoter was constructed with the AdEasy System (Quantum Biotechnologies; Ref. 18). Briefly, the 1.2-kb \textit{XhoI}-\textit{XhoI} fragment of \textit{mst2} cDNA was subcloned into \textit{XhoI}-digested shuttle vector \textit{pAdTrack-CMV}. This vector (Ad.CMV.sst2.GFP) also independently expressed enhanced GFP (Clontech). The adenoviral vector Ad.CMV.GFP expressing GFP alone was a gift from B. Coudere (INSEMe U563, Institut Claudius Regaud, Toulouse, France). Ad.CMV.sst2.GFP vector was produced by the Vector Core of the University Hospital of Nantes, France (Ph. Moullier and N. Ferry).

**Tumor Cell Lines**

The hamster pancreatic carcinoma PC1-0 cells [derived from pancreatic ductal carcinoma induced by N-nitrosobis(2-oxopropyl)amine in hamsters] were cultured in RPMI 1640 containing 5% FCS, fungizone, penicillin, streptomycin, and L-glutamine (Invitrogen, Cergy Pontoise, France) as described previously (17).

**Animal Models and Experimental Protocol**

Five-week-old male Syrian golden hamsters (weighing 70–80 g) were obtained from Harlan France (Gannat, France). The animals were acclimatized in a temperature-controlled room under a 12-h light/12-h dark schedule and received pelleted diet and water. Hamsters were anesthetized for all surgical procedures by an i.p. injection of pentobarbital (60 mg/kg, i.p.).
Imaging Transgene Expression in Allograft Tumors after Adenovirus-mediated Gene Transfer. The adenoviral recombinant vector independently encodes both mouse sst2 and enhanced GFP genes. Frozen Ad.CMV.sst2.GFP-transduced tumor tissues were cryosectioned and fixed in freshly prepared paraformaldehyde solution [2% in PBS (pH 7.4)] for 10 min at room temperature. The slides were rinsed three times with PBS and mounted under a fluorescent mounting medium (Fluoroprep; Dako Corp., Carpinteria, CA). GFP reporter gene expression was observed using a fluorescence microscope coupled to an image analyzer (Biocom, France).

RNA Isolation and RT-PCR Assays. Tumor tissue was homogenized mechanically in RNAlute, and total RNA was extracted by the modified procedure of Chomczynski and Sacchi as described previously (12). RNase-free Dnase I treatment and PCR were performed using specific sense and antisense primers for mouse sst2 receptor as described previously (12, 15).

Immunohistochemical Staining of Proliferative Cells

After tumor growth, the half of each tumors were harvested and fixed in formalin. Four-μm-thick sections were prepared from paraffin-embedded specimens. Immunohistochemical reactions were performed as described previously (16) using the horseradish peroxidase-conjugated system and anti-PCNA (mouse anti-PCNA, clone PC10; purchased from Dako Corp.). Immunoperoxidase immunostaining using the DAKO EnVision+ system (Dako Corp) was performed to augment the PCNA immunostaining intensity. The immunostaining was developed with the use of 3,3'-diaminobenzidine solution (Sigma) as chromogen. Slides were counterstained with Mayer’s hematoxylin. Negative control slides were prepared by replacing the primary antibody with PBS.

In Situ TUNEL Staining for the Detection of Apoptotic Cells

TUNEL staining was performed using in situ cell death detection kit apopDETekt (Enzo Diagnostics, Inc., Farmingdale, NY), according to the manufacturer’s instructions. Briefly, 6 days after gene transfer, primary tumors were removed and mechanically homogenized in lysis buffer. DNA was then isolated, and the resulting DNA ladder was visualized on a 1.5% agarose/ethidium bromide gel.

Assessment of DNA Fragmentation

DNA fragmentation was detected by gel electrophoresis using the Apoptotic DNA Ladder Kit (Roche) according to the manufacturer’s instructions. Briefly, 6 days after gene transfer, primary tumors were removed and mechanically homogenized in lysis buffer, DNA was then isolated, and the resulting DNA ladder was visualized on a 1.5% agarose/ethidium bromide gel.

Image Processing

The histological cuts after immunohistochemistry labeling (PCNA) or TUNEL reaction are visualized with an optical microscope coupled to a VisiLab2000 image analyzer (Biocom Paris, France). This system allows the quantification of proliferating cells or cells undergoing apoptosis. Measurements are made at high magnification (×40) on 15–20 fields representing at least 5000 cells/tumor for apoptosis and on 8–10 fields representing at least 1000 cells for proliferation index (16).

Immunoblotting Analysis of Caspase-3 Activation and Cleavage of PARP

Primary tumors were dissected mechanically in liquid nitrogen, and aggre-gates were solubilized in lysis buffer (50 mM HEPES, 150 mM NaCl, 10 mM EDTA, 10 mM Na3PO4, 100 mM NaF, and 2 mM sodium orthovanadate (pH 7.4)) containing 0.01% soybean trypsin inhibitor and 1 mM phenylmethylsulfonyl fluoride in the presence of 20 μg/ml aprotinin and 20 μg/ml leupeptin (Sigma, Saint-Quentin Fallavier, France). The mixture was gently agitated for 20 min at 4°C and centrifuged at 12,000 rpm for 10 min. For immunoblotting, 100 μg of soluble proteins were resolved through 7.5% or 12% SDS-polyacrylamide gels, transferred to a nitrocellulose membrane, and immunoblotted with goat polyclonal antihuman caspase-3 (sc-1224; Santa Cruz Biotechnology) or rabbit polyclonal anti-PARP (Roche) antibodies. Immunoreactive proteins were visualized by the enhanced chemiluminescence immunodetection system (Super Signal kit; Pierce) as described in Refs. 17 and 21.

Statistical Analysis

The results are expressed as mean ± SE. Paired or unpaired Student’s t test was used to compare data. P < 0.05 was considered significant.

RESULTS

In Vivo Gene Transfer Efficiency Using Adenoviral- or PEI-mediated Transfection in Pancreatic Primary Tumors

To examine the transduction efficiency of PEI- or adenovirus-mediated in vivo gene transfer into tumors, 107 pfu of Ad.RSV.LacZ adenosviruses or PEI:DNA pCMV.LacZ complexes were injected directly in primary pancreatic tumor mass. Tumors were removed from animals at days 1, 2, and 6 after gene transfer, and then we quantitatively evaluated LacZ expression in primary tumors by measuring the total β-galactosidase activity using a chemiluminescence assay. As shown in Fig. 1, using PEI at the optimal PEI:DNA ratio (20), β-galactosidase activity was maximal at day 1, and it was detectable up to day 6 in tumor extracts. Levels of β-galactosidase activity were higher in tumors of animals injected with adenoviruses as compared with tumors in animals injected with PEI (maximal at day 2); β-galactosidase levels were 5-fold higher and detected up to day 6 (P < 0.01). These quantitative results were confirmed by X-Gal staining: in animals that received an intratumoral injection of PEI-DNA pCMV.LacZ complexes both in primary and metastatic pancreatic cancer models, quantification revealed that about 2% of tumors cells were stained. In contrast, marked X-Gal staining-positive areas were observed in tumors that received an intratumoral injection of adenovirus Ad.RSV.LacZ, with 10–15% positive transduced tumor cells (data not shown).

Detection of Transgene Expression after in Vivo Adenovirus- and PEI-mediated Gene Transfer

We were able to easily identify tumor cells successfully infected by adenovector by detecting expression of enhanced GFP on primary...
Fig. 2. Detection of mouse sst2 transgene expression in primary and metastatic pancreatic adenocarcinoma models. A, light-based detection of reporter GFP after gene transfer with adenovector Ad.CMV.sst2.GFP. Primary tumors were removed from animals on day 6 and frozen in nitrogen. Light emission was achieved after excitation of the GFP protein with blue light on frozen tissue sections. The micrograph is representative of at least five different Ad.RSV.LacZ or Ad.CMV.sst2.GFP-transduced tumors (magnification, ×200). Left panel corresponds to Ad.RSV.LacZ-transduced tumor section, right panel corresponds to Ad.CMV.sst2.GFP-transduced tumor section, and GFP expression is reflected by green-colored cytoplasm. B, tumors were removed from animals on days 2, 6, and 5 in primary carcinoma and hepatic metastatic models, respectively. Total RNA was extracted, and RT-PCR analysis was performed from LacZ and sst2 tumors. The PCR products resulting from specific primers for mouse sst2 (expected length, 661 bp) were analyzed on polyacrylamide ethidium bromide-stained gel. Lane M, DNA size markers (pGEM markers; Promega). RT-PCR carried out in the absence of reverse transcriptase during reverse transcription procedure were negative. Results are representative at least of six different PEI-transduced tumors.

Antitumoral Effect of Adenovirus-mediated sst2 Gene Transfer in the Orthotopic Pancreatic Carcinoma Model

On day 9 after intrapancreatic inoculation of PC1-0 cells, adenoviruses carrying sst2 and/or GFP genes were administered intratumorally, and tumor volume was measured 6 days later (day 15 after tumor implantation). As shown in Fig. 3, TGP after Ad.CMV.sst2.GFP injection was slowed down as compared with those of control tumors injected with Ad.GFP or with vehicle ($P < 0.005$). Primary pancreatic tumor volume in hamsters treated with Ad.CMV.sst2.GFP ($529 \pm 98 \text{ mm}^3$) was significantly reduced as compared with those in hamsters treated with Ad.CMV-GFP ($972 \pm 123 \text{ mm}^3$) or in untreated animals ($1385 \pm 282 \text{ mm}^3$; data not shown; $P < 0.01$). In addition, we observed that 40% of tumors transferred with sst2 were stabilized without any tumor volume increase.

Antitumoral Effect of PEI-mediated sst2 Gene Transfer in Both Primary and Metastatic Pancreatic Carcinoma Models

To examine the usefulness of synthetic vector administration, the PEI-DNA complexes were also administered intratumorally to the hamsters at day 9 after intrapancreatic tumor cell inoculation, and animals were sacrificed 6 days later. For our newly created metastatic model, PEI-DNA complexes were administered intratumorally at 15 days after intraportal tumor cell inoculation, and treatments were sacrificed 5 days later. The volume of transplanted pancreatic tumors or hepatic metastasis was measured before and after 

In vivo sst2 gene transfer. No significant difference was
observed for initial tumor volume (before gene transfer) among untreated, control LacZ, and sst2 groups (Fig. 4, A and B). In contrast, as shown in Fig. 4A, mean primary pancreatic tumor volume was significantly reduced in hamsters treated with PEI-DNA sst2 as compared with that measured in hamsters treated with PEI-DNA LacZ or in nontreated animals. Intratumoral injection of the sst2 gene resulted in a significant inhibition of tumor progression as compared with tumors injected or not injected (untreated group) with the LacZ gene (Fig. 4A, inset). Fig. 4B represents the same results obtained in the pancreatic carcinoma hepatic metastasis model. We also found a significant inhibition of tumor volume and tumor progression (Fig. 4B, inset) at day 20 after cell implantation in the sst2 group as compared with the control LacZ group. Animals injected only with PEI solution did not show any differences in tumor progression compared with other control animals (data not shown).

Mechanisms Involved in the Antitumoral Effect of PEI-mediated sst2 Gene Transfer in Both Primary and Metastatic Pancreatic Carcinoma Models

Antiproliferative Effect of in Vivo sst2 Gene Transfer. PCNA has been recognized as an auxiliary protein for DNA polymerase δ and as an endogenous histological marker for the late G1-S phase of the cell cycle. We then analyzed the proliferative index on histological sections from pancreatic cancer allograft and tumor metastases using nuclear immunodetection of the PCNA antibody. As shown in Fig. 5, a significant decrease of immunodetected PCNA in cell cycling tumor cells (PCNA labeling index) was observed in tumors (primary or metastatic) injected with the sst2 gene compared with control tumors injected with the LacZ gene. Representative micrographs of PCNA labeling in pancreatic hepatic metastases are illustrated in Fig. 6, A and B.

Proapoptotic Effect of in Vivo sst2 Gene Transfer. To investigate whether the sst2 gene therapy-induced antitumorigenic effect results from an induction of apoptosis, we attempted to detect typical DNA ladders, a hallmark of apoptotic cells, on purified nucleic acids from primary tumor samples. DNA fragmentation was observed in both sst2- and control LacZ-expressing tumors (Fig. 7A). A quantification of apoptosis was then performed using TUNEL in situ labeling on histological sections from pancreatic cancer allograft and metastases. As shown in Fig. 7B, the percentage of apoptotic cells was significantly increased in both primary and metastatic tumors injected with the sst2 gene as compared with control tumors injected with the LacZ gene. Representative micrographs of TUNEL analysis in pancreatic primary tumor are shown in Fig. 6, C and D.

To characterize whether caspases, which are the major protease mediators of the classical apoptosis pathways, are involved in the sst2 gene transfer-induced proapoptotic effect, we measured the modulation of caspase-3 activity and the expression of PARP caspase target proteins of the DNA repair system by Western blot. Western blot analysis showed an activation of caspase-3 in 67% of sst2-expressing tumors (six of nine tumors were positive). The M₆, 32,000 precursor form of caspase-3 was more efficiently cleaved into the active M₄, 11,000 shorter fragment in sst2-versus control LacZ-expressing tumors (22%, two of nine tumors were positive), indicating that the effector proapoptase-3 is specifically activated in tumors that received the PEI-DNA sst2 complexes. Representative results are shown in Fig. 7C (top panel). Consistently, in the same tumor samples, the caspase-3 substrate PARP is also cleaved into shorter fragments in 75% of sst2 tumors versus 25% of LacZ tumors (Fig. 7C, bottom panel).

![Fig. 4. Effect of in vivo PEI-mediated sst2 gene transfer on growth of primary pancreatic cancer (A) and hepatic metastasis (B) established in hamsters. A, PCI-0 cells were inoculated orthotopically into the tail of the pancreas of hamsters (5 × 10⁴). Pancreatic tumor volumes were measured at day 9, injected or not injected with sst2 or LacZ gene, and measured again at day 15. Open bars, untreated animals (animals that did not receive any gene); hatched bars, animals receiving LacZ gene; black bars, animals receiving sst2 gene. The inset represents the percentage of tumor progression 6 days after in vivo PEI-mediated gene transfer. Values were from a total of 12 animals from three separate comparative experiments. *P < 0.05 (paired t test); **P < 0.02, difference between sst2 group and control animals (untreated or LacZ) at day 15. B, volume of one representative hepatic metastasis was measured at day 15 after PCI-0 cells intraportal injection. Gene transfer was then performed, and tumor volume was subsequently evaluated at day 20. Hatched bars, animals receiving LacZ gene; black bars, animals receiving mouse sst2 gene. Tumor volume is significantly increased in LacZ control group (hatched bars) between day 15 and day 20. #P < 0.01, but not in the sst2 group. **P < 0.02, difference between sst2 group (filled bars) and control group (hatched bars) at day 20. Inset, a significant inhibition of TGF was also observed at day 20 between the sst2 group and LacZ control group. **P < 0.02 (paired t test). Values were from 8–10 animals/group from three separate experiments.

