Targeted Virus Replication Plus Immunotherapy Eradicates Primary and Distant Pancreatic Tumors in Nude Mice

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Abstract

Pancreatic cancer is an aggressive neoplasm with no current viable, effective treatment options. In the majority of cases, at first diagnosis, pancreatic cancer has already become metastatic so that conventional treatment regimens provide minimal, if any, clinical benefit in prolonging life or ameliorating the negative prognosis of this disease. These harsh realities underscore the need for developing improved treatment paradigms for this cancer, with gene therapy and immunotherapy currently being evaluated as potential therapeutic options. We currently describe an adenovirus-based therapy for successfully managing pancreatic cancer, the cancer terminator virus (CTV), which is founded on targeted induction of viral replication from a cancer-specific promoter (PEG-3) and immune modulation by IFN-γ. The PEG-Prom functions selectively in cancer cells of diverse lineages compared with their normal cellular counterparts. In the CTV, the PEG-Prom drives expression of the adenoviral early region 1A (E1A) gene, necessary for virus replication, with IFN-γ simultaneously being expressed from the E3 region. Infection of normal cells and pancreatic cancer cells with the CTV confirmed cancer cell–selective adenoviral replication, robust IFN-γ production coupled with virus replication, growth inhibition, and apoptosis induction. Infection of established pancreatic tumors in nude mice with the CTV promoted viral replication, IFN-γ production, and activation of antitumor immunity resulting in complete eradication of both primary and distant tumors, curing animals of disease. The CTV provides a novel reagent for treating pancreatic and other human cancers with potential for eliminating both primary tumors and metastatic disease. (Cancer Res 2005; 65(19): 9056-63)

Introduction

Pancreatic cancer is the fourth leading cause of cancer deaths and it is estimated that >30,000 new cases will be diagnosed in the United States in 2005 and essentially all of these patients will die (1). The prognosis of pancreatic cancer is extremely poor, owing to the lack of effective markers for early diagnosis, inability to achieve complete surgical resection, and lack of effective systemic therapy for metastatic disease (2). Less than 10% of patients present with locally resectable tumors and nearly 50% of patients already have metastatic disease. The overall 5-year survival for all stages of pancreatic cancer is ≤4%, with median survival of <20 months (3). Considering its incidence and almost universal fatality, enhanced research efforts are mandated to comprehend, prevent, and effectively treat this devastating disease.

Although studied intensively, crucial molecular determinants of pancreatic cancer and effective therapies remain elusive (4–6). Pancreatic cancer is a complex disorder in which multiple subsets of genes undergo genetic change, either activation or inactivation, during tumor development and progression (3, 6, 7). Common genetic modifications in pancreatic carcinomas include activation of the K-ras oncogene (85–95%); overexpression of specific growth factors and their associated receptors; and inactivation of the p16/RBI (>90%), p53 (75%), DPC4 (55%), and BRCA2 tumor suppressor genes (3, 6, 7). These findings accentuate the complexity of this heterogeneous cancer and may underlie the aggressiveness and inherent resistance of this neoplasm to conventional therapies, including chemotherapy and radiation (2, 8–10).

Gene therapy and immunotherapy are currently being evaluated to treat pancreatic cancer (11, 12). One approach being tested clinically uses ONYX-015, a replication-competent adenovirus that propagates preferentially in p53 mutant cells (13). A similar replication-competent adenovirus, ONX-411, exploits defects in the Rb pathway, a frequently altered phenotype in tumor cells (14). The rationale for using such adenoviruses is that because of intact p53 or Rb pathways, these adenoviruses will not replicate in normal cells while selectively reproducing and inducing cytolysis in tumor cells containing intrinsic defects in p53 or Rb pathways. A problem confounding this approach relates to the multitude of changes occurring in primary tumors that are frequently exacerbated during tumor progression, thereby limiting the effectiveness of these types of viruses. To overcome this barrier to effective therapy, we created a conditionally replication-competent adenovirus (CRCA) using a cancer-specific promoter (15), from rat progression elevated gene-3 (PEG-3; refs. 16, 17), that replicates selectively in both rodent and human cancer cells irrespective of tumor suppressor status. The mechanism underlying this cancer specificity involves defined transcription factor binding sequences in the PEG-3 promoter (PEG-Prom), activator protein-1 (AP-1), and polyoma enhancer activator protein-3 (PEA-3), expressed at elevated levels in the vast majority of cancer cells (15, 18, 19). Nonreplicating adenoviruses containing the PEG-Prom specifically targeted expression of reporter genes (luciferase and green fluorescence protein) and tumor suppressor apoptosis-inducing genes (wild-type p53 and mda-7/IL-24) in a spectrum of human and rodent tumor cells, with minimal expression in normal cellular counterparts (15). Based on these considerations and to improve
therapeutic efficacy, we constructed a bipartite CRCA in which the expression of early region 1A (E1A) and E1B genes of adenovirus, necessary for replication, is regulated by the PEG-Prom with simultaneous IFN-γ production from the E3 region (Ad.PEG-E1A-IFN-γ), the cancer terminator virus (CTV). We hypothesized that this virus should elicit a profound antitumor effect not only in primary tumors but also in distant metastases by provoking direct cancer-specific killing by cytolysis combined with an augmented anticancer immunologic effect elicited by IFN-γ (20).

Materials and Methods

Cell lines, culture conditions, and viability assays. MIA Paca-2, Panc-1, AsPC-1, and BxPC-3 pancreatic cancer cells were obtained from American Type Culture Collection (ATCC, Manassas, VA) and cultured in RPMI 1640 and FM-516-SV (FM-516), IM-PHFA, and human embryonic kidney-293 cells were cultured in DMEM supplemented with 10% fetal bovine serum, 50 units/mL penicillin G, and 50 μg/mL streptomycin at 37°C in 5% CO2 and 100% relative humidity. All cells were routinely screened for Mycoplasma infection. For viability assays, 1.5 × 106 cells were plated in each well of a 96-well plate and next day were either uninfected or infected with different adenovirus at a multiplicity of infection (MOI) of 100 plaque-forming unit (pfu)/cell. Cell viability was determined by standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay 2, 4, and 6 days after infection.

Construction of conditionally replication-competent adenovirus. To construct the CTV (Ad.PEG-E1A-IFN-γ) the AdenoQuick cloning system from OD 260, Inc. (Boise, ID) was used. This system utilizes two shuttle vectors (pE1.2 and pE3.1) in which the transgenes must be inserted before being transferred into a large adenoviral plasmid (Fig. 1). The expression cassette in which the PEG-Prom drives E1A of adenovirus was inserted into the multiple cloning site of pE1.2. The other expression cassette, in which cytomegalo virus (CMV) promoter drives the expression of IFN-γ, was inserted into the multiple cloning site of pE3.1. In both shuttle plasmids, the multiple cloning sites are flanked by two sets of restriction sites. Within one set, the sticky ends generated by these enzymes are identical to each other, but are incompatible with those generated in the other set (GAG versus AGA in pE1.2; CCA versus ATG in pE3.1). The adenoviral plasmid has two pairs of SfiI sites, one in the E1 region the other in the E3 region. The SfiI sites at the E1 region generate sticky ends that are incompatible with each other but are compatible with those generated by digesting pE1.2 with AlwNI, BstAPI, DraIII, or PfiMI. The SfiI sites at the E3 region generate sticky ends that are incompatible with each other and with those present in the E1 region but are compatible with those generated by digesting plasmid pE3.1 with AlwNI, BstAPI, DraIII, or PfiMI. Upon ligation of the expression cassettes to the respective plasmid, the cassettes were released by digestion and were ligated to SfiI-digested adenoviral plasmid in a four-fragment ligation. The ligation product was transformed into Escherichia coli and clones were selected for resistance to ampicillin (ampicillin resistance gene provided by adenoviral plasmid) and kanamycin (kanamycin resistance gene provided by the fragment from the shuttle vector). The cosmid DNA was amplified by standard large-scale preparation using a CaCl2 gradient, digested with PacI restriction enzyme, and transfected into human embryonic kidney-293 cells for in vivo recombination. The adenovirus was purified by BD AdenoX Virus Purification kit (BD Biosciences, Palo Alto, CA) and viral titer was determined by measuring absorbance at 260 nm and using BD AdenoX rapid titer kit (BD Biosciences). Similarly strategies were used to generate AdCMV-E1A-IFN-γ, AdCMV-IFN-γ and AdPEG-IFN-γ were constructed by the Massey Cancer Center Virus Vector Shared Resource. Adenovirus infection was done 24 hours after cell plating in one fifth the volume of the original culture medium in a serum-free condition for 2 hours with rocking the plates several times.

Annexin V binding assay. Cells were trypsinized, washed twice with PBS, and stained with Annexin V-APC (BD Biosciences) according to the protocol of the manufacturer. Flow cytometry was done immediately after staining using a FACS Calibur flow cytometer and data was analyzed using CellQuest software (Becton Dickinson, San Jose, CA).

Preparation of whole cell lysates and Western blot analysis. Cells were harvested in radioimmunoprecipitation assay buffer (1× PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS) containing protease inhibitor cocktail (Roche, Mannheim, Germany), 1 mmol/L Na3VO4, and 50 mmol/L NaF and centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatant was used as total cell lysate. Thirty micrograms of total cell lysate were used for SDS-PAGE and transferred to a nitrocellulose membrane. The primary antibodies used were anti-E1A (1:1,000; mouse monoclonal; Upstate Biotechnology, Waltham, MA), anti-c-jun (1:1,000; rabbit polyclonal; Santa Cruz Biotechnology, Santa Cruz, CA), anti-PEA-3 (1:500; mouse monoclonal; Santa Cruz), and anti-EF1α (1:1,000; mouse monoclonal; Upstate).

Human IFN-γ ELISA. Human IFN-γ either in the culture supernatant or in mouse sera was analyzed by ELISA using human IFN-γ ELISA kit (Pierce, Rockford, IL) according to the instructions of the manufacturer.

Human pancreatic cancer xenograft in athymic nude mice. AsPC-1 cells (2 × 106) were injected s.c. in 100 μL of PBS in both flanks of athymic nude mice (NCRnu/nu; 4 weeks old; 20 g body weight). The establishment of visible tumors of 75 mm3, requiring 4 to 5 days, intratumoral injections of different adenovirus were given only to the tumors on the left flank at a dose of 1 × 109 pfu in 100 μL. No injection was given to the right-sided tumors. The injections were given thrice a week for the first week and then twice a week for 2 more weeks to a total of seven injections or only three injections during the first week. At least five animals were used per experimental point. Tumor volume was measured twice weekly with a caliper and calculated using the formula π/6 × larger diameter × (smaller diameter)2. At the end of the experiment, the animals were sacrificed and the tumors were removed, snap frozen in liquid nitrogen, and weighed. Additionally, blood samples were also collected from the mice for ELISA assays.

RNA extraction and reverse transcription-PCR. Total RNA was extracted from the tumor tissues using Qiagen RNAeasy mini kit (Qiagen, Hilden, Germany) according to the protocol of the manufacturer. Two micrograms of total RNA were used for reverse transcription-PCR (RT-PCR) according to standard methods. The primers used were as follows: E1A sense: 5′-CCCCAGTGACGACGAGGATGAA-3′ and antisense: 5′-CCACC-CAACCTCTCAGGGCAACT-3′; EF1-α sense: 5′-GCCCGAGGAACACAGA- GACCTT-3′ and antisense: 5′-CTTACGGTTGACTTTCATCC-3′.
In vitro cytotoxicity assay. AsPC-1 cells or IM-PHFA target cells were incubated at 37°C in 5% CO₂ for 2 hours with 500 μCi Na²⁵CrO₄ (Amersham, Arlington Heights, IL) and then washed thrice in HBSS. Effector cells were obtained by isolating spleen cells from the mice. Labeled target cells (10⁴) were cultured with effector cells at a target-to-effector ratio of 1:12.5, 1:25, 1:50, and 1:100 in a total volume of 200 μL in a 96-well plate at 37°C in 5% CO₂ for 4 hours. Scintillation counter. Specific ⁵¹Cr release was determined as [(experimental release/spontaneous release) / (maximal release/spontaneous release)] × 100. Maximum release and spontaneous release were determined from well containing 100 μL of 2% Triton X-100 and medium alone, respectively, in the place of the effector cells.

