Improved Antitumor Effect of Adenovirus-Mediated Interferon Therapy in Combination with Chemoradiotherapy in a Syngeneic Immunocompetent Hamster Model

Department of Surgery
Basic and Translational Research

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BTR, DIVISION OF BASIC AND TRANSLATIONAL RESEARCH

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Abstract

Despite the emergence of adjuvant interferon-α (IFN) in recent clinical studies as a powerful treatment strategy for pancreatic ductal adenocarcinoma (PDAc), the excess toxicity and insufficient level of IFN in tumor site remain as serious challenges. The application of adenovirus (Ad) as a vector system enables local production of IFN at therapeutic concentration. We hypothesize that the conditionally replicative adenovirus (CRAd) expressing IFN in combination with chemotherapy and radiotherapy would significantly enhance anti-cancer effect of existing IFN modalities while reducing systemic toxicity of IFN. We designed the oncolytic adenoviruses expressing human or hamster IFN (Ad-IFN). To increase adenovirus potency, we enhanced oncolysis through overexpression of adenoviral death protein (ADP) and genetic modifications of the viral capsid (Ad5/Ad3 or RGD).

The significance of our design strategy was exemplified through analysis of the cytocidal effect and tumor specificity in human and hamster PDAc cell lines. The addition of IFN and ADP overexpression to the Ad structure resulted in remarkably improved oncolysis. The IFN concentration increased in a time- and dose-dependent manner. *In vivo* therapeutic experiments were performed in immunodeficient mice bearing human PDAc xenografts and immunocompetent hamster bearing syngeneic pancreatic tumors with a single *i.t.* dose of Ad expressing the same species IFN. Therapeutic effect in immunocompetent hamsters was more evident than in immunodeficient mice, suggesting indirect antitumor effect of IFN as an immunomodulator. In hamster survival studies, a single *i.p.* Ad-IFN injection exhibited significant improvement in survival rate in an extremely aggressive peritoneal dissemination model.

Next, we analyzed potential of IFN as a chemo- and radiotherapy sensitizer and the antitumor effect of combination therapy. *In vitro* assays in human and hamster PDAc cells revealed that combination of Ad-IFN with either 5-FU or radiation killed cancer cells better than either of the single treatments. Furthermore, we established syngeneic pancreatic tumors in hamsters and treated them with a single dose of Ad-IFN followed by radiation (8 and 20 Gy). Ad-IFN combined with radiotherapy showed remarkable decrease in tumor volume and was significantly superior to single treatments of radiation and virus. At day 42, the tumors in the combination group nearly disappeared.

Currently, the combination of Ad-IFN and chemotherapy (5-FU) *in vivo* is being assessed along with the effect of the triple-modal therapy (Ad-IFN + 5-FU + Radiation). Thus, we have the first report of the improved combination effect of Ad-IFN with radiotherapy and 5-FU. These results reinforce the impact of adenovirus-induced IFN expression to sensitize anti-tumor effect of chemotherapy and radiation. Such a strategy will hopefully lead to a more powerful yet better tolerated means of IFN administration in PDAc patients.

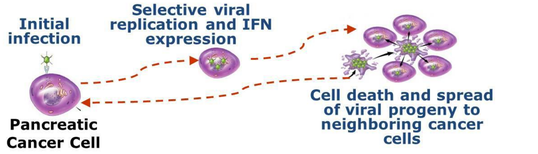
IFNα-based Adjuvant Chemoradiation Therapy Could Be a Powerful Tool for Pancreatic Adenocarcinoma

- Virginia Mason Medical Center: an **improved 5-year survival rate of 55%** in a phase II trial evaluating adjuvant chemotherapy, immunotherapy and external-beam radiation (Picozzi VJ, et al. Am J Surg 2003; Picozzi VJ, et al. Ann Oncol 2011)
- Washington University Medical Center: Phase II study resulted in 56% 2- year survival (Linehan DC et al, Ann Surg. 2008)
- University of Heidelberg, Germany: Phase III trial for adjuvant treatment of pancreatic adenocarcinoma; the first immunomonitoring data (Schmidt J, Marten A. J, et al. Immunother 2007)

Major Concerns Impairing the Clinical Utility of IFNα:

1. **Systemic toxicity of IFNα**
2. Insufficient delivery and unsustainable levels of IFNα in the tumor site

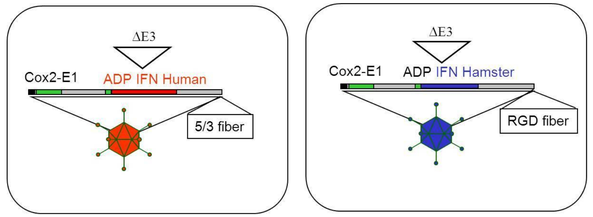
Conditionally-Replicative Adenovirus (CRAd) Expressing IFNα



Main Hypotheses:

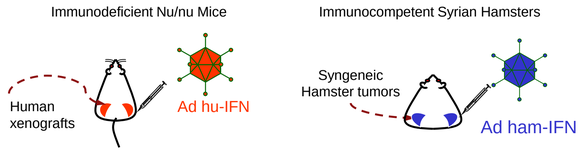
- Adenovirus-mediated IFNα delivery combined with chemoradiation will mitigate the major drawbacks of adjuvant IFN therapy (e.g. systemic toxicity and inefficient delivery of IFNα).
- IFNα may profoundly improve the oncolytic effect of viral therapy when it is combined with chemoradiation.

Conditionally Replication Competent Adenovirus (CRAd) Expressing IFNα for Pancreatic Cancer



- CRAds express syngeneic IFNα. The IFNα gene is linked to the Ad major late promoter.
- Adenoviral E1 is controlled by Cox2 promoter.
- Infectivity-enhanced through fiber modification and over-expression of adenoviral death protein (ADP).

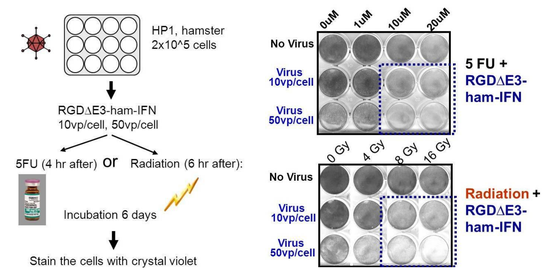
Comparison of Syngeneic IFNα Biological Activity in Two Different Cancer Models



- Ability to establish human cancer xenografts
- Does not permit human Ad replication
- Impossible to study host immune response

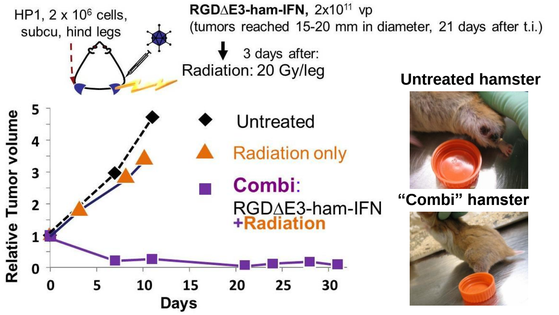
- Hamster tumor only
- Permits Human Ad replication
- Ability to analyze immunomodulatory effect of IFN-expressing Ads

In Vitro Combination Therapy in Hamster Pancreatic Cancer Cells



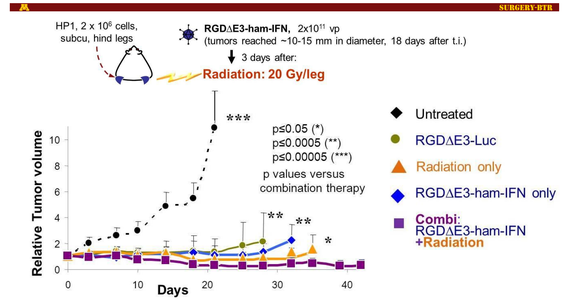
- RGDΔE3-ham-IFN combined with either 5-FU or radiation resulted in improved cytolysis in hamster pancreatic cancer cells

Combination Therapy *In Vivo* (Pilot Study)



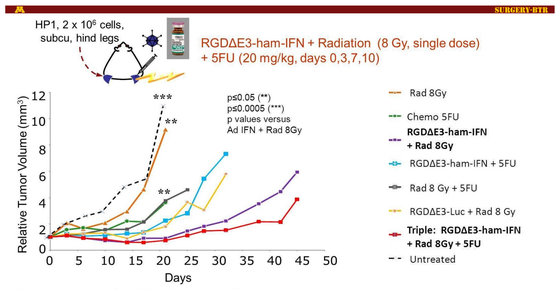
- RGDΔE3-ham-IFN combined with radiation resulted in remarkable HP1 tumor shrinkage

In Vivo Combination Therapy with Radiation (20Gy)



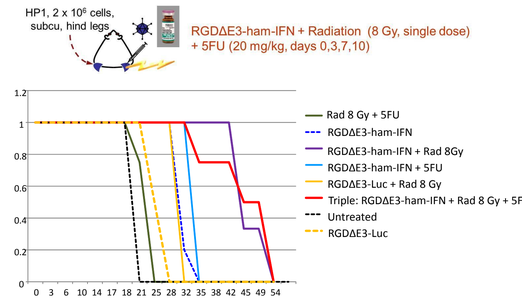
- Combination therapy was significantly superior to either monotherapies

In Vivo Combination Therapy with Radiation (8Gy) and 5FU



- Combination of RGDΔE3-ham-IFN and radiation was superior to radiation and chemotherapy alone or both of these combined.
- Triple-therapy outperformed all dual-therapy groups

Improved Survival Rate with Combination Therapies



- The evaluation of the survival rate showed great improvement in groups treated with dual (RGDΔE3-ham-IFN+Rad 8 Gy) and triple therapies.

SUMMARY

1. **IFN-expressing oncolytic Ad exhibits multiple, integrated antitumor effect.**
2. **IFN-expressing oncolytic Ad sensitizes chemo- and radiotherapy.**
3. **Infectivity-enhanced, tumor-selective oncolytic adenovirus expressing syngeneic IFNα is a promising therapeutic modality for pancreatic cancer**

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"The project described was supported by P50CA101955 from the National Cancer Institute (UAB-UMN SPORE in Pancreatic Cancer Career Development Award, Davydova), NIH R01CA094084 (Yamamoto), and by NIH/NIMHD through the P60 MD003422 "Center for Health Equity".