![Fig. 5. Antiproliferative effect after in vivo sst2 gene transfer in pancreatic primary and metastatic tumors. Histological sections of tumors were analyzed for proliferation by PCNA immunostaining (PCNA labeling index). The percentage of labeled nuclei was measured at high magnification (>40) using the VisioLab 2000 analysis system on 8–10 successive high-power fields representing at least 1000 cells/tumor. Values are means ± SE (5 tumors/group were analyzed). Hatched bars, primary pancreatic tumors; dotted bars, hepatic metastasis (*, P < 0.0001; #, P < 0.0001, respectively).

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**DISCUSSION**

Transfer of molecules carrying antioncogenic properties could be a promising therapeutic strategy for exocrine pancreatic cancer. Gene therapy approaches for pancreatic cancer should be aimed at the treatment of not only primary tumors but also the proximal and distant metastases that often occur in this type of carcinoma. sst2, the expression of which is frequently lost in human pancreatic carcinomas, evoked an antioncogenic effect when reexpressed in vitro or in vivo in pancreatic exocrine cancer cells (15, 17). In the present study, we used transplantable models of primary and metastatic pancreatic carcinoma established in Syrian Golden hamsters to evaluate the therapeutic efficacy of in vivo sst2 gene transfer on pancreatic carcinoma. We demonstrated that the direct intratumoral sst2 gene transfer is responsible for a significant antitumor effect in both models of pancreatic carcinoma.

Despite a low transgene expression rate, we observed a significant inhibition of tumor growth in sst2-transduced tumors. A sst2-induced bystander effect might therefore be responsible for this therapeutic effect. A bystander effect has been described in gene-directed enzyme prodrug therapy. The mechanism is not fully characterized, but we can put forward some hypothesis for our gene therapy approach with the sst2 gene. The first explanation is that apoptosis can contribute to local bystander killing effect. Indeed, in ex vivo experiments conducted in nude mice xenografted with human pancreatic cancer cells, we observed that mixed tumors (containing only 25% sst2-expressing cells) displayed a significant inhibition of tumor growth as compared with control tumors (16). Interestingly, we detected a similar increase of apoptosis in both mixed tumors and tumors containing 100% of sst2-expressing cells (16). We therefore concluded that apoptosis can diffuse from the transduced cells to the untransduced neighboring cells. In the present work conducted on orthotopic primary pancreatic cancers, we also detected apoptosis in tumor injected with sst2 gene. A significant increase of the apoptotic index was measured by the TUNEL reaction, demonstrating an activation of caspase-3 and PARP pathways that are major protease mediators of the late phase of apoptosis. These pathways could be specifically activated in sst2-expressing cells as a result of recombinant sst2 receptor occupation by mature somatostatin. Indeed, sst2 gene transfection in the pancreatic cancer cell lines BxPC-3, Capan-1, and PC1-0 (which lack the sst2 receptor) has been shown to induce a sst2-activated negative autocrine loop, through the activation of endogenous somatostatin, which consequently activates sst2 (15, 17). Finally, bystander effect might result from an uptake by the neighboring untransduced tumor cells of apoptotic vesicles that contain hydrolases or enzymes activated by programmed cell death, including caspase-3 and PARP (22).

However, mechanisms other than apoptosis might be involved in sst2-mediated bystander killing. We observed that in vivo, sst2 gene transfer was accompanied by a significant decrease in the PCNA proliferative index. This is in agreement with our previous ex vivo experiments conducted in nude mice xenografted with mixed tumors. This inhibition could be explained in two ways; (a) the receptor sst2 is known to mediate G1-G2 cell cycle arrest in transduced cells and inhibits proliferation (21); and (b) the secreted somatostatin by sst2-expressing cells could also inhibit, via a paracrine or endocrine pathway, local angiogenesis and/or secretion of growth factors that positively control the untransduced cell proliferation. Moreover, as we described previously, locally produced somatostatin may also up-regulate the somatostatin receptor subtypes sst1 or sst5 in untransduced cells; these subtypes are also known to mediate the antiproliferative effect of the native peptides somatostatin 14 and 28 (11, 16).
In addition, we observed previously that both the antioncogenic effect and the amount of somatostatin autocrine secretion depend on the sst2 expression level (15). Indeed, as compared with PEI-sst2-treated animals, adenoviral vector-based transfer resulted in a higher expression of sst2 transgene (5-fold) and a more significant reduction of tumor growth. The expression level and/or number of sst2-expressing cells within the tumors transduced with sst2 adenoviral vector could be increased versus PEI-sst2-transduced tumors. This might be another argument to support that in vivo sst2 gene transfer specifically generated an antitumor effect.

PEI has been chosen because of its high efficiency for gene delivery in pancreatic cancer cells both in vitro (17) and in vivo (23). In our study, we performed gene transfer using a mode of delivery of DNA-PEI complexes adapted to intratumoral injection. Gene intratumoral delivery was realized by means of a micropump, allowing low delivery of complexes into tumor mass. Indeed, Coll et al. (20) analyzed the influence of the mode of intratumoral delivery using a micropump versus a normal, rapid injection using a syringe. In contrast to syringe-injected PEI complexes, micropump-injected PEI complexes produced a high and long-lasting expression of the transgene (20).

Using the LacZ reporter gene, we estimated that about 2% of tumor cells were transfected and expressed transgene for at least 6 days. Such experimental in vivo data were also observed in s.c. tumors of lung carcinoma, and the level of reporter gene was constant for 15 days (20). Furthermore, we were able to detect transgene expression by RT-PCR in both primary and metastatic tumors 5–6 days after injection. In a similar manner, Aoki et al. (23) detected the presence of injected DNA in various organs 7 days after the i.p. injection, and transgene expression decreased in all organs except for the brain 3 months later. In the present model, due to the fast growth of primary tumors and their metastases (17), we could not perform long-term experiments because control animals had to be sacrificed before day 20 after cell inoculation. However, in the nude xenograft pancreatic cancer model, we recently observed that in vivo PEI-sst2 gene transfer generated a significant and sustained antitumor effect over 12 days after intratumoral gene delivery.

Despite their low efficiency in comparison with viral vectors, cationic vectors retain high attractiveness in gene therapy because of their theoretical safety profile. PEI should fulfill both requirements for gene therapy in humans. (a) It is easy to prepare and handle, and it is an inexpensive in vivo transfection reagent. (b) It is an inert and innocuous compound with no or low toxicity; no mortality was observed in injected animals, and no necrosis was observed at the site of injection (24, 25). (c) It raises none of the concerns of viral vectors for human gene therapy and is not associated with the possibility of generating a replication-competent virus, and there is no immune reaction such as those experienced for adenovirus vector. (d) Multiple injections will be less problematic than virus-based gene transfer because repeated intrapancreatic adenovirus injection increases cytotoxic, lymphoproliferative, and cytokine release activity (26). (e) Finally, contrary to cationic lipids, PEI may better protect DNA
because of its best stability in pancreatic environment, known to be rich in enzymes such as cholesterol esterase that could digest other nonviral lipidic vectors.

In conclusion, PEI-formulated DNA can efficiently and stably transfect tumor pancreatic cells in vivo. Because of the efficient expression and absence of toxicity, the intratumoral injection of sst2-PEI complexes is expected to play an important role in future treatment of both primary and metastatic pancreatic tumors.

In summary, we have demonstrated that transfer of sst2 into both primary and metastatic pancreatic cancer models resulted in a significant inhibition of tumor growth in vivo. The antioncogenic effect that results from sst2 reexpression is accompanied by a strong bystander effect on untransduced cells, which undergo apoptotic cell death and inhibition of cell proliferation. sst2 gene therapy as a new strategy for pancreatic cancer requires long-term and stable expression of the sst2 receptor to obtain a durable antitumor effect. Repeated in vivo intra-tumor transfer of sst2 gene using cationic vector should be performed because of the absence of toxicity.

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