INTRODUCTION
Prostate Cancer as a Public Health Problem
- Prostate cancer (PCa) is the second most common cancer in American men.
- It is predicted that in 2013 there will be 238,990 new cases and 32,270 men will die of the disease.
- Survival rate is nearly 100% when caught in early stages but falls to only 28% by the time it progresses to metastatic stage IV PCA.
- Identification of prognostic indicators of this aggressive disease will decrease mortality and improve patient outcomes.

TGF-β Pathway as a Potential Target for PCa
- The TGF-β pathway targets many downstream genes and transcription factors that control the cell cycle as well as cycle arrest and apoptosis. This makes it a strong target for aggressive stage IV metastatic cancer.
- Additionally, limited studies suggest miRNA 885-5p functions as a tumor suppressor. It has been suggested that this miRNA targets SMAD2 altering its expression. SMAD2 is also a target of the TGF-β signaling pathway.

OBJECTIVES
- To measure the expression of the downstream targets of the TGF-β pathway in various prostate cancer cell lines compared to normal prostate epithelial cells.
- To determine the effect of overexpression of miRNA 885-5p on levels of expression of end targets of the TGF-β signaling pathway in PCa cell lines.

HYPOTHESIS
- We hypothesize that expression levels of p300, p107, SP1, E2F4, E2F5, Casp8, and Casp9 compared to normal prostate epithelium will be decreased in stage IV prostate cancer since these targets control the cell cycle and apoptosis.
- Additionally, we believe that overexpression of miRNA 885-5p in stage IV PCa will restore normal levels of expression of those same targets.

CLINICAL RELEVANCE
- The results of this study will serve to further the knowledge of PCa progression and yield further insight into targets for treatment of this aggressive disease.

METHODS
Total RNA Isolation

RESULTS
Figure 1. Gene expression of TGF-β downstream targets

Figure 2. Flow cytometry of transfected and untransfected cells

Figure 3. Overexpression of mir885-5p in PC-3 cells

Table 1. Cell Lines

Table 2. TGF-β Pathway Targets

CONCLUSIONS
Relative to normal prostate epithelial cells:
- p107, SP1, E2F4 were all down-regulated in Stage IV PC-3 prostate cancer cell line.
- This was expected as these genes are transcription factors involved in the regulation of cell cycle and apoptosis signaling. Down-regulation of these targets explains the progression from normal prostate epithelium to tumorigenic cell lines.
- However, E2F5 and Casp8 were significantly up-regulated in stage IV prostate cancer cell lines.
- These results are contrary to what was expected. To fully understand the meaning of these results, further investigation will be required.
- In conclusion, it does appear that the TGF-β pathway does play a role in the progression of normal prostate epithelium to stage IV metastatic prostate cancer. Additionally, transfection with mir885-5p did not yield differential expression of cell-cycle signaling and apoptosis genes.

Future Directions
- Transfect E006AA, DU145, and LNCaP cell lines with mir885-5p and measure the expression of the previously studied downstream targets of the TGF-β pathway.
- Perform western blot analysis of protein expression since gene expression is not a reliable indicator of protein expression.
- Perform motility assays of transfected and untransfected cell lines.
- Develop and perform an apoptosis assay to compare survival of transfected and untransfected cells.

ACKNOWLEDGEMENTS
- NCI R25 Cancer Education Grant to D.W. Hein (CA134283)
Developing Computational Tools for Molecular Comparison and Metabolic Placement of Detectable Uncharacterized Metabolites

Joshua M. Mitchell and Hunter N.B. Moseley
Department of Chemistry, University of Louisville, KY USA

Abstract

Design and identification of metabolites is key to modeling and understanding complex cellular and extracellular metabolic networks. Allostery in metabolism, especially in ultra-low resolution mass spectrometry (MS) analysis of the metabolome, presents a major challenge in recognizing a large number of metabolites. We present a novel approach to this problem by developing a new class of fingerprinting methods for accurate, rapid, and cost-effective identification of metabolites.

Materials and Methods

Project development has been divided into 2 phases (Figure 3).

1. Development of MOL file parser to convert MOL files into a common internal representation.

2. Development of a binary fingerprinting method to identify functional groups in databases and predict the likelihood of a metabolite sharing those groups.

Development of MOL file parser to convert MOL files into a common internal representation.

- This phase of the project will leverage the work of our collaborators from Checkmol and KEGG Compