A Novel Gene Therapy in Combination with Chemotherapeutic to Treat Pancreatic Cancer

Joohee Han, Leonard Armstrong, M.D., Eric Brown, B.S., Selwyn Vickers, M.D., Masato Yamamoto, M.D., Ph.D., and Julia Davydova, M.D., Ph.D.

Division of Basic and Translational Research, Department of Surgery, University of Minnesota, Minneapolis, USA

Introduction

Pancreatic cancer is one of the deadliest diseases without a cure. In 2010, an estimated 43,140 people were diagnosed with pancreatic cancer and 36,800 death occurred in the U.S.[1] Approximately 85-90% of newly diagnosed patients have an inoperable pancreatic cancer due to invasion and metastasis[2]. Those few patients eligible for surgery, the median length of survival is 13.8 months. Diagnosed with unresectable pancreatic cancer, the average length of survival is less than one year.

Even after treatments with the well-established chemotherapeutic agent, 5-Fluorouracil (5-FU), 95-97% of the patients will not be alive within five years.

Demonstrated in recent clinical studies, interferon-α (IFN) therapy in conjunction with 5-FU improved survival and emerged as a promising treatment strategy. However, systemic toxicity and unsustainable level of IFN in tumor sites remain serious challenges.

To further analyze the efficacy of combination therapy, we designed and cloned a novel, Cox2-promoter-dependent adenovirus (Ad) expressing IFN as a vector to deliver high dosage of IFN to only the pancreatic cancer cells, leaving normal cells intact.

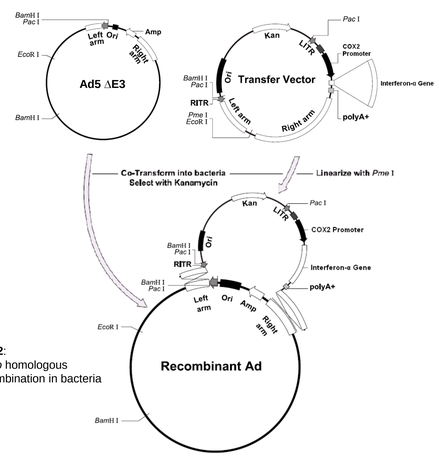
Hypothesis

We hypothesize that the combination therapy with 5-FU and Ad expressing IFN will significantly enhance the selectivity and anti-cancer effect of existing IFN-based regimens, while reducing toxicity to healthy tissues.

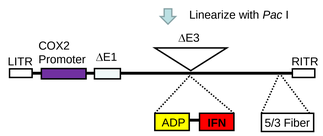
Methodology

Molecular Cloning of Recombinant Ad Expressing IFN

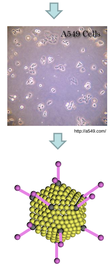
Step 1:
cDNA cloning in transfer plasmid



Step 2:
In vivo homologous Recombination in bacteria

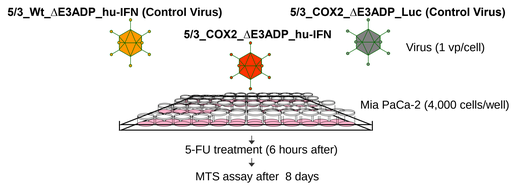


Step 3:
Virus production in A549 cells



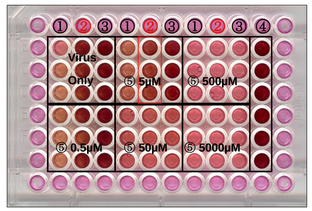
Ready to be amplified recombinant adenovirus

MTS Assay Showed Superior Anti-cancer Effect of Ad expressing IFN Combined with 5-FU



Results

Quantifying Cytotoxicity by MTS Assay



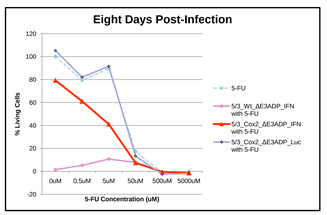
① 5/3_Wt_ΔE3ADP_IFN

② **5/3_COX2_ΔE3ADP_IFN (IFN-Ad)**

③ 5/3_COX2_ΔE3ADP_Luc

④ Pancreatic Cancer MiaPaCa2 Cells Only

⑤ 5-Fluorouracil



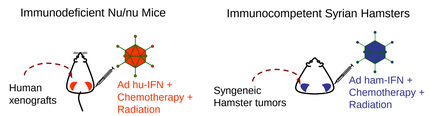
- The lone treatment of 5μM of 5-FU resulted in 10% of cell death.
- The lone IFN-Ad therapy resulted in 21% of cell death.
- In combination, the IFN-Ad with 5μM of 5-FU killed 59% of pancreatic cancer cells.

Conclusions

- Successfully cloned, amplified, and purified Ad expressing IFN, 5/3_COX2_ΔE3ADP_IFN.
- The novel adenovirus has been proven to be superior compared to the identical adenovirus expressing Luciferase in killing pancreatic cancer cells.
- Adenovirus expressing interferon-α in combination with 5-Fluorouracil exhibited the greatest oncolysis than either of the treatments alone.

Future Direction

- *In vitro* efficacy on various pancreatic cancer cell lines employing different chemotherpeutics, such as Gemcitabine and Cisplatin.
- *In vivo* studies with immunodeficient Nude Mice and immunocompetent Syrian Hamsters.
- Assess multimodal therapy by combining IFN-Ad virus therapy, chemotherapy, and radiation therapy.



References

1.Cancer facts & figures 2010 [Internet]. Atlanta: American Cancer Society; 2010. Available from: http://www.cancer.org/Research/CancerFactsFigures/CancerFactsFigures/cancer-facts-figures-2010-rev.
2.Merchant NB, Parikh AA, Liu EH. Adjuvant chemoradiation therapy for pancreas cancer: Who really benefits? Adv Surg. 2010;44:149-64.

Acknowledgments

Funding for this project was provided by NIH/NIMHD through the P60 MD003422 "Center for Health Equity", UAB/UMN SPORE in Pancreatic cancer CDA UCMED09060 (Davydova), NIH R01CA094084 (Yamamoto)

CENTER FOR HEALTH EQUITY

UNIVERSITY OF MINNESOTA
Driven to Discover℠