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Immunocompetent syngeneic cotton rat tumor models for the assessment of replication-competent oncolytic adenovirus

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Abstract

Oncolytic adenoviruses as a treatment for cancer have demonstrated limited clinical activity. Contributing to this may be the relevance of preclinical animal models used to study these agents. Syngeneic mouse tumor models are generally non-permissive for adenoviral replication, whereas human tumor xenograft models exhibit attenuated immune responses to the vector. The cotton rat (*Sigmodon hispidus*) is susceptible to human adenovirus infection, permissive for viral replication and exhibits similar inflammatory pathology to humans with adenovirus replicating in the lungs, respiratory passages and cornea. We evaluated three transplantable tumorigenic cotton rat cell lines, CCRT, LCRT and VCRT as models for the study of oncolytic adenoviruses. All three cells lines were readily infected with adenovirus type-5-based vectors and exhibited high levels of transgene expression. The cell lines supported viral replication demonstrated by the induction of cytopathogenic effect (CPE) in tissue culture, increase in virus particle numbers and assembly of virions seen on transmission electron microscopy. *In vivo*, LCRT and VCRT tumors demonstrated delayed growth after injection with replicating adenovirus. No *in vivo* antitumor activity was seen in CCRT tumors despite *in vitro* oncolysis. Adenovirus was also rapidly cleared from the CCRT tumors compared to LCRT and VCRT tumors. The effect observed with the different cotton rat tumor cell lines mimics the variable results of human clinical trials highlighting the potential relevance of this model for assessing the activity and toxicity of oncolytic adenoviruses.
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Introduction

The concept of using replicating lytic viruses as a treatment for cancer is not new. A study of wild-type adenovirus as a potential treatment for carcinoma of the uterine cervix was reported in 1956, 3 years after the initial discovery of the virus (Smith et al., 1956). Intratumoral injections of adenovirus resulted in necrosis and cavity formation in 65% of treated tumors and live adenovirus was recovered from two-thirds of these patients suggesting that viral replication was occurring *in vivo*. In contrast, no virus was isolated from the tumors of patients treated with heat-inactivated virus. A second clinical trial targeting a variety of tumors using adenovirus administered by various routes was less successful, with only 2 of 14 patients showing transient tumor regression and virus recovered from only a single subject (Southam et al., 1956). Shortly thereafter, advances in chemotherapy coupled with low viral yields due to inefficient production techniques, and concerns over the risk of viral dissemination and illness lead to the abandonment of virotherapy.

Over the last decade, new understanding and developments in molecular biology, virology and genetic engineering have led to the reemergence of virotherapy as a potential treatment for cancer. A number of replicating oncolytic adenovirus-based

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vectors have entered into clinical trials. These include the conditionally replicating ONYX-015 (*dl*1520), CV706, CG7870, Ad5-CD/TKrep and the Ad-OC-E1a vectors (Benjamin et al., 2001; Bischoff et al., 1996; DeWeese et al., 2001; Freytag et al., 2002; Small et al., 2006). Despite promising preclinical studies, much of the initial excitement surrounding oncolytic adenoviruses has dampened with reports of a lack of tumor specificity and few observed clinical responses when used as single agents (Kirn, 2001).

The animal models used to evaluate these vectors may be a significant factor in this discordance. Human adenoviruses are unable to generate productive infections in most non-human tissues limiting the usefulness of syngeneic immunocompetent animal tumor models for the preclinical evaluation of these agents. To date, the model of choice to study these agents has been human tumor xenografts grown immunodeficient mice. While immunodeficient mouse models are permissive to adenoviral replication occurring in the xenograft, they do not adequately assess the effect of viral dissemination and replication on other tissues, and the host immune responses to the virus and related toxicity. The inflammatory responses induced by adenovirus are attenuated in these animals (Zhang et al., 2001). In order to better assess factors important to the efficacy of oncolytic adenovirus vectors, a preclinical model that utilizes a syngeneic transplantable tumor in an immunocompetent host would be advantageous.

