Targeting Mesothelioma Using an Infectivity Enhanced Survivin-Conditionally Replicative Adenoviruses

Zeng B. Zhu, MD, Sharmila K. Makhija, MD,† Baogen Lu, MD,* Minghui Wang, MD,* Shuyi Wang, MD,‡ Koichi Takayama, MD,§ Gene P. Siegal, MD, PhD,‖ Paul N. Reynolds, MD,¶ and David T. Curiel, MD, PhD**

Mesothelioma is a highly malignant neoplasm with no effective treatment. Conditionally replicative adenoviruses (CRAds) represent a promising new modality for the treatment of cancer in general. A key contribution in this regard is the introduction of tumor-selective viral replication for amplification of the initial inoculum in the neoplastic cell population. Under ideal conditions following cellular infection, the viruses replicate selectively in the infected tumor cells and kill the cells by cytolysis, leaving normal cells unaffected. However, to date there have been two limitations to clinical application of these CRAd agents; viral infectivity and tumor specificity have been poor. Herein we report on two CRAd agents, CRAd-S.RGD and CRAd-S.F5/3, in which the tumor specificity is regulated by a tumor-specific promoter, the survivin promoter, and the viral infectivity is enhanced by incorporating a capsid modification (RGD or F5/3) in the adenovirus fiber region. These CRAd agents effectively target human mesothelioma cell lines, induce strong cytoxicity in these cells in vitro, and viral replication in a H226 murine xenograft model in vivo. In addition, the survivin promoter has extremely low activity both in the non-transformed cell line, HMEC, and in human liver tissue. Our results suggest that the survivin-based CRAds are promising agents for targeting mesothelioma with low host toxicity. These agents should provide important insights into the identification of novel therapeutic strategies for mesothelioma.

Departments of *Medicine, Pathology, and Surgery, †Division of Gynecologic Oncology, ‡Howard Hughes Medical Institute and Departments of Medicine, University of Alabama at Birmingham, Birmingham, Alabama; §Research Institute for Diseases of the Chest, Kyushu University, Fukuoka, Japan; ‖Departments of Pathology, Cell Biology, and Surgery and the Gene Therapy Center, University of Alabama at Birmingham, Birmingham, Alabama; ¶Royal Adelaide Hospital Chest Clinic, Adelaide, South Australia.

Supported by the National Institute of Health Grants: R01 CA83821, R01 CA 94084, K12 HD01261-02, R01 HL67962, R01 CA93796; the Mesothelioma Applied Research Foundation, Inc.; a grant from the UAB Mesothelioma Center of the UAB Comprehensive Cancer Center; and Medical Research and Compensation and MHMRC, Australia.

Address for correspondence: David T. Curiel, Division of Human Gene Therapy, Gene Therapy Center, BMR II 508, 2172, 901 19th Street S., University of Alabama at Birmingham, Birmingham, AL 35291. E-mail: curiel@uab.edu

ISSN: 1556-0864/06/0107-0701

Key Words: Survivin gene, Tumor-specific promoter, Transcriptional targeting, Adenoviral vector and mesothelioma.

(*J Thorac Oncol.* 2006;1: 701–711)

Mesotheliomas are neoplasms of the serosal membranes of body cavities, arising principally from the pleura, peritoneum, and tunica vaginalis. Eighty percent of mesotheliomas involve the pleural space, and they represent the most common primary tumor of the pleural cavity. Despite its relative rarity in the United States, mesothelioma remains an area of special interest in pulmonary medicine because of its increasing frequency, dismal prognosis, and attendant medicolegal issues related to asbestos exposure. Mesotheliomas are classified into three general categories: diffuse malignant, localized benign, and localized malignant. Ten percent of localized mesotheliomas are malignant, but they are often low-grade and potentially resectable.[1,2] Diffuse pleural mesothelioma account for the preponderance of primary pleural tumors. No particular therapy has proven reliably superior to supportive therapy alone in terms of survival. The median survival for patients without treatment is 6 to 8 months, quite similar to the survival for those who received therapy, including chemo- or radiotherapy and surgery.[3–12] There is no widely accepted newer treatment strategy for patients with pleural mesothelioma. To that end, a novel therapeutic approach to pleural mesothelioma is warranted.

Gene therapy has shown potential for the treatment of other solid cancers. In a recent summary of gene therapy clinical trials worldwide (1989 to 2004), 66% of such trials addressed cancers.[13] Strategies for anti-mesothelioma therapies by gene therapy include: (1) molecular chemotherapy based on the use of "suicide genes," such as the herpes simplex thymidine kinase gene[14,15] and cytosine deaminase,[16] in which DNA that encodes an enzyme capable of generating a toxic metabolite is transferred to tumor cells followed by the administration of a nontoxic enzyme substrate; (2) genetic immunopotentiation based on the capacity to destroy tumor cells either via T lymphocytes, natural killer cells, or macrophages[17] or by use of immunomodulators, such as IL-2[18] and TGF-β[19]; (3) mutation compensation based on correction of oncogenes or tumor-suppresser genes, such as K-*ras*, p53, P16 ^{INK4A}, *P14*, and *NF2* genes.[20–24] Thus, in this regard, a number of gene therapy approaches for mesothelioma have

been developed. Furthermore, gene therapy of mesothelioma may be useful in combination with conventional therapies.