Transient transfection and luciferase assays. The minimum region of PEG-Prom (−118 to +194) was cloned into pGL3-basic Vector (Promega) to generate pPEG-luc. Mutant pPEG-luc constructs containing mutation either in PEA-3 or in AP-1 or in both sequences were generated using the Altered Sites II In vitro Mutagenesis System (Promega, Madison, WI). Transient transfection was done using LipofectAMINE 2000 (Invitrogen) transfection reagent according to the instructions of the manufacturer. Luciferase and β-galactosidase assays were done using commercial kits (Promega and Tropix, respectively) 48 hours after transfection.

Statistical analysis. Statistical analysis was done using one-way ANOVA, followed by Fisher’s protected least significant difference analysis. P < 0.05 was considered as significant.

Results

to test our hypothesis of cancer-specific activity and therapeutic effectiveness of the CTV (Ad.PEG-E1A-IFN-γ), we created a series of additional adenoviruses, including Ad.CMV-E1A-IFN-γ, in which viral replication is controlled by the CMV promoter and which also expresses human IFN-γ, Ad.CMV-E1A, and Ad.PEG-E1A, in which viral replication is controlled by the CMV promoter or the PEG-Prom, respectively, and Ad.CMV-IFN-γ and Ad.PEG-IFN-γ, replication-incompetent adenovirus in which IFN-γ expression is driven by the CMV promoter or the PEG-Prom, respectively. A replication-incompetent empty adenovirus, Ad.vec, was used as control. The strategy used to create Ad.PEG-E1A-IFN-γ is depicted in Fig. 1. Experiments were done in four pancreatic cancer cell lines, Mia Paca-2, PanC-1, AsPC-1, and BxPC-3, and two normal immortal cell lines, FM-516-SV, normal human melanocytes immortalized by SV40 TAg, and IM-PHFA, primary human fetal astrocytes immortalized by the catalytic subunit of human telomerase. The functionality of these constructs was ascertained following adenovirus infection by monitoring IFN-γ production by ELISA and E1A protein levels, a marker for adenoviral replication, by Western blot analysis after appropriate viral infection. The latter assay detects multiple E1A gene products ranging from 36 to 50 kDa (Fig. 2).

Infection of FM-516-SV or IM-PHFA cells with Ad.CMV-E1A or Ad.CMV-E1A-IFN-γ, but not Ad.PEG-E1A or Ad.PEG-E1A-IFN-γ (CTV), resulted in the production of E1A proteins, whereas in pancreatic carcinoma cells infection with all four replication-competent adenovirus generated E1A proteins (Fig. 2A). No E1A proteins were detected in any cell line following infection with replication-incompetent adenoviruses. In FM-516-SV and IM-PHFA cells, infection with Ad.CMV-E1A-IFN-γ (CTV) resulted in a 4- to 5-fold more IFN-γ production when compared with infection with Ad.CMV-IFN-γ, whereas infection with Ad.PEG-IFN-γ or Ad.PEG-E1A-IFN-γ (CTV) resulted in very low level of IFN-γ production (Fig. 2B). In pancreatic cancer cells, infection with Ad.CMV-IFN-γ and Ad.PEG-IFN-γ generated significant IFN-γ production, whereas Ad.CMV-E1A generated significant E1A production.

