A Novel Conditionally Replicative Adenovirus (CRAd) Increases Infectivity Efficiency and Oncolytic Activity in a Human Pancreatic Adenocarcinoma Cell Line

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Abstract

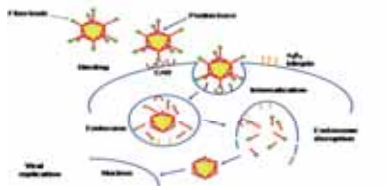
Introduction: Cancer virotherapy has become an area of focus in developing new therapeutic approaches for pancreatic and other adenocarcinomas. We replaced the fiber gene of the Ad serotype 5 vector with an Ad serotype 3 fiber, to increase cancer cell infectivity by a CAR independent pathway. We also used the CXCR4 chemokine receptor gene promoter to regulate expression of the Ad E1A gene, restricting virus replication and oncolytic activity to cells that over express CXCR4. We then tested this novel modified CRAd agent using a human pancreatic cancer cell line. We hypothesize that the novel oncolytic virus will efficiently infect and preferentially lyse pancratic tumor cells.

Methods: The human pancreatic cancer line CFPAC-1 was obtained from ATCC and cultured in DMEM containing 10% FBS. Expression of CXCR4 was determined by Western blot analysis. To determine infection efficiency, CFPAC-1 cells were infected with increasing titers of unmodified and fiber modified Ads expressing the green fluorescence protein (GFP) marker (Ad5-CMV-GFP, Ad5/3-CMV-GFP, Ad5-CMV-GFP-RGD), and after 48 h, GFP expression was determined by flow cytometry. Oncolytic activity in CFPAC-1 was determined using an oncolytic assay. The cells were infected with increasing doses of CRAds from 0.1 to 1000 infectious units/cell. Five days after infection, the percentage of adherent live cells was evaluated by crystal violet staining.

Results: The CFPAC-1 pancreatic cancer cell line demonstrated over expression of CXCR4. The serotype 3 fiber-modified Ad (Ad5/3-CMV-GFP) was most efficient in infecting CFPAC-1 cells compared to an Ad containing an unmodified fiber (Ad5-CMV-GFP) or an Ad containing an RGD motif in the fiber knob (Ad5-CMV-GFP-RGD). A CRAd construct incorporating both the CXCR4 promoter and the serotype 3 fiber (Ad5/3-CXCR4) demonstrated highly effective oncolytic activity in CFPAC-1 cells compared to a CRAd construct containing an unmodified fiber (Ad5-CXCR4).

Conclusion: By modifying the Ad fiber gene and restricting viral replication with the CXCR4 promoter, we demonstrated that our novel CRAd (Ad5/3-CXCR4) had a marked increase in infectivity and oncolytic potential over an unmodified CRAd. Thus, this construct could be broadly exploited for virotherapy of pancreatic cancer.

Adenovirus Infection Cycle



Introduction

Virotherapy using conditionally replicative adenoviruses (CRAds) is an innovative approach, in which a replicating virus itself is the anticancer agent. Ideally, cancer specific replication of CRAds result in viral-mediated oncolysis of infected tumor tissues and release of the virus progeny, capable of further propagating in surrounding tumor cells but not in those of normal tissues. A number of characteristics of the adenovirus (Ad) serotype 5 make it an optimal virotherapy vector. Despite these advantages, overall efficacy of CRAd based cancer virotherapy approaches remains limited by suboptimal vector delivery efficiency in cancer tissues and nonspecific delivery to normal tissues. To circumvent these problems cancer specific Ad fiber modifications as well as tumor specific Ad replication control were tested.

Hypothesis

Substituting the knob domain of the Adenovirus (Ad) serotype 5 with a serotype 3 knob domain and incorporating the CXCR4 promoter to regulate AdE1A gene expression will efficiently transduce and lyse pancreatic tumors.