Cotton rats (*Sigmodon hispidus*) are susceptible to infection with a number of human respiratory viruses including Group C adenoviruses (Niewiesk and Prince, 2002). Cotton rats exhibit similar inflammatory pathology to humans with the virus actively replicating in the lungs, tracheobronchial tree, nasal passages and cornea (Pacini et al., 1984; Prince et al., 1993; Tsai et al., 1992). This has resulted in their use to assess the *in vivo* spread and toxicity of adenoviral-based vectors prior to their use in human gene therapy trials (Rojas-Martinez et al., 1998). Until recently syngeneic transplantable cotton rat tumor cell lines were unavailable. We studied three recently isolated tumorigenic cell lines derived from spontaneous tumors of cotton rats, CCRT, LCRT (Toth et al., 2005) and VCRT. Subcutaneous implantation of these cell lines leads to the formation of tumors in immunocompetent cotton rats. We evaluated each of the tumor cell lines for permissiveness for adenoviral infection and replication and assessed their efficacy as an *in vivo* model for human adenovirus virotherapy for cancer.

Results

CCRT, LCRT and VCRT cotton rat tumor cells are readily infected with adenovirus type-5 and express encoded transgenes

The *in vitro* ability of the cotton rat tumor cell lines to be infected with adenovirus was examined using Ad.GFP, an E1-deleted replication-deficient vector expressing green fluorescent protein. The cotton rat tumor cells were compared to mouse 15-12RM sarcoma cells and TS/A mammary carcinoma cells and human Saos-2 osteosarcoma and A549 lung cancer cells (Fig. 1). LCRT cells were the most readily infected of the cotton rat tumors lines, with 99.3% of the cells expressing GFP at an MOI of 100 PFU/cell as detected by flow cytometry. This was similar to the human cell lines examined, with 99.5% of Saos-2 cells and 98% of A549 cells expressing GFP. At MOI 100 PFU/cell, VCRT and CCRT cells also showed high levels of expression with 90.6% and 88.5% cells expressing GFP, respectively. At

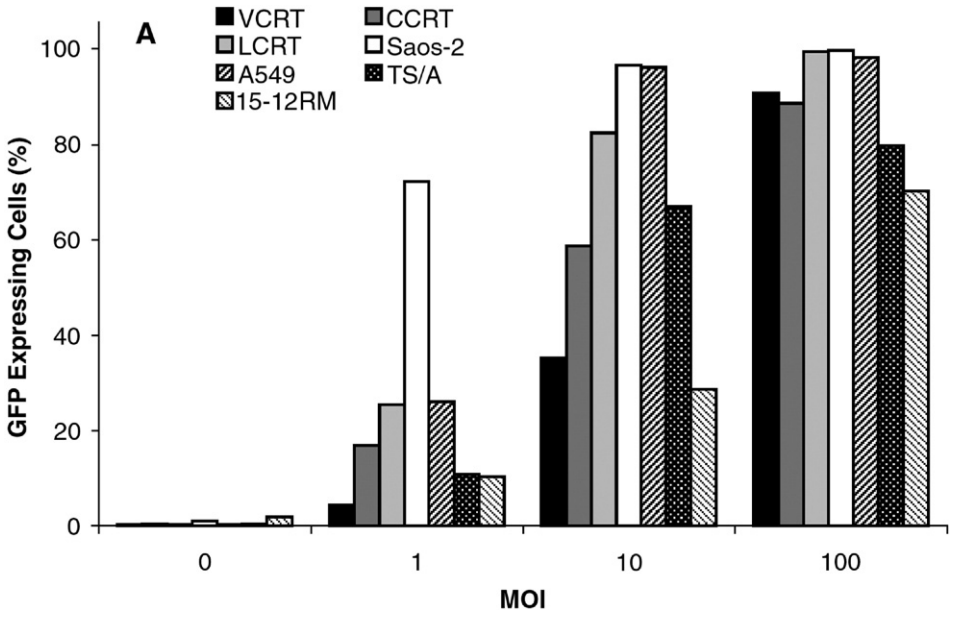

Fig. 1. Transgene expression in cotton rat tumor cells infected with a non-replicating adenovirus expressing GFP. CCRT, LCRT and VCRT cotton rat tumor cells, A549 and Saos-2 human cancer cell lines and TS/A and 15-12RM murine cancer cells were infected with Ad.GFP at MOI 1, 10 and 100 and the percentage of cells expressing GFP was determined at 48 h by flow cytometry.