Figure 2. PEG-Prom promotes adenovirus replication and transgene expression selectively in pancreatic cancer cells. The indicated cells were uninfected (control) or infected with the indicated adenovirus (as described at the bottom of the figure) at a MOI of 100 pfu/cell for 48 hours. A, the expressions of E1A and EF-1α were analyzed by Western blot analysis. B, IFN-γ in culture supernatants was determined by ELISA. Columns, mean of two independent experiments each done in triplicates; bars, SD.
However, infection with Ad.CMV-E1A-IFN-γ or Ad.PEG-E1A-IFN-γ (CTV) resulted in an 10 to 20-fold increase in IFN-γ production when compared with Ad.CMV-IFN-γ or Ad.PEG-IFN-γ infection. No IFN-γ production could be detected in control uninfected cells or following infection with Ad.vec, Ad.CMV-E1A, or Ad.PEG-E1A. These findings document that the PEG-Prom facilitates cancer cell–selective replication of adenovirus and transgene expression. Of added significance, when transgene expression is coupled with replication, IFN-γ expression is greatly amplified.

Studies were done to establish potential selective effects on growth and viability of normal and pancreatic tumor cells when replication was controlled by the PEG-Prom versus the CMV promoter. In FM-516-SV and IM-PHFA cells, infection with only Ad.CMV-E1A and Ad.CMV-E1A-IFN-γ (CTV) resulted in an ~10 to 20-fold increase in IFN-γ production when compared with Ad.CMV-IFN-γ or Ad.PEG-IFN-γ infection. No IFN-γ production could be detected in control uninfected cells or following infection with Ad.vec, Ad.CMV-E1A, or Ad.PEG-E1A. These findings document that the PEG-Prom facilitates cancer cell–selective replication of adenovirus and transgene expression. Of added significance, when transgene expression is coupled with replication, IFN-γ expression is greatly amplified.

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To investigate the mechanism of growth inhibition, Annexin V staining assays, which permit differentiation between apoptotic and necrotic cells, were done (Fig. 4). Infection with only Ad.CMV-E1A and Ad.CMV-E1A-IFN-γ elevated the percentage of early apoptotic and late apoptotic (necrotic) FM-516-SV and IM-PHFA cells. However, all of the adenoviruses, except for Ad.vec, resulted in significant apoptosis in the pancreatic cancer cell lines. Infection with the replication-competent adenoviruses resulted in predominantly necrosis as evidenced by an increase in late apoptotic cells, whereas infection with Ad.CMV-IFN-γ and Ad.PEG-IFN-γ resulted predominantly in apoptosis as evidenced by an increase in early apoptotic cells.

To expand on the in vitro studies, in vivo assays were done using nude mice containing established AsPC-1 s.c. xenografts on both right and left flanks. After palpable tumors of ~75 mm³ developed, in ~4 to 5 days, seven intratumoral injections with different adenoviruses, thrice per week for the first week, and twice per week for an additional 2 weeks, were administered to the tumors on the left flank at a dose of 1 × 10⁸ pfu in 100 μL. No injections were given to the right-sided tumors. The experiment was terminated after 4 weeks because with injections of Ad.CMV-E1A-IFN-γ or production. However, infection with Ad.CMV-E1A-IFN-γ or Ad.PEG-E1A-IFN-γ (CTV) resulted in an ~10 to 20-fold increase in IFN-γ production when compared with Ad.CMV-IFN-γ or Ad.PEG-IFN-γ infection. No IFN-γ production could be detected in control uninfected cells or following infection with Ad.vec, Ad.CMV-E1A, or Ad.PEG-E1A. These findings document that the PEG-Prom facilitates cancer cell–selective replication of adenovirus and transgene expression. Of added significance, when transgene expression is coupled with replication, IFN-γ expression is greatly amplified.

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Ad.PEG-E1A-IFN-γ (CTV) tumors on both sides showed regression only after three injections and with seven injections they were completely eradicated (Fig. 5). Whereas Ad.CMV-E1A or Ad.PEG-E1A inhibited the growth of tumors on the left flank, they had some inhibitory effect on the tumors on the right side, which was not statistically significant. Ad.CMV-IFN-γ or Ad.PEG-IFN-γ significantly inhibited tumor growth on both flanks, emphasizing the importance of immune stimulation, but these treatment regimens did not completely destroy the tumors. The observation that intratumoral injection of Ad.PEG-E1A-IFN-γ (CTV) completely eradicated the primary tumor and dramatically inhibited or eliminated (four of five animals) the distant tumor (comparable with a metastasis) provides confidence that this strategy may prove amenable for successfully treating aggressive cancers.

