Eradication of Therapy-Resistant Human Prostate Tumors Using a Cancer Terminator Virus

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Abstract

Terminal prostate cancer is refractory to conventional anticancer treatments because of frequent overexpression of antiapoptotic proteins Bcl-2 and/or Bcl-xL. Adenovirus-mediated delivery of melanoma differentiation associated gene-7/interleukin-24 (mda-7/IL-24), a secreted cytokine having cancer-selective apoptosis-inducing properties, profoundly inhibits prostate cancer cell growth. However, forced overexpression of Bcl-2 or Bcl-xL renders prostate cancer cells resistant to Ad.mda-7. We constructed a conditionally replication-competent adenovirus in which expression of the adenoviral E1A gene, necessary for replication, is driven by the cancer-specific promoter of progression elevated gene-3 (PEG-3) and which simultaneously expresses mda-7/IL-24 in the E3 region of the adenovirus (Ad.PEG-E1A-mda-7), a cancer terminator virus (CTV). This CTV generates large quantities of MDA-7/IL-24 as a function of adenovirus replication uniquely in cancer cells. Infection of Ad.PEG-E1A-mda-7 (CTV) in normal prostate epithelial cells and parental and Bcl-2- or Bcl-xL-overexpressing prostate cancer cells confirmed cancer cell-selective adenoviral replication, mda-7/IL-24 expression, growth inhibition, and apoptosis induction. Injecting Ad.PEG-E1A-mda-7 (CTV) into xenographs derived from DU-145-Bcl-xL cells in athymic nude mice completely eradicated not only primary tumors but also distant tumors (established in the opposite flank), thereby implementing a cure. These provocative findings advocate potential therapeutic applications of this novel virus for advanced prostate cancer patients with metastatic disease. [Cancer Res 2007;67(11):5434–42]

Introduction

Prostate cancer represents one of the most important health problems in industrialized countries (1, 2). It is the most common cancer and the second leading cause of cancer-related deaths in men in the United States. In 2006, the estimated new prostate cancer cases were 234,460, of which 27,350 men were estimated to die predominantly from metastatic prostate cancer in the United States. Therapeutic options vary according to the stage of the disease at the time of presentation and diagnosis. Patients with localized disease may be treated with surgery or radiation, whereas the treatment for patients with metastatic disease is purely palliative (1, 2). Hormonal treatment with antiandrogens is the standard therapy for stage IV prostate cancer, but patients ultimately become nonresponsive to androgen ablation (1, 2). Current therapy options for patients with hormone-refractory prostate cancer include radiotherapy and cytokotoxic chemotherapeutic agents, such as mitoxantrone, estramustine, and taxanes (1–3). Despite a palliative benefit, none of these approaches engender a beneficial effect on the overall survival of patients. Consequently, no consistently effective therapy exists for these patients mandating the development of novel, more efficacious, and innovative treatment approaches, especially those targeting metastasis.

Overexpression of antiapoptotic proteins Bcl-2 and Bcl-xL is a frequent occurrence in prostate cancer development and progression (4–6). Bcl-2 immunostaining increases with Gleason grade of prostate cancer and Bcl-2 expression is a predictor of poor prognosis in prostate cancer patients (7–10). Moreover, Bcl-2 expression is augmented following androgen ablation and correlates with progression of prostate carcinomas from androgen dependence to androgen independence (11–13). Bcl-xL overexpression associates with resistance to drug-induced apoptosis in prostate cancer cells (14–16). Forced overexpression of Bcl-2 has been shown to desensitize prostate cancer cells to apoptotic stimuli (17, 18). Accordingly, strategies that overcome apoptosis resistance afforded by either Bcl-2 or Bcl-xL overexpression would clearly have important therapeutic implications in treating patients with prostate cancer.