Methods and Materials

The human pancreatic cancer line CFPAC-1 was obtained from ATCC and cultured in *Iscove's* Modified Dulbecco's Medium containing 10% FBS and 1% fungizone-penicillin-streptomycin. All cells were maintained at 37°C and 5% CO2. Expression of CXCR4 was determined by Western blot analysis. To determine infection efficiency, CFPAC-1 cells were infected with increasing titers of unmodified and fiber modified Ads expressing the green fluorescence protein (GFP) marker (Ad5-CMV-GFP, Ad5/3-CMV-GFP, Ad5-CMV-GFP-RGD). The multiplicity of infection (m.o.i.) was determined at titers of 0.1, 1, 10, 100, and 1000 ifu/cell. A normal cell line was used as a control. After 24 hours the cells were checked under a fluorescent microscope and several pictures were taken. After 48 h, GFP expression was determined quantitatively by flow cytometry. Oncolytic activity in CFPAC-1 was determined using an oncolytic assay. Cells were grown in 96 well plates in media 10% FBS. After 16 hours the cells were infected with increasing titers of unmodified and fiber modified Ads (Ad[GFP], Ad5wT, Ad5[Δ24RGD], Ad5[CXCR4], Ad5/3[CXCR4], Ad5[CXCR4-UTR], Ad5/3[CXCR4-UTR]). The m.o.i.'s were 0.1, 1, 10, 100, and 1000 ifu/cell. A normal cell line was used as a control. The infected cells were incubated for two hours before the media was aspirated and replaced. After five days the experiment was ended and the media was removed. The cells were stained with 30uL of 0.1% crystal violet solution per well for one minute. The plate was then washed three times in distilled water and dried for 48 hrs. Finally, the crystal violet was dissolved in a 200mL methanol/HCL solution. The optical densities were read at the 595nm wavelength, and the percentage of adherent live cells was evaluated.

Oncolytic Virotherapy

- Selective replication in tumor cells constitutes basis of therapeutic effect
- Post-transduction amplification provides means to potentially circumvent non-quantitative tumor transduction

Results

The CFPAC-1 pancreatic cancer cell line demonstrated over expression of CXCR4. The serotype 3 fiber-modified Ad (Ad5/3-CMV-GFP) was most efficient in infecting CFPAC-1 cells compared to an Ad containing an unmodified fiber (Ad5-CMV-GFP) or an Ad containing an RGD motif in the fiber knob (Ad5-CMV-GFP-RGD). A CRAd construct incorporating both the CXCR4 promoter and the serotype 3 fiber (Ad5/3-CXCR4) demonstrated highly effective oncolytic activity in CFPAC-1 cells compared to a CRAd construct containing an unmodified fiber (Ad5-CXCR4).

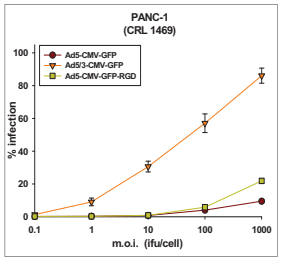
Adenovirus Infectivity of Pancreatic Cancer Cell Lines

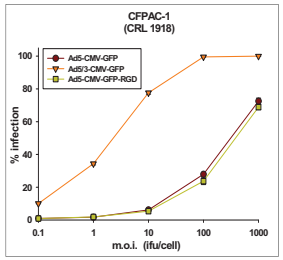
Ad5-CMV-GFP
Ad5/3-CMV-GFP
Ad5-CMV-GFP-RGD

Infect 1h

Cell lines

Flow cytometry after 48 hrs



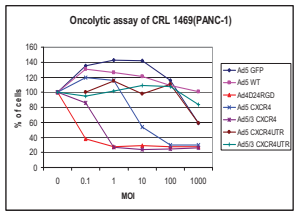


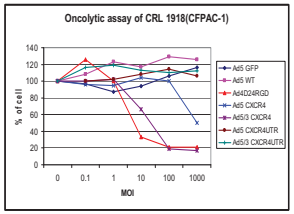
Oncolytic Assay of Pancreatic Cancer Cell Lines

Ad5 GFP
Ad5 WT
Ad4 D24-RGD
Ad5 CXCR4
Ad5/3 CXCR4
Ad5 CXCR4 UTR
Ad5/3 CXCR4 UTR

Infect 1h

Crystal violet staining after 5 days





Conclusions

Modifying the knob domain of the adenovirus serotype 5 with a serotype 3 knob domain resulted in the highest viral infectivity when compared to a wild-type Ad serotype 5 fiber and an RGD motif incorporated into the HI loop of the fiber knob.

Incorporating the CXCR4 gene promoter into the modified CRAd resulted in preferential killing of pancreatic tumors.

Several things to keep in mind when you prepare a poster:

1. You need to have all of the following elements:
   (a) Abstract
   (b) Introduction
   (c) Materials and Methods
   (d) Results
   (e) Conclusion.
2. Some authors will even include references, although this is optional
3. You need to emphasize and make it clear why you did the study such that someone who is not in your field of expertise can understand your study
4. In your introduction, you need to have a HYPOTHESIS
5. Spend the bulk of your poster on Results
6. If applicable, you need to include statistics
7. The first and last authors are the two most important members of the study. Generally, the last author is the senior author (i.e. your mentor)
8. Go over the poster with your mentor(s)