Recently, the use of replicative viral delivery represents a novel approach to such neoplastic diseases, including malignant mesothelioma.[25] In this strategy, target tumor cell killing by the viral agent is achieved via direct consequence of the viral replication.[26] It is apparent that the specificity of the viral agent for achieving tumor cell killing via replication ("oncolysis") is the functional key to successful exploitation of these agents. To this end, an ideal viral agent would possess two characteristics: (1) high infectivity, in that viral vectors would have the capacity to infect tumor versus non-tumor cells; and (2) tumor specificity, in that viral vectors would possess a relative preference for replication in tumor versus non-tumor cells. However, both viral infectivity and specificity have been poor when using current applicable conditionally replicative viral vectors (CRAds). To develop infectivity enhanced and tumor-specific CRAd agents for mesotheliomas, better constructs are needed.

To overcome these two disadvantages of poor infectivity and specificity, many approaches have been described. Important in the consideration of efficient infection is the knowledge that cells may be resistant to Ad infection because of their lack of the Coxsackie adenovirus receptor (CAR) on their cell surfaces that result in poor infectivity.[27] To circumvent this, genetic and immunologic alterations to the virus fiber that use CAR-independent pathways have been identified. An example of this is the use of the RGD motif in the fiber knob of the Ad. This capsid modification seems to facilitate Ad binding and entry into tumor cells via integrin receptors that are abundantly expressed on many solid tumor cells.[27] Additional capsid modifications have been explored to obtain infectivity enhancement of Ads, including AdF5/3,[28]Ad5-pk7,[29] and Ad5-CK.[30] Alternately, transcriptional targeting exploits promoters that display preferentially in tumor cells but not in normal host cells.[26] An ideal tumor-specific promoter (TSP) for transcriptional targeting exhibits selective high activity in tumor cells (termed a "tumor on" phenotype). To mitigate hepatoxicity on systemic delivery, candidate promoters additionally exhibit low activity in the liver (termed a "liver off" phenotype). To develop TSP-CRAds, one of the most widely used methods is to drive Ad E1 gene expression with a selected promoter, as Ad E1 is the main element that drives viral replication. In this method, a CRAd replicates only in tumor cells, killing cells by oncolysis, but not in normal host cells, thereby avoiding the toxicity of the CRAd agent. Many TSPs have been explored for specific cancers, such as the prostate-specific antigen (PSA) for prostate cancer and the α-fetoprotein (AFP) promoter for hepatocarcinoma.[31,32] Recently we reported a novel TSP, the survivin promoter, which exhibited a tumor on/liver off phenotype in vitro and in vivo in a wide range of neoplastic tumors.[33] This promoter has also been reported to exhibit a radiation-responsive promoter and cisplatin-sensitive capabilities.[34,35] Therefore, the survivin promoter is an excellent candidate to drive E1 expression in the development of a new CRAd agent.

In this study, we further constructed conditionally replicative adenoviral vectors, in which the Ad E1 gene was regulated by using the survivin promoter as a TSP and viral infectivity was enhanced with a capsid modification (RGD or F5/3) by which Ad vector target to mesothelioma cells occurred via a CAR-independent pathway. We verified that these infectivity enhanced and tumor-selected vectors, especially CRAD-S.F5/3, replicated in mesothelioma cells and in a H226 xenograft murine model. We also showed that the survivin promoter had very low activity compared with the other TSPs in human liver slices. Our data thus indicate that the CRAd-S.F5/3 is an excellent candidate for translation into a clinical trial for the treatment of malignant mesothelioma in humans.

MATERIALS AND METHODS

Cells, Tissues, and Animals

Human mesothelioma cell lines, H226 (purchased from ATCC), H 2373, mmp4, and BC286-2b (gifts from Dr. J. Kolls, Pittsburgh, PA) were used in this study. All cells were cultured in RPMI 1640 complete medium supplemented with 10% fetal calf serum, penicillin (100 IU/ml), and streptomycin (100 μg/ml). Cells were incubated at 37°C in a 5% CO_2 environment under humidified conditions. Non-transformed human mammary epithelial cells (HMEC), used as a survivin expression-negative control, were purchased from Cambrex BioScience Company (Walkersville, MD) and cultured in the medium specially purchased from the same company.

Human liver samples were obtained from hepatectomy remnants not needed for diagnostic purpose after liver transplantation after institutional review board approval. To generate liver tissue slices, tissue was cut in consecutive 0.5-mm slices using the Krumdieck tissue slicer (Alabama Research Development, Munford, AL). Sequential slices were then cultured in 24-well plates in RPMI supplemented with 10% bovine fetal serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 μg/ml insulin. Cultures were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Three tissue slices were examined per group.

Female BALB/c nude mice, 6 to 8 weeks of age (Charles River, Wilmington, MA), were used for in vivo experiments. All animals received humane care based on the guidelines set by the American Veterinary Association. All of the experimental protocols involving live animals were reviewed and approved by the institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Recombinant Adenoviruses

Ad5-CMV, Ad5-Cox-2, Ad5-CXCR4, Ad5-EGP-2, Ad5-HPR, Ad5-SLPI, Ad5-MsLn, and Ad5-Survivin are replicative-defective Ads containing a luciferase reporter gene driven by the TSP—Cox-2, CXCR4, EGP-2, HPR, SLPI, MsLn, surviving, and a control CMV promoter, respectively—in the E1 region, as has been previously described.[33,36–40] These Ads were used in this study for evaluating the transcriptional activities of the TSPs by means of the expression of a luciferase reporter gene in mesothelioma cells.