Melanoma differentiation associated gene-7/interleukin-24 (mda-7/IL-24) was originally identified as a gene associated with terminal differentiation and irreversible growth suppression of metastatic human melanoma cells (19, 20). mda-7 belongs to the IL-10 family of cytokines that include IL-19, IL-20, IL-22, mda-7/IL-24, and IL-26 (21, 22). A unique property of mda-7/IL-24, when delivered by an adenoviral expression system (Ad.mda-7), is selective induction of growth suppression and apoptosis in a broad spectrum of human cancers, including prostate carcinoma, without exerting any deleterious effects to their normal counterparts (reviewed in refs. 23–26). In addition to its direct apoptosis-inducing properties, Ad.mda-7 also shows antiangiogenic, radiosensitizing, immunostimulatory, and potent “bystander” antitumor activity (27–32). A phase I clinical trial evaluating Ad.mda-7 (INGN 241) activity by intratumoral injection in patients with advanced solid tumors was done, and the results indicate that mda-7/IL-24 is safe and could induce as much as 70% apoptosis in tumors following a single injection of recombinant virus, and multiple
injections promote an objective clinical response (23, 33, 34). These exciting results provide direct support for using mda-7/IL-24 in potentially developing an effective gene-based therapy for cancer.

In multiple cancer subtypes, Ad.mda-7 infection reduces the levels of antiapoptotic proteins, including Bcl-2 and/or Bcl-xL, and enhances expression of proapoptotic proteins, including Bax and/or Bak, thus shifting the balance towards an apoptotic phenotype and tumor cell death (24–26, 35). Although parental prostate cancer cells are highly susceptible to Ad.mda-7-induced apoptosis, stable overexpression of Bcl-2 and Bcl-xL renders prostate cancer cells resistant to the apoptotic effects of Ad.mda-7 (36). A differential protective effect of Bcl-2 and Bcl-xL is evident in human prostate cancer cells. Although Bcl-2 is primarily not only in rodent but also in human cancer cells of diverse origin when compared with normal cells (39–41). The cancer cell specificity of the PEG-Prom is governed by two transcription factors (activator protein-1 and polyoma enhancing activator-3) that are expressed at elevated levels, each singly or in combination, in virtually all types of cancers (37, 38). PEG-3 was cloned as an up-regulated transcript from a transformation progression rodent cancer model, and attractively, the activity of its promoter (PEG-Prom) was found to be significantly and often markedly higher than expression selectively in prostate cancer cells as well as in malignant glioma cells (41). These observations prompted us to investigate the use of the PEG-Prom to drive expression of the E1A gene, necessary for adenovirus replication, to create cancer cell–specific CRCA (37, 38). One engineered CRCA simultaneously expresses mda-7/IL-24 in the E3 region (Ad.PEG-E1A-mda-7; CTV), thereby mediating robust production of this cytokine as a function of adenoviral replication (37). We now show that Ad.PEG-E1A-mda-7 (CTV) effectively circumvents Bcl-2– and Bcl-xL–dependent resistance of prostate cancer cells and eradicates both primary and distant tumors in a nude mouse xenograft model. These observations establish that Ad.PEG-E1A-mda-7 (CTV) might prove to be an efficient tool for elimination of terminal metastatic and therapy-refractory prostate cancers in patients.

Materials and Methods

Cell lines, culture conditions, and viability assays. DU-145, PC-3, and LNCaP prostate cancer cells were obtained from the American Type Culture Collection and cultured as described (36). The generation and characterization of DU-145-Bcl-xL, PC-3-Bcl-xL, and LNCaP-Bcl-2 have been described previously (36). P69 cells are normal human prostate epithelial cells immortalized by SV40 T/t antigen and are cultured as described (32). Cell viability was determined by standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays (35).

Construction of a CRCA. To construct the CRCA Ad.PEG-E1A-mda-7, a CTV, the AdenoQuick cloning system from OD260, Inc. was employed (37, 38). This system uses two shuttle vectors (pE1.2 and pE3.1) in which the transgene cassettes, PEG-Prom driving E1A and cytomegalovirus (CMV) promoter driving mda-7/IL-24, were inserted, respectively, before being transferred into a large adenoviral plasmid (pAd). Adenoviral amplification, purification, titration, and infection were done as described (37, 38). Similar strategies were used to generate Ad.CMV-E1A-mda-7. Ad.CMV-mda-7 and Ad.PEG-mda-7 were constructed as previously described (37).

Annexin V binding assay. Annexin V binding assays were done as described (36).

Preparation of whole-cell lysates and Western blot analyses. Preparation of whole-cell lysates and Western blot analyses was done as described (37). The primary antibodies used were anti-E1A (1:1,000; mouse
monoclonal; Upstate Biotechnology), anti-MDA-7 (1:2,000; rabbit polyclonal), and anti-EF1α (1:1,000; mouse monoclonal; Upstate Biotechnology).

**Human prostate cancer xenografts in athymic nude mice.** DU-145-Bcl-xL cells (2 × 10⁶) were injected s.c. in 100 µL of PBS in both flanks of male athymic nude mice (NCRnu/nu, 4 weeks old, ~20 g body weight; refs. 37, 38). After establishment of visible tumors of ~75 mm³, requiring ~4 to 5 days, intratumoral injections of different adenoviruses were given only to the tumors on the left flank at a dose of 1 × 10⁸ plaque-forming units 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 two more weeks for a total of seven injections. A minimum of five animals was used per experimental point. Tumor volume was measured twice weekly with a caliper and calculated using the formula: \( \pi/6 \times \text{larger diameter} \times (\text{smaller diameter})^2 \). At the end of the experiment, the animals were sacrificed, and the tumors were removed and weighed.

**Immunofluorescence analysis.** Tumors were harvested from the animals, fixed in formalin, and embedded in paraffin. The sections were deparaffinized and were permeabilized with 0.1% Triton X-100 in PBS for 30 min. After fixation, the sections were blocked for 1 h at room temperature with 2% goat serum and 1% bovine serum albumin in PBS and incubated with anti-E1A antibody (1:100) or anti-MDA-7 antibody (1:100) overnight at 4°C. Sections were then rinsed in PBS and incubated with Alexa 488–conjugated anti-mouse or anti-rabbit IgG (Molecular Probes), respectively, for 1 h at room temperature. The sections were then rinsed in PBS and incubated with Alexa 488–conjugated anti-mouse or anti-rabbit IgG (Molecular Probes), respectively, for 1 h at room temperature. The sections were then mounted in VectaShield fluorescence mounting medium containing 4,6-diamidino-2-phenylindole (Vector Laboratories). For CD31 staining, FITC–conjugated rat anti-mouse CD31 monoclonal antibody (BD Pharmingen) was used. A confocal laser scanning microscope analyzed the images.

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

Figure 2. PEG-Prom–driven CRCA (Ad.PEG-E1A-mda-7; CTV) selectively kills prostate cancer cells. The indicated cells were uninfected (control) or infected with the indicated adenovirus (as described at the top) at the indicated multiplicity of infection (viral particles per cell). Cell viability was analyzed by standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay after 2, 4, and 6 d of infection. Columns, mean; bars, SD.
Results

PEG-Prom targets selective expression of Ad E1A and MDA-7/IL-24 in prostate cancer cells. Apart from Ad.PEG-E1A- mda-7 (CTV), a series of additional adenoviruses were created, such as Ad.CMV-E1A-mda-7, in which viral replication is controlled by the CMV promoter and which also expresses mda-7/IL-24, and Ad.CMV-E1A and Ad.PEG-E1A, in which viral replication is controlled by the CMV promoter or the PEG-Prom, respectively, to compare and contrast their relative efficacy. We also employed Ad.CMV-mda-7 and Ad.PEG-mda-7, replication-incompetent adenoviruses in which the CMV or the PEG promoter drives mda-7/IL-24 expression, respectively. A replication-incompetent empty adenovirus, Ad.vec was used as a control. Experiments were done in three prostate cancer cell lines, androgen-nonresponsive DU-145 and PC-3 cells, and androgen-responsive LNCaP cells and their Ad.mda-7-resistant variants (i.e., DU-145-Bcl-x<sub>L</sub>, PC-3-Bcl-x<sub>L</sub>, and LNCaP-Bcl-2). These Bcl-2– or Bcl-xL–overexpressing clones and their resistance to Ad.mda-7 have been extensively characterized in previous studies (36). As a control, P69, a normal prostate epithelial cells immortalized by SV40 T/antigen, was employed.