The question naturally arises as to how Ad.PEG-E1A-IFN-γ (CTV) destroys tumors on the right flank when the therapeutic adenovirus was injected only on the left flank. A possible explanation could be that this adenovirus enters the circulation and replicates in the tumors of the right side and/or the secreted IFN-γ induces a potent antitumor immune response. To address this question, tumor xenografts from both left and right flanks were collected from mice after only three injections with the different adenoviruses and the expressions of E1A was analyzed by RT-PCR in these samples. Of relevance, E1A expression could be detected in tumors on both left and right flanks when injected with the replication-competent, but not with the replication-incompetent adenoviruses (Fig. 6A). These findings confirm that the replication-competent adenoviruses have the ability to migrate and replicate at distant sites in the animals. In this context, the ability of the PEG-Prom in Ad.PEG-E1A-IFN-γ (CTV) to drive replication is extremely important for ensuring adenovirus replication in cancer cells while sparing harmful effects in normal cells.

ELISA quantified the levels of human IFN-γ in the circulation of mice after three adenovirus injections, confirming expression only after injection with IFN-γ–expressing adenoviruses (Fig. 6B). The level of IFN-γ produced by Ad.CMV-E1A-IFN-γ or Ad.PEG-E1A-IFN-γ (CTV) was approximately three to four times more than that produced by Ad.CMV-IFN-γ or Ad.PEG-IFN-γ, indicating that even in in vivo contexts, the replication-competent adenoviruses produce significantly higher levels of IFN-γ compared with replication-incompetent adenoviruses.

The activation of immunocompetent cells, such as natural killer (NK) cells, in athymic nude mice was confirmed by the capacity of...
spleen cells, isolated from mice injected with different adenoviruses, to lyse AsPC-1 cells in an in vitro $^{51}$Cr release assay. Spleen cells from control mice or mice injected with Ad.vec, Ad.CMV-E1A, or Ad.PEG-E1A induced little cytolysis of AsPC-1 cells, whereas those isolated from Ad.CMV-IFN-γ, Ad.PEG-IFN-γ, Ad.CMV-E1A-IFN-γ, or Ad.PEG-E1A-IFN-γ (CTV) injected mice exerted significant cytolysis of AsPC-1 cells that increased with increasing target-to-effector cell ratio (Fig. 6C). Conversely, IM-PHFA cells were resistant to lysis by spleen cells isolated from any of the treated mice (Fig. 6D), indicating that a specific antitumor effect was elicited toward AsPC-1 cells, but not toward normal IM-PHFA cells.

In the minimum effective element of PEG-Prom (−118 to +194), there are two important transcription factor–binding sites, one for PEA-3 at −104 and another at +8 for AP-1 (Fig. 7A). These two transcription factors play an essential role in regulating PEG-Prom activity in prostate and breast cancer cell lines and in rodent cell system (15, 18, 19). Based on this consideration, the potential involvement of PEA-3 and AP-1 in regulating PEG-Prom activity was evaluated in pancreatic cancer cell lines. PEG-Prom activity was ~5- to 8-fold higher in pancreatic cancer cells than that in FM-516 or IM-PHFA cells (Fig. 7A). Mutation in PEA-3 site reduced the promoter activity by ~50% in all cell types except FM-516 cells in which the effect was marginal. Mutation in AP-1 site reduced the promoter activity by ~50%, whereas mutation in both PEA-3 and AP-1 sites reduced the activity by ~75% in all the cell lines. To further strengthen the role of PEA-3 and AP-1 in regulating PEG-Prom activity, the relative abundance of these proteins was analyzed in pancreatic cancer cell lines and FM-516 and IM-PHFA cells (Fig. 7B). Markedly high PEA-3 expression was detected in MIA Paca-2, PANC-1, and AsPC-1 cells, whereas in BxPC-3 cells PEA-3 expression was moderate. PEA-3 expression was undetected in FM-516 cells whereas it was very low in IM-PHFA cells. c-JUN expression was high in MIA Paca-2 and PANC-1 cells, moderate in AsPC-1 and BxPC-3 cells, and low in FM-516 and IM-PHFA cells. These expression levels of the PEA-3 and c-JUN correlate with the PEG-Prom activity in different cell lines and explain the cancer cell–selective activity of the PEG-Prom in pancreatic adenocarcinomas.

**Discussion**

We presently describe a novel therapeutic virus that produces complete remission of primary and distant pancreatic tumors in vivo in animals. The replication-competent adenoviruses have the ability to migrate to distant sites and replicate, suggesting utility in treating both primary and distant tumors. However, the replication of adenovirus must be controlled in a strict cancer-specific manner, preventing replication in normal cells when migrating from the primary site of injection to distant anatomic sites. Administration of replication-competent adenovirus alone induces significant growth inhibition of primary tumors but not distant tumors, indicating that at those new sites replication of adenovirus may not be sufficiently robust to be bioactive. As such, an additional antitumor strategy must be combined with replication-competent adenovirus to provide therapeutic benefit. An oncolytic adenovirus expressing human IFN consensus gene has been evaluated for human breast cancer therapy providing benefit (21). Immunotherapy is being evaluated as a therapeutic option for pancreatic cancer and administration of cytokines, such as IFN-α, IL-2, IL-15, or IL-12, elicit significant growth inhibitory effects on pancreatic cancer cells, both in animal models and in clinical trials (12). IFN-γ has not been evaluated stringently as a therapeutic
cancer patients is well tolerated and it can be administered directly to the primary tumor under the guidance of endoscopic ultrasound (13, 23). Moreover, whereas antiadenovirus neutralizing antibodies significantly attenuate the activity of a replication-incompetent adenovirus, they have no apparent effect on the activity of replication-competent adenovirus, indicating that patients with preexisting adenovirus immunity would still be promising candidates for i.v. administration of oncolytic adenovirus (24). These observations suggest that the CTV could be safely administered to patients with pancreatic cancer thereby providing an effective therapy for a currently incurable malignant disease. The mechanism by which human IFN-γ stimulates an antitumor immune response in nude mice is not clear. There could be some level of cross-reactivity of human IFN-γ with mouse IFN-γ receptors or human IFN-γ might stimulate the release of immunostimulatory factor(s) from cancer (or normal mouse) cells that bind to cognate mouse receptors to stimulate an immune response. We are currently evaluating the CTV in severe combined immunodeficient mice humanized with human peripheral blood lymphocytes and in immunocompetent mouse models of pancreatic cancer (25) to address these relevant issues.

Tissue-specific and cancer cell–selective promoters can facilitate conditional adenovirus replication (26). The human telomerase promoter has been used successfully for this purpose (27, 28). Additionally, the insulin promoter, driven by PDX-1, has been used for delivery of transgenes in a pancreatic cancer cell–specific manner (29). The advantage of using the PEG-Prom in these cancer contexts is its apparent ubiquitous cancer specificity. PEA-3 and AP-1 transactivation and subsequently PEG-Prom activity are positively regulated by the Ras-dependent signaling cascade (30–32). Because activation of the Ras pathway is a frequent event in diverse cancers, including pancreatic and colorectal cancers (33), the efficacy of the PEG-Prom to drive transgene expression in these cancers will be vigorous and specific. Additionally, PEA-3 and AP-1 are frequently overexpressed in diverse cancers, thereby conferring ubiquitous cancer-specific activity of the PEG-Prom, suggesting that the CTV will also provide benefit to patients with additional types of histologically distinct cancers. Further studies are essential in established animal models of pancreatic and other cancers that mimic the human disease followed by clinical trials to effectively translate this approach into a mainstream, viable therapy for primary and metastatic pancreatic and other cancers.

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References

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