A Fiber-Modified Mesothelin Promoter – Based Conditionally Replicating Adenovirus for Treatment of Ovarian Cancer

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Abstract

Purpose: Recently, virotherapy has been proposed as a new therapeutic approach for ovarian cancer. Conditionally replicative adenoviruses (CRAd) may contain tumor-specific promoters that restrict virus replication to cancer cells. Mesothelin, a cell surface glycoprotein, is overexpressed in ovarian cancer but not in normal ovarian tissues. The purpose of this study was to explore the therapeutic utility of a mesothelin promoter – based CRAd in a murine model of ovarian cancer, using noninvasive in vivo imaging.

Experimental Design: We constructed a mesothelin promoter – based CRAd with a chimeric Ad5/3 fiber (AdMSLNCRAd5/3) that contains an Ad5 tail, Ad5 shaft, and an Ad3 knob. Previously, a chimeric Ad5/3 fiber has shown improved infectivity in many ovarian cancer cells. Viral replication and oncolysis were assessed in a panel of ovarian cancer cell lines. To test the oncolytic efficacy of AdMSLNCRAd5/3 in a murine model, bioluminescence imaging of tumor luciferase activity and survival analysis were done.

Results: AdMSLNCRAd5/3 achieved up to a 10,000-fold higher cell killing effect and up to 120-fold higher levels of viral replication in all human ovarian cancer cells, compared with wild-type Ad5. AdMSLNCRAd5/3 significantly inhibited tumor growth as confirmed by in vivo imaging (P < 0.05). Survival with AdMSLNCRAd5/3 was significantly enhanced when compared with no virus or with a wild-type Ad5-treated group (P < 0.05).

Conclusions: The robust replication, oncolysis, and in vivo therapeutic efficacy of AdMSLNCRAd5/3 showed that this CRAd is a promising candidate for treating ovarian cancer. Importantly, we have applied in vivo imaging that has allowed repeated and longitudinal measurements of tumor growth after CRAd treatment.

Ovarian cancer is the most fatal gynecologic malignancy in the United States, and the incidence rate is reported to be increasing (1). The majority of patients present with peritoneally disseminated disease, which is associated with a poor prognosis. Although a number of advances in ovarian cancer treatment have occurred in the last decade, most patients will experience a recurrence after standard surgical and chemotherapeutic approaches. Therefore, new therapeutic approaches are necessary for ovarian cancer. Recently, virotherapy has been proposed as a therapeutic approach for many kinds of malignancies, including ovarian cancer (2). Virotherapy uses replicating viral agents that accomplish selective replication and lysis of tumor cells. In this regard, virotherapy agents have been derived from a variety of parent virus types including herpes virus, measles virus, adenovirus, and others. Adenovirus has shown particular promise as a conditionally replicative adenovirus (CRAd) and has been rapidly translated into human clinical trials where efficacy has been noted, at least in selective cases. For cancer virotherapy, one major class of CRAd agents is designed to achieve tumor-specific replication and oncolysis based on incorporation of tumor-specific promoters into the adenovirus genome to control the expression of the adenovirus E1 gene that is essential for adenovirus replication. The utility of this approach, however, is subservient to the identification of promoters that exhibit the appropriate inducitivity/specificity profile. For adenovirus vectors, a secretory leukoprotease inhibitor promoter–based CRAd and a cyclooxygenase-2 promoter–based CRAd have been reported to exhibit a “tumor on/liver off” replicative phenotype (3–6). To date, however, few ovarian cancer–relevant promoter elements have been suggested and well-characterized for use in CRAds. One of the...
most attractive candidates for targeted therapy of ovarian cancer is Mesothelin, a glycosylphosphatidylinositol-linked cell surface glycoprotein, which is overexpressed in ovarian cancer, mesotheliomas, and selected squamous cell carcinomas. Mesothelin is not expressed in normal tissues except mesothelial cells. We have previously reported the expression of mesothelin mRNA and Mesothelin surface protein in ovarian cancer cells (7). In addition, we found that the mesothelin promoter was activated in ovarian carcinoma cells but showed significantly reduced activity in normal control cells. In this regard, we constructed a mesothelin promoter–based CRAd that also contained a modified fiber previously shown to improve infectivity of many ovarian cancer cells.