The replication-incompetent adenoviruses were infected at a multiplicity of infection of 5,000 viral particles per cell, whereas the replication-competent adenoviruses were infected at 1,000 viral particles per cell. The functionality of these constructs was ascertained following adenovirus infection by monitoring protein levels of MDA-7/IL-24 and E1A, a marker for adenoviral replication, by Western blot analysis after appropriate viral infection (Fig. 1). Western blot detection multiple E1A gene products ranging from 36 to 50 kDa and multiple glycosylated forms of MDA-7/IL-24 protein ranging from 21 to 28 kDa (37, 38).

Infection of normal immortal human P69 prostate epithelial cells with Ad.CMV-E1A or Ad.CMV-E1A-mda-7, but not Ad.PEG-E1A or Ad.PEG-E1A-mda-7 (CTV), resulted in production of E1A proteins, whereas in prostate carcinoma cells, infection with all four replication-competent adenoviruses generated E1A proteins (Fig. 1). No E1A proteins were detected in any cell line following infection with replication-incompetent adenoviruses. In P69 cells, infection with Ad.CMV-E1A-mda-7 and Ad.CMV-mda-7 resulted in MDA-7/IL-24 production, whereas infection with Ad.PEG-mda-7 or Ad.PEG-E1A-mda-7 (CTV) resulted in barely detectable levels of MDA-7/IL-24 production (Fig. 1). In prostate cancer cells, infection with Ad.CMV-mda-7, Ad.PEG-mda-7, Ad.CMV-E1A-mda-7, or Ad.PEG-E1A-mda-7 (CTV) generated significant MDA-7/IL-24 production. No MDA-7/IL-24 protein 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 mda-7/IL-24 expression.

Ad.PEG-E1A-mda-7 (CTV) selectively kills prostate cancer cells, without harming normal prostate cells. The effects of the engineered adenoviruses on cell viability and apoptosis were evaluated in the various prostate cell lines. Cells were infected with replication-competent adenoviruses at 10, 100, and 1,000 viral particles per cell and with replication-incompetent adenoviruses at 1,000, 2,000, and 5,000 viral particles per cell. In P69 cells, infection with only Ad.CMV-E1A or Ad.CMV-E1A-mda-7, but not with Ad.PEG-E1A, Ad.CMV-mda-7, Ad.PEG-mda-7, or Ad.PEG-E1A-mda-7 (CTV), induced profound growth inhibition (Fig. 2). In contrast, in all prostate cancer cells, both parental and mda-7/IL-24–resistant, Ad.CMV-E1A-mda-7, Ad.PEG-E1A-mda-7 (CTV), Ad.CMV-E1A, and Ad.PEG-E1A infection resulted in significant growth inhibition. Infection with Ad.CMV-mda-7 and Ad.PEG-mda-7 inhibited growth of parental DU-145, PC-3, and LNCaP cells but not their resistant counterparts. It should be noted that the level of growth inhibition observed with 5,000 viral particles per cell of Ad.CMV-mda-7 and Ad.PEG-mda-7 was equivalent to that observed with only 100 viral particles per cell of replication-competent adenovirus, indicating that the replication-competent adenoviruses are much more potent than the replication-incompetent adenoviruses in cell growth inhibition. These findings indicate that the PEG-Prom allows adenovirus replication specifically in cancer cells, protecting normal cells from growth inhibition because of adenovirus replication. The observation that mda-7/IL-24 exerted no direct growth inhibitory effect on normal cells confirms the cancer cell selectivity of this therapeutic approach. Most importantly, Ad.PEG-E1A-mda-7 was able to overcome the resistance of Bcl-2– and Bcl-xL–overexpressing...
clones to mda-7/IL-24, indicating its potential therapeutic application in prostate cancer patients frequently showing Bcl-2 and Bcl-xL overexpression.

Ad.PEG-E1A-mda-7 (CTV) selectively induces apoptosis in prostate cancer cells. To investigate the mechanism of growth inhibition, Annexin V staining assays, which permit differentiation between apoptotic and necrotic cells, were done (Fig. 3). Infection with only Ad.CMV-E1A and Ad.CMV-E1A-mda-7 (CTV) elevated the percentage of early-apoptotic and late-apoptotic (necrotic) P69 cells. However, all of the adenoviruses, except for Ad.vec, resulted in significant apoptosis in DU-145, PC-3, and LNCaP parental prostate cancer cells. Infection with the replication-competent adenoviruses resulted predominantly in necrosis as manifested by an increase in late apoptotic cells, whereas infection with Ad.CMV-mda-7 and Ad.PEG-mda-7 resulted in predominantly apoptosis as evidenced by an increase in early-apoptotic cells. Although Ad.CMV-mda-7 and Ad.PEG-mda-7 had no apoptotic effect on DU-145-Bcl-xL, PC-3-Bcl-xL, and LNCaP-Bcl-2 cells, Ad.CMV-E1A-mda-7 and Ad.PEG-E1A-mda-7 (CTV) could override this resistance and induce significant apoptosis and necrosis in these cells. It is important to mention that whereas the replication-incompetent adenoviruses were used at 5,000 viral particles per cell, replication-competent adenoviruses were used at 1,000 viral particles per cell.

Ad.PEG-E1A-mda-7 (CTV) eradicates both primary and distant Bcl-xL–overexpressing prostate tumors in nude mice. To expand on the in vitro studies, in vivo assays were done using nude mice containing established DU-145-Bcl-xL 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 × 10¹⁰ viral particles in 100 μL. No injections were given to the right-sided tumors. The experiment was terminated after 6 weeks with injections of Ad.CMV-E1A-mda-7 or Ad.PEG-E1A-mda-7 (CTV) because tumors on both sides showed regression after only three injections, and with seven injections they were completely eradicated (Fig. 4). Further studies are required to determine if a single or double injection with the CTV elicits any discernible antitumor activity in this animal model. Additionally, both control and Ad.vec–infected DU-145-Bcl-xL tumor xenografts reached tumor volumes of ~2,000 mm³ requiring them to be sacrificed. Although Ad.CMV-E1A or Ad.PEG-E1A inhibited the growth of tumors on the left flank, they had some inhibitory effect on tumors on the right side, which was not statistically significant. Ad.CMV-mda-7 or Ad.PEG-mda-7 also displayed marginal effects on the growth of both left- and right-sided tumors, which correlates with the in vitro findings of resistance of DU-145-Bcl-xL cells to

**Figure 4.** PEG-Prom–driven CRCA (Ad.PEG-E1A-mda-7; CTV) eradicates primary and distant tumors. S.c. tumor xenografts from DU-145-Bcl-xL cells were established in athymic nude mice in both right and left flanks, and only tumors on the left side were injected with PBS (control) or with the indicated adenovirus for 3 wks (total of seven injections). **A**, measurement of tumor volume. Points, mean (with a minimum of five mice in each group); bars, SD. **B**, photograph of the animals of each representative group. White arrow, tumor. **C**, left, photograph of the tumor at the end of the study; right, measurement of tumor weight at the end of the study. Columns, mean (with at least five mice in each group); bars, SD.
The observation that intratumoral injection of Ad.PEG-E1A-mda-7 (CTV) completely eradicated the primary and the distant tumor (comparable with a metastasis) provides confidence that this strategy may prove amenable for successfully treating aggressive cancers.

