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Treatment of ovarian cancer with a novel dual targeted conditionally replicative adenovirus (CRAd)

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Abstract

Objectives. Current virotherapy strategies for ovarian cancer have been hampered by limitations in target cell infectivity and nonspecific tissue replication. In an effort to circumvent these limitations, we evaluated various CRAds modified to incorporate novel capsid targeting motifs (RGD and chimeric Ad5/3) with a novel tissue-specific promoter (CXCR4).

Methods. Two novel CRAds (Ad5-CXCR4-F5/3 and Ad5-CXCR4-RGD) were constructed via homologous recombination and verified by PCR and DNA sequencing. The infectivity and viral replication rates of these two CRAds were analyzed via quantitative real-time PCR (QRT-PCR) in cell line experiments using three ovarian cancer cell lines (SKOV3.ip1, Hey, and OV4) and compared to that achieved with a clinical grade CRAd (Δ24-RGD) to be evaluated in a Phase I trial. Cytocidal effects were determined by crystal violet staining in these same cell lines infected with different concentrations of viral particles per cell (0, 0.1, 1, 10, 100, and 500). Additionally, viral replication was evaluated by QRT-PCR in primary ovarian cancer tissue slices from multiple patients with ovarian cancer as well as in primary human normal liver tissue slices in order to establish CRAd selectivity. All experiments incorporated appropriate controls and repeated in triplicate.

Results. Compared to RGD-capsid CRAds (Δ24-RGD and CXCR4-RGD), the F5/3-capsid CRAd (CXCR4-F5/3) demonstrated significant improvements in infection rates ($p=0.025$, 0.006, and 0.006) in all ovarian cancer cell lines tested (SKOV3.ip1, Hey, and OV4, respectively). In addition to improved transduction of virus into the cells, the TSP CXCR4-based CRAds demonstrated improved viral replication. Specifically, CXCR4-F5/3 further enhanced viral replication 89-fold ($p=0.009$, 0.010, 0.003) in the same cancer cell lines. Furthermore, CXCR4-F5/3 showed a 4-log improvement in oncolytic potential over Δ24-RGD. In the *ex vivo* primary ovarian tissue slices, CXCR4-F5/3 showed a 58-fold improvement in viral replication ($p=0.005$) compared to the clinical grade Δ24-RGD. Both CXCR4-F5/3 and CXCR4-RGD demonstrated significant reduction of viral replication in normal liver slices ($p=0.001$).

Conclusions. These data suggest that a dual targeted approach is feasible for the combined enhancement of infectivity and replication in ovarian cancer with a specificity that was attenuated in normal liver tissues. In fact, CXCR4-F5/3 outperformed our best CRAd agent to date nearly 60-fold in our most stringent *ex vivo* model of primary ovarian cancer tissue slices and suggests that this novel agent could be useful for the treatment of ovarian cancer.

Keywords: Ovarian cancer; Gene therapy; Virotherapy; Adenovirus; CXCR4; Tumor-specific promoter; Capsid modification

Introduction

Greater than 20,000 women are expected to be diagnosed with ovarian cancer during the year 2006 in the United States alone [1]. Although the majority of patients will present with advanced disease, most will respond to cytoreductive surgery and first-line platinum-based combination chemotherapy.

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Despite this response, effective curative therapy has yet to be determined and thus many patients with advanced disease will experience a recurrence and unfortunately succumb to progressive disease [2–4]. In this regard, novel therapeutics, such as virotherapy, are currently being sought for the treatment of advanced ovarian cancer. Specifically, virotherapy utilizing conditionally replicative adenoviruses (CRAd) represents one such advanced modality for the treatment of ovarian cancer.

Despite the promise of CRAds, clinical outcomes with virotherapy have been disappointing to date. Two limitations restricting the clinical efficacy include imprecise tumor cell replicative specificity and inefficient tumor cell infectivity. The recognition of these limits has lead to strategies designed to address these barriers. On this basis, advanced generation CRAd agents have been proposed, which seek to achieve improved replicative specificity and/or enhanced infectivity.

For improving tumor cell replication specificity, tumor-specific promoters (TSPs) selectively drive adenoviral E1 gene expression in tumor cells and thereby accomplish transcriptional targeting based upon the induced specificity of viral replication [5]. The optimal TSP restricts replication to tumor cells alone, thereby having the highest activity in tumor cells ("tumor on") and lowest activity in normal cells ("normal off"). Of note, adenoviral vectors possesses a predilection for hepatocytes thereby making hepatotoxicity the dose-limiting toxicity for these vectors. In this regard, the ideal TSP-directed adenovirus would have the highest "tumor on/liver off" ratio and thus possess the optimal therapeutic index. One such promoter, CXCR4 has been recently investigated as a good candidate for cancer gene therapy. Several authors have demonstrated that CXCR4 gene expression has been undetectable in normal ovarian epithelial cells but markedly upregulated in ovarian cancer cell lines and primary ovarian cancers [6–8]. In our previous work, we have reported that the CXCR4 promoter had a "tumor on" and "liver off" phenotype in both *in vitro* and *in vivo* experiments in ovarian cancer [9]. In other studies, the therapeutic index of the CXCR4 promoter was evaluated with other promoters commonly evaluated in ovarian cancer (survivin, SLPI, and Cox-2) (Makhija, personal communication). In these studies, the CXCR4 promoter demonstrated a superior therapeutic index with greater activity in ovarian cancer cell lines and primary ovarian tumor, while demonstrating low levels of activity in human liver tissue. Thus, the CXCR4 promoter appears to be an excellent candidate for use in transcriptional targeting for ovarian cancer gene therapy.

For enhancing tumor cell infectivity, the targeting adenoviral vector to tumor cells via alternative pathway is of importance. Native Ad5 tropism is mediated by two capsid proteins: the fiber and the penton base. These proteins bind to the primary high affinity cellular receptor, coxsackie–adenovirus receptor (CAR), and to the integrins $\alpha v\beta 3$ and $\alpha v\beta 5$, respectively. Tumor is resistant to adenovirus infection due to a relative paucity of the primary receptor CAR on tumor cell surface [10–12]. Based on these molecular interactions, a concerted effort has been made to modify Ad5 tropism, resulting in enhanced tumor cell transduction by retargeting cellular entry through heterologous pathway or CAR-independent pathway. An example of this is the utilization of RGD motif in the fiber knob of the Ad. This capsid modification appears to facilitate Ad binding and entry into tumor cells via integrin receptors that are abundantly expressed on tumor cells [13,14]. Additional capsid modifications have been explored to obtain infectivity enhancement of Ads including AdF5/3, which substituted the Ad5 fiber with the Ad3 fiber [15,16], Ad5-pk7 in which a motif polylysine (pk7) was genetically inserted at C terminus of Ad fiber and bound to heparin sulfate-containing receptor [17], and Ad5-CK in which canine Ad knob was used to replace Ad5 knob [18]. In this study, we utilized two capsid modifications (RGD and F5/3) that target to integrins and CD80, 86, and 46, respectively. These modifications enhance the viral infectivity via a CAR-independent pathway [19,20].

Our objective was to improve the viral-specific replication in tumor cells and enhance tumor cell infectivity via an optimal CRAd for the treatment of ovarian cancer. We constructed two novel CRAds (Ad5-CXCR4-RGD and Ad5-CXCR4-F5/3) in which the viral replication is under the control of the CXCR4 TSP and the viral infectivity enhanced via the capsid modifications (RGD and F5/3). Both tumor specificity and infectivity of these novel CRAd agents were evaluated and compared with Δ24RGD. Δ24RGD is a first-generation CRAd that utilizes a partial deletion (24 base pair) of the E1 region which allows a relative selectivity by depending upon the target cells' machinery to complete replication. This CRAd has been approved for use in an NIH-funded Phase I trial.

Materials and methods

Cells and tissues

Human ovarian tumor cell lines, SKOV3.ip.1, OV4, and Hey were cultured in medium suggested by ATCC. 911 cells (a kind gift from Dr. Van Der Eb, Leiden University, The Netherlands) were maintained in Dulbecco's modified Eagle medium. Each medium was 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.

Following IRB approval, human ovarian specimens were obtained for tissue slices from epithelial ovarian cancer remnants not needed for diagnostic purposes during primary cytoreductive surgery performed at the University of Alabama at Birmingham (UAB). Time from harvest to tissue slicing was kept at an absolute minimum (<2 h). To generate tissue slices, a coring device (Alabama Research Development, Munford, AL) was used to create an 8-mm diameter core of tissue from the ovarian cancer. This core was cut in consecutive 0.25-mm-thick slices using the Krumdiek tissue slicer (Alabama Research Development, Munford, AL) with the reciprocating blade at 30 rpm. Sequential slices were then cultured in 12-well plates in RPMI medium supplemented with 10% bovine fetal serum, 100 U/ml of penicillin, and 100 μg/ml of streptomycin.

Human liver samples were obtained (Department of Surgery, University of Alabama at Birmingham) from three seronegative donor livers prior to transplantation into recipients. All liver samples were flushed with University of Wisconsin (UW) solution (ViaSpan, Barr Laboratories, Inc., Pomona, NY) before harvesting and kept on ice in UW solution until slicing with the Krumdiek tissue slicer. Time from harvest to slicing was kept at an absolute minimum (<2 h). Liver slices were placed into 6-well plates (1 slice per well) containing 2 mL of complete culture media (William's Medium E with 1% antibiotics, 1% L-glutamine, and 10% bovine fetal serum).