The main obstacle of virotherapy agents is the lack of a tumor-volume monitoring system that allows the evaluation of CRAd effect on cancer treatment. Therefore, methods for analyzing CRAd efficacy and tumor response are required. Noninvasive imaging techniques can provide fundamental information on efficacy and virus replication with CRAd therapies in animal models. Furthermore, another important feature of noninvasive imaging is the possibility of performing repeated and longitudinal measurements (8). We, therefore, explored the therapeutic utility of a mesothelin promoter–based CRAd in a murine model of ovarian cancer, using a noninvasive in vivo imaging system, thus providing a powerful tool to determine the therapeutic index for clinical translation. Development of improved CRAds that overcome transductional and specificity limits noted in human clinical trials, and evaluation of CRAd efficacy in an updated model system could provide full realization of the potential benefits of the CRAd approach for ovarian cancer.

Materials and Methods

Cell lines. The 293 cells were purchased from Microbix. Human ovarian cancer cell lines SK-OV-3 and OV-3, and HeLa cells derivative GH329 cells were obtained from the American Type Culture Collection. Human ovarian adenocarcinoma cell lines OV-4 and Hey were a kind gift from Dr. T. J. Eberlein (Harvard Medical School, Boston, MA) and Dr. J. Wolf (M. D. Anderson Cancer Center, Houston, TX), respectively. The firefly luciferase–expressing ovarian adenocarcinoma cell line SK-OV-3-luc was kindly provided by Dr. R. Negrin (Stanford Medical School, Stanford, CA). A nontransformed human fibroblast cell line HBFC was a kind gift from Dr. S. Boppana (Children Hospital, University of Alabama at Birmingham, Birmingham, AL). Cells line were cultured in medium recommended by suppliers (Mediatech and Irvine Scientific). Fetal bovine serum was purchased from Hyclone. All cell lines were cultured in medium recommended by suppliers (Mediatech and Irvine Scientific). Fetal bovine serum was purchased from Hyclone. All cells were grown at 37°C in a humidified atmosphere of 5% CO2.

Generation of shuttle plasmids for the mesothelin promoter–based Ad5 genome. The current shuttle vector was based on a previously created plasmid pSE1-luc, which was generated by introducing into the corresponding restriction sites of pShuttle (Qbiogene, Inc.), a fragment containing a XhoI restriction site followed by the Ad5 genome sequence from nucleotide 522 to the MfeI restriction site at position 3924 (nucleotide positions refer to Genbank sequence AD5001; ref. 9). To create the backbone fragment of the shuttle vector, pScswt was incompletely digested with HindIII and then completely digested with NotI. Plasmid pShuttle.MSLN.Luc is a previously constructed shuttle vector, which contains the luciferase gene under the control of mesothelin promoter (7). The coding sequence of the mesothelin promoter was amplified from plasmid pShuttle.MSLN.Luc with the primers 5′-AGCCGTTAATACGCTGTCGTTTTCATCATT and 5′-TTAC-GAACTTACGGAAATGCCAACGTTGA. The PCR product was cloned into NotI/HindIII-digested plasmid pScswt, resulting in pSE1-MSLN. The sequence of pSE1-MSLN was confirmed by DNA sequencing. The expression of the luciferase gene was determined by thoroughly analyzing CRAd efficacy and tumor response with CRAd therapies in animal models. Furthermore, another important feature of noninvasive imaging is the possibility of performing repeated and longitudinal measurements (8). We, therefore, explored the therapeutic utility of a mesothelin promoter–based CRAd in a murine model of ovarian cancer, using a noninvasive in vivo imaging system, thus providing a powerful tool to determine the therapeutic index for clinical translation. Development of improved CRAds that overcome transductional and specificity limits noted in human clinical trials, and evaluation of CRAd efficacy in an updated model system could provide full realization of the potential benefits of the CRAd approach for ovarian cancer.
nonreplicative luciferase expression vector; ref. 14) and AdCMVSDF (E1-deleted nonreplicative vector, created by pShuttle-CMV and pAdEasy-1 in the AdEasy Vector System, Qbiogene, Inc.) were used as replication-incompetent control viruses with wild-type fiber. These control vectors were propagated in 293 cells and purified using a standard protocol (13). Viral particle concentration was determined by the method of Maizel, et al. (15). An infectious titer was determined according to the AdEasy Vector System (Qbiogene, Inc.).

**PCR amplification of viral genome fragments.** Viral DNA was amplified using the Taq PCR Core kit (Qiagen, Inc.). The sequences of the primers were as follows: Ad5 fiber sense, 5’-ATGAAGGCCCAGAACCCGTCGGAAGATA; the chimeric Ad5/3 fiber sense, 5’-ATGAAGGCCCAGAACCCGTCGGAAGATA; the chimeric Ad5/3 fiber antisense, 5’-GTATGTGCAAGGGAACTGTT; Mesothelin promoter sense, 5’-TGTGACGGTTCCAGCCCGTTCG; Mesothelin promoter antisense, 5’-TCCTGAGGGGCTTCTGCCCAGAT; E1 promoter site sense (pre-E1 promoter site), 5’-GGCGTACACCGGTGAAGATTTGGCC; E1 promoter site antisense (the E1 sequence), 5’-AACGACTGCCGGCCATTTCTTCGG; Ad5 wild-type E1 promoter sense, 5’-CATTCTGTACGCCGTGAGT; Ad5 wild-type E1 promoter antisense, 5’-CATTACACCGCCATGAGAT.

**Quantitative virus replication.** The ovarian cancer cell line, Hey, was cultured in 24-well plates (1 x 105 cells per well) and infected with 10 viral particle per cell of viruses. Then, the growth medium was harvested at 0, 2, 4, and 6 d after infection. DNA purification and quantitative PCR of E4 gene copy number were essentially done as described previously (16). To quantify the total E4 copy number, DNA was purified from the growth medium using QIAamp DNA Blood Mini kit (Qiagen, Inc.). The primers used to amplifying the E4 were sense 5’-GGATGCGCCGGCACAC and antisense 5’-ACTACTGTGCCTCGTTCCAT and detected with probe 5’-TGGCATGACACTACGACCC.

**In vitro cytotoxicity assay.** Cells were cultured on 24-well plates (1 x 105 cells per well) and infected with 100, 10, 1, 0.1, 0.01, or 0 viral particle per cell of viruses. When positive control virus (Ad5wt) showed clear clearing with the lowest amount of virus, oncolysis was evaluated by crystal violet staining. Briefly, the cells were fixed with 10% buffered formalin containing 0.2% crystal violet for 30 min, followed by 3 washes in tap water and air dried.

**Therapeutic ovarian cancer model.** Female CB17 severe combined immunodeficient mice (Taconic Farm) were obtained at ages 6 to 8 wk and quarantined for 2 wk. Mice were kept under pathogen-free conditions according to the American Association for Accreditation of Laboratory Animal Care guidelines. Animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. On day 0, mice were injected i.p. with 5 x 105 SK-OV-3-luc cells in 500 μL PBS. On day 8, mice were injected i.p. with 5 x 104 viral particle per injection of AdMSLNCRAdwt (n = 5), AdMSLNCRAd5/3 (n = 5), Ad5wt (n = 5), AdCMVSDF (n = 5), or no virus (n = 5) in 500 μL PBS. Mice were sacrificed upon evidence of pain or distress. *In vivo* optical imaging was done at the small animal imaging core facility in the Comprehensive Cancer Center of the University of Alabama at Birmingham as described previously (16). Three animals from each group were imaged for bioluminescence on days 8, 17, 24, and 31 after establishing tumors and followed to record survival times. Statistical differences of tumor size among groups were assessed with Student’s t test. A P value of <0.05 was considered statistically significant. Data are presented as mean values ± SD. All mice (n = 5) were followed daily to record survival times. Survival data were plotted on a Kaplan-Meier curve, and different groups of data were compared using the Log-rank χ2 test (GraphPad Prizm Software v.4).

**Results**

**Vector construction of mesothelin promoter–based CRAds.** Figure 1 depicts the genomes of the recombinant adenoviruses generated. The mesothelin gene is highly expressed in a majority of ovarian epithelial cancers but has low expression in mesothelial cells. The 1850-bp human mesothelin gene promoter has previously been shown to mediate highly specific transcription within plasmids (7). To generate a replication-competent adenovirus targeting ovarian cancer, we replaced the E1 promoter with the transcriptional control sequence of the mesothelin gene. In this setting, E1 expression was designed to be regulated with the mesothelin promoter inserted in front of the E1 sequence. We introduced the mesothelin promoter sequence into pShuttle, a shuttle vector for recombination into the E1 promoter region (9). This resulted in pSE1-MSLN, a shuttle vector for deriving mesothelin promoter–based CRAds. Using the shuttle vector pSE1-MSLN, we generated the adenovirus recombinant genomes, pAdMSLNCRAdwt (derived by pTG3602, containing a wild-type fiber) and pAdMSLNCRAd5/3 (derived by pAdback5/3, containing a chimeric Ad5/3 fiber). The corresponding mesothelin promoter–based CRAds, AdMSLNCRAdwt and AdMSLNCRAd5/3, were generated after transfection of the recombinant genomes into GH329 cells.

![Figure 2. Analysis of adenovirus components in rescued viral particles. A, detection of the Ad5 fiber gene in adenovirus genomes. Rescued viral particles were analyzed by PCR, using Ad5 fiber primer pairs (lanes 1-4). B, detection of the chimeric Ad5/3 fiber gene in adenovirus genomes. Rescued viral particles were analyzed by PCR, using the chimeric Ad5/3 fiber primer pairs (lanes 5-8). C, detection of the mesothelin promoter gene in adenovirus genomes. Rescued viral particles were analyzed by PCR, using pairs of the mesothelin promoter primers (lanes 9-12). D, detection of the E1 promoter size in adenovirus genomes. Rescued viral particles were analyzed by PCR, using pairs of the E1 promoter site sense (pre-E1 promoter site) and the E1 promoter site antisense (the E1 sequence, lanes 13-15). E, detection of the native E1 promoter gene in adenovirus genomes. Rescued viral particles were analyzed by PCR, using pairs of the native E1 promoter primers (lanes 16-20). Lanes 1, 5, 9, 13, and 17, AdMSLNCRAdwt; lanes 2, 6, 10, 14, and 18, AdMSLNCRAd5/3; lanes 3, 11, and 15; pAdMSLNCRAdwt, lane 7, pAdMSLNCRAd5/3, lane 19, Ad5wt; lanes 4, 8, 12, 16, and 20, no PCR template.](image)
Structure confirmation of mesothelin promoter–based CRAds. The vector structure was confirmed by PCR of viral DNA. In the fiber configuration analyses, the Ad5 fiber fragment was amplified in AdMSLNCRAdwt, the mesothelin promoter–based CRAd with a wild-type Ad5 fiber (Fig. 2A, lane 1). On the other hand, the chimeric Ad5/3 fiber fragment was amplified by AdMSLNCRAd5/3, the mesothelin promoter–based CRAd with a chimeric Ad5/3 fiber (Fig. 2B, lane 6). As expected, the mesothelin promoter region was amplified in both mesothelin promoter–based vectors (AdMSLNCRAdwt and AdMSLNCRAd5/3; Fig. 2C, lanes 9 and 10, respectively). We also confirmed that the size of E1 promoter site was appropriate in both vectors, using primer pairs that amplified from the pre-E1 promoter site to the beginning of the E1 region (Fig. 2D, lanes 13 and 14). Both mesothelin promoter–based vectors (AdMSLNCRAdwt and AdMSLNCRAd5/3) lacked the wild-type E1 promoter sequences of Ad5 (Fig. 2E, lanes 17 and 18). These data confirmed the structural accuracy of the CRAd constructs.

A mesothelin promoter–based CRAd with a chimeric Ad5/3 fiber displays efficient replication of ovarian cancer Hey cells. We analyzed adenovirus replication in the Hey ovarian cancer cells. Monolayers of Hey cells were infected with four adenovirus vectors: mesothelin promoter–based CRAds (AdMSLNCRAdwt and AdMSLNCRAd5/3), a replicative control vector (Ad5wt), and a nonreplicative control vector (Ad5Luc1). Growth medium was collected at the indicated time points for quantitative PCR–based measurements of the E4 gene copy numbers. Based on the same fiber configuration, the viral copy number of the mesothelin promoter–based CRAd (AdMSLNCRAdwt) resulted in a 10-fold increase from day 0 to day 6 in Hey cells, whereas the viral copy number of the native E1 promoter–based vector (Ad5wt) increased only 3-fold during 6 days of cell culture (Fig. 3). Remarkably, the viral copy number of the mesothelin promoter–based CRAd with a chimeric Ad5/3 fiber (AdMSLNCRAd5/3) increased 350-fold from day 0 to day 6 (Fig. 3). In fact, AdMSLNCRAd5/3 achieved up to 120-fold higher viral replication levels compared with the replicative control vector (Ad5wt) in Hey cells (Fig. 3). These results thus indicate the excellent replication ability of a mesothelin promoter–based CRAd with a chimeric Ad5/3 fiber (AdMSLNCRAd5/3) in a human ovarian cancer cell line.

Mesothelin promoter–based CRAds gained in vitro oncolytic potency and specificity. To analyze the oncolytic potency and specificity of mesothelin promoter–based CRAds, we infected viruses in monolayers of OV-3, SK-OV-3, OV-4, and Hey cells, which have been reported to express high levels of mesothelin (7). HFBC cells, which express low levels of the mesothelin gene, were used as the negative control (7). The cell killing assay was done by the crystal violet staining. Importantly, the mesothelin promoter–based CRAd with a chimeric Ad5/3 fiber (AdMSLNCRAd5/3) caused dramatic cell killing in the mesothelin-positive ovarian cancer cell lines but did not cause oncolysis in the mesothelin-negative control cell line, HFBC (Fig. 4). Similarly, the mesothelin promoter–based CRAd with a wild-type fiber (AdMSLNCRAdwt) gained moderate oncolysis in ovarian cancer cell lines but not in HFBC (Fig. 4). As a
positive control, the replicative control vector with a wild-type fiber (Ad5wt) has shown moderate oncolysis in all cell lines tested regardless of mesothelin expression (Fig. 4). Ad5Luc1 was a nonreplicative control and did not cause oncolysis (Fig. 4). These experiments show the specific cell killing effect of AdMSLNCRAd5/3 and AdMSLNCRAdwt in mesothelin-positive cells.

Therapeutic effect of a mesothelin promoter–based CRAd with a chimeric Ad5/3 fiber in vivo. To test the oncolytic efficacy of mesothelin promoter–based CRAds in vivo, we generated xenografts of human ovarian cancer in severe combined immunodeficient mice by injecting SK-OV-3-luc cells. We used this line to visualize the therapeutic effects of our treatments because it stably expresses luciferase, which allows noninvasive estimates of tumor burden in vivo. Xenografts were introduced in mice (4 groups; n = 5) by injection with 5 x 10⁶ cells. Eight days after administration of tumor cells, mice received 5 x 10⁹ viral particle per injection of therapeutic viruses, AdMSLNCRAdwt or AdMSLNCRAd5/3. Control groups received replicative control virus (Ad5wt), nonreplicative control virus (AdCMVSDF), or PBS. Three randomly selected mice were imaged after i.p. injections of β-luciferin on days 8, 17, 24, and 31. A panel of representative images is shown in Fig. 5A. Low bioluminescent signals of SK-OV-3-luc cells were detected as early as 8 days after i.p. injection, which greatly increased by day 17. At the last imaging time point (31 days), the bioluminescent signals from two mice in the PBS injection group exceeded the camera's limit of detection, which is indicative of a bulky tumor growth in the peritoneal space. The rate of tumor development correlated with the tumor mass detected by image intensity. Lower light intensities were detected in the groups that received mesothelin promoter–based CRAds (AdMSLNCRAdwt or AdMSLNCRAd5/3) compared with nonreplicative AdCMVSDF or PBS injection groups, indicating a slower rate of tumor development in the groups exposed to the oncolytic adenovirus. The level of light intensities obtained in mouse cohorts treated with mesothelin promoter–based CRAds (AdMSLNCRAdwt or AdMSLNCRAd5/3) were significantly lower than those obtained in the cohort treated with the nonreplicative AdCMVSDF on day 24 and 31 (P < 0.05; Fig. 5B). Furthermore, tumor growth was significantly suppressed in cohorts treated with AdMSLNCRAd5/3, the mesothelin promoter–based CRAd with a chimeric Ad5/3 fiber, compared with AdMSLNCRAdwt, the mesothelin promoter–based CRAd with a wild-type fiber (P = 0.0094; Fig. 5B). Animals in the imaging groups were also followed to determine the length of survival. The median survival time increased from 35 days, for the PBS injected cohort, to 53 days in the cohort injected with the mesothelin promoter–based CRAd with a wild-type fiber.
In vivo, the mesothelin promoter–based CRAd with a chimeric Ad5/3 fiber was able to exert an enhanced oncolytic effect on tumor cells. The cohort injected with the mesothelin promoter–based CRAd with a chimeric Ad5/3 fiber (AdMSLNCRAd5/3) increased the median survival time to 68 days (Fig. 5C). Moreover, statistically significant differences between AdMSLNCRAd5/3 and the replicative control virus (Ad5wt) groups were calculated using Log-rank \( \chi^2 \) test \( (P = 0.033) \). In summary, the mesothelin promoter–based CRAd with a chimeric Ad5/3 fiber was able to exert an enhanced oncolytic effect on tumor cells in vivo.

**Discussion**

In our previous study, we evaluated the utility of mesothelin for transcriptional gene therapy to direct adenoviral vectors to mesothelin-expressing targets (7). We tested a nonreplicative adenovirus containing the mesothelin promoter driving reporter gene expression in ovarian cancer substrates. We reported that the mesothelin promoter was activated in ovarian cancer cells and in primary ovarian cancer cells but showed significantly reduced activity in nontransformed cell lines and normal murine organs (7). At the same time, transductional targeting of adenoviruses via an antimesothelin antibody had also shown increased transgene expression in ovarian carcinoma cells.

Because we had already proven the utility of mesothelin for transcriptional as well as transductional targeting strategies for ovarian cancer gene therapy, we created mesothelin promoter–based CRAds that target mesothelin gene expressing ovarian cancer cells. We confirmed that the mesothelin promoter–based CRAds resulted in maximum replication specificity in these cells.

In general, adenovirus gene therapy has been attributed to insufficient transduction of tumor cells. A major obstacle to overcome in Ad5-based cancer gene therapy is the paucity of the primary receptor, CAR, on human primary tumor cells. Variable, but usually low expression of CAR has been documented in many cancer cell types including glioma, rhabdomyosarcoma, and ovarian carcinoma (17–19). It has been suggested that the adenovirus serotype 3 receptor is expressed at high levels in ovarian cancer cells (17). Ad5 vectors that express a chimeric fiber consisting of the tail and shaft domains of adenovirus serotype 5 and the knob domain of serotype 3 (Ad5/3) have been shown to significantly increase infectivity in ovarian cancer cells (16, 17, 20, 21).

To increase the infectivity of the Ad5 vector and to gain more specific infectivity for ovarian cancer, we modified vector tropism using a chimeric Ad5/3 fiber in addition to the current mesothelin targeting strategy. We confirmed that the mesothelin promoter–based CRAd with a chimeric Ad5/3 fiber could replicate in vitro and obtain maximum oncolysis in ovarian cancer cells in vitro and in vivo.

Tissue specific promoter–based CRAds have been used for preclinical studies in cancer gene therapy and have achieved therapeutic efficacy in many cases (11, 22–24). In fact, a secretory leukoprotease inhibitor promoter–based CRAd, a cyclooxygenase-2 promoter–based CRAd, and a CXCR4 promoter–based CRAd have been created and tested in our group. Among the tested CRAd vectors, the mesothelin promoter–based CRAd is a promising candidate for ovarian cancer because strong mesothelin reactivity has been exhibited in majority of clinical specimens from ovarian cancer (25, 26).

To determine the efficacy of the newly developed vector in vivo, we used noninvasive bioluminescence imaging techniques. Optical imaging by bioluminescence or fluorescence is a powerful tool for high-throughput longitudinal monitoring of tumor load in small animals. Optical imaging also allows the implementation of tumor models in therapy intervention studies with almost the same simplicity as when measuring traditional s.c. models with calipers (27). Compared with other modern imaging tools, i.e., positron emission tomography, single photon emission computed tomography, X-ray volumetric computed tomography, magnetic resonance imaging or ultrasound, bioluminescence imaging has advantages and disadvantages (27, 28). Bioluminescence imaging is less suited for the determination of absolute tumor mass in an animal because of quenching of bioluminescence by tissue components and for the determinations of the exact location of tumors because its spatial resolution is limited. In fact, we have experienced bioluminescent signals exceeding the camera’s limit of detection when bulky tumors grow in the peritoneal space of nontreated mice at the later imaging time points. However, bioluminescence imaging is rapid, easy to perform, sensitive, safe, and cost effective. It can be used to detect tumor load shortly after inoculation, even when relatively few cancer cells are present. Furthermore, we have clearly showed in this submission the therapeutic efficacy of the mesothelin promoter–based CRAd, with a chimeric Ad5/3 fiber incorporating noninvasive bioluminescence imaging, in assisting preclinical evaluation.

The limited expression of mesothelin on normal human tissues combined with high cell surface expression in several tumors including malignant mesothelioma, ovarian and pancreatic carcinomas, as well as some squamous cell carcinomas, makes mesothelin an attractive candidate for targeted therapy (25, 29, 30). These therapies include agents that target cell surface mesothelin or elicit an immune response against mesothelin. For example, a recombinant immunotoxin against mesothelin, SS1P, consisting of an antimesothelin Fv linked to a truncated *Psudomonas* exotoxin, has been shown to mediate cell killing (31, 32). Based on preclinical studies, animal models had shown possible synergy when SS1P was combined with chemotherapy (33), and mice treated with radiation and SS1P had a significant prolongation in tumor doubling or tripling times, compared with controls (34). Two phase I studies have shown safety and limited antitumor efficacy of SS1P (35). An issue has been raised however, about the high level of SS1P neutralization after initial treatment, which might limit repeated administration of SS1P in the clinical setting.

Another mesothelin-targeting strategy uses MoRAb-009, a chimeric (mouse/human) monoclonal IgG1/κ with high affinity and specificity for mesothelin. Because of the mouse sequences that recognize human mesothelin, MoRAb-009 should be less immunogenic and allow repeat administration to patients. Currently, a phase I clinical trial of MoRAb-009 has been initiated. Mesothelin has also been used as a tumor vaccine, based on studies showing that mesothelin can elicit a strong CD8+T-cell response in patients (36). Preclinical studies have been completed and a phase I clinical trial of the
mesothelin-based cancer vaccine for the treatment of patients with mesothelin-expressing cancers is about to start. A similar vaccine study has been proposed that supports the potential utility of mesothelin in peptide and/or vector-mediated immunotherapy for treatment of cancers that highly express mesothelin (37, 38). Mesothelin as a target for radioimmuno-therapy was also reported in vivo using nude mice bearing mesothelin-expressing xenografts (39).

Thus, as one of the mesothelin-targeting strategies, we created and tested a mesothelin promoter–based CRAds in vitro and in an in vivo cancer model. This is the first vector using a mesothelin promoter in CRAds combined with a tropism modification strategy. The mesothelin promoter–based CRAd with a chimeric Ad5/3 fiber should be a promising candidate for virotherapy of ovarian cancer in the clinical setting.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. V. Krasnykh (M. D. Anderson Cancer Center, Houston, TX) for providing plasmids, pNBE.PK.F5/3 and pkK500; Dr. D. M. Nettelleck (University of Erlangen-Nürnberg, Germany) for providing a plasmid pScwv; Dr. K. R. Zinn (University of Alabama at Birmingham) for technical support in bioluminescent imaging; and Dr. J. C. Roth for his critical reading of the manuscript.

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