**Ad.PEG-E1A-mda-7 (CTV) replicates at distant tumor sites and generates MDA-7/IL-24 protein.** Because Ad.CMV-E1A- mda-7 or Ad.PEG-E1A-mda-7 (CTV) eradicated both the left-sided injected and right-sided uninjected tumors, we analyzed the replication efficiency and transgene delivery by these adenoviruses (Fig. 5). Tumors were harvested from the animals after three injections and formalin-fixed, paraffin-embedded sections were stained for E1A and MDA-7/IL-24 and analyzed by a confocal laser scanning microscopy. The replication-competent adenoviruses, and not the replication-incompetent adenoviruses, could effectively replicate in the left-sided tumors as evidenced by robust staining for E1A protein (Fig. 5). Interestingly, staining for E1A could also be detected in the right-sided tumors, albeit at a much lower level than their left-sided counterparts, indicating that the adenovirus could enter into the circulation and replicate in the right-sided tumor cells. Staining for MDA-7/IL-24 supported these findings (Fig. 5). Although all of the mda-7/IL-24 expressing adenoviruses effectively generated MDA-7/IL-24 protein in the left-sided tumors, only Ad.CMV-E1A- mda-7 and Ad.PEG-E1A-mda-7 (CTV), and not Ad.CMV- mda-7 and Ad.PEG-mda-7, generated MDA-7/IL-24 protein in the right-sided tumors. These data indicate that the combination of adenovirus replication and robust MDA-7/IL-24 generation resulted in a significant response that could effectively eliminate both the primary and distant tumor.

**Ad.PEG-E1A-mda-7 (CTV) inhibits angiogenesis.** MDA-7/IL-24, as a secreted cytokine, is known to inhibit angiogenesis (34). Based on this consideration, we analyzed the microvessel density in the tumor by staining for CD31 (Fig. 6). After three injections, Ad.CMV-E1A and Ad.PEG-E1A showed marginal effects on angiogenesis inhibition on the left-sided tumors with no effect on the right-sided uninjected tumors. Ad.CMV-mda-7 and Ad.PEG- mda-7 significantly inhibited angiogenesis in both left- and right-sided tumors. Because these two adenoviruses did not affect on the growth of the tumors, it might be inferred that inhibition of angiogenesis alone may not be sufficient to inhibit the growth of the tumors, and inhibition of growth of the cancer cells themselves is mandatory to provoke an enduring antitumor effect. Ad.CMV- E1A-mda-7 and Ad.PEG-E1A-mda-7 (CTV) profoundly inhibited angiogenesis in both left- and right-sided tumors, indicating that the MDA-7/IL-24 protein generated exhibits its complete spectrum of biological activities (Fig. 6). The summation of these findings strongly implies that Ad.PEG-E1A-mda-7 (CTV) might be an effective therapeutic for efficiently eliminating aggressive and resistant prostate cancers.
Cancer is a complicated process involving abnormalities in multiple genetic and signal transduction pathways. As such, effective eradication of cancer requires a combinatorial treatment approach. CRCAs are being evaluated for treatment of prostate cancers (43–45). Although administration of a CRCA alone in phase I/II clinical trials showed their safety, very limited objective clinical responses have been attained using this strategy (43–45). Additionally, most currently employed CRCAs are based on the ONKX-015 backbone, which is dependent on the p53 status of the cancer cells, thus limiting their universal applicability (46). Our approach is unique and superior to other viral therapy approaches for several reasons. First, we employ the PEG-Prom that functions in all types of cancer cells, irrespective of their p53 or Rb status, with very limited to no activity in normal cells (38, 40, 41). Consequently, the PEG-Prom may provide a generic tool for ensuring cancer selectivity. Second, our approach involves the unique tumor suppressor molecule mda-7/IL-24 that not only has cancer-selective apoptosis-inducing properties but also displays a plethora of indirect antitumor "bystander" activities (23–26). The finding that Ad.PEG-E1A-mda-7 (CTV) eradicated not only primary injected tumors, but also distant non-injected tumors derived from a resistant prostate cancer cell line in a nude mouse xenograft model support the anticancer potency of this cancer therapeutic virus. This powerful genetic tool is now ready for further evaluation in animal models, such as Syrian hamsters that facilitate adenovirus replication and therefore analysis of toxicity (47) and transgenic mouse models that recapitulate prostate cancer development, and eventually in phase I/II clinical trials.

The question is how does Ad.PEG-E1A-mda-7 (CTV) induce complete elimination of right-sided non-injected tumors. Our immunofluorescence studies show that Ad.PEG-E1A-mda-7 (CTV) is able to enter into the circulation and replicate and generate MDA-7/IL-24 protein in distant tumors. This observation is exciting because it indicates that the universality of PEG-Prom will allow adenovirus replication and transgene expression, wherever there is any tumor tissue in the animal. Additionally, we have observed that Ad.PEG-E1A-mda-7 (CTV) also effectively inhibited angiogenesis in right-sided tumors. Although Ad.CMV-mda-7 or Ad.PEG-mda-7 also inhibited angiogenesis in both left- and right-sided tumors, these adenoviruses had very limited effect on tumor growth owing to the inherent resistance of DU-145-Bcl-xL cells to mda-7/IL-24. Accordingly, it might be inferred that the combination of adenovirus replication with generation of robust amounts of MDA-7/IL-24, that can override resistance and evoke a profound "bystander effect," is essential for promoting a clinically significant effect, especially in the case of metastatic, end-stage, and therapy-resistant cancers.

As a secreted cytokine, MDA-7/IL-24 induces IL-6, IFN-γ, tumor necrosis factor-α, IL-1β, IL-12, and granulocyte macrophage colony-stimulating factor, potent immunomodulatory agents, from peripheral blood mononuclear cells (30, 31). These secondary cytokines induced by MDA-7/IL-24 might activate antigen-presenting cells to present tumor antigens, thereby triggering an antitumor immune response. Studies in a phase I clinical trial involving intratumoral injection of Ad.mda-7 (INGN 241) suggest that these in vitro effects are recapitulated in the context of patients, supporting the immune-modulating properties of this cytokine (32, 33). Experiments done in syngeneic mouse tumor models also support this hypothesis (48). Although current studies were done in athymic nude mice that are immunocompromised, these mice still have a spleen and a liver and display potent natural
killer cell activity (49). Therefore, in addition to inhibition of angiogenesis, augmentation of an antitumor immune response might also be a potential mechanism of the “bystander effect” of Ad.PEG-E1A-mda-7 (CTV). This function of mda-7/IL-24 is especially significant in a patient with an intact immune system where the effect of Ad.PEG-E1A-mda-7 (CTV) might be further augmented. In this context, the balance between clearance of adenovirus by the immune system and the modulation of the immune system by mda-7/IL-24 will likely represent major determinants in the antitumor potency of Ad.PEG-E1A-mda-7 (CTV) in patients. The robust activity of this adenovirus suggests a need for only limited administration, which in principle will preclude the activation of the immune system promoting viral clearance. Additionally, the observation that neutralizing antiadenovirus antibodies do not inhibit replicating adenoviruses indicates that Ad.PEG-E1A-mda-7 (CTV) might engender a profound effect in inducing complete eradication of metastatic and possibly inducing a cure. The feasibility of intratumoral injection of Ad.PEG-E1A-mda-7 (CTV) into prostate cancers, so that it is shielded from immune system-mediated clearance while evoking a potent antitumor response, makes this genetic vector an ideal therapeutic for prostate cancer. Current studies are ongoing for stringent evaluation of this CTV in immunocompetent animal models for eventual transition into clinical trials in patients with localized and metastatic prostate cancer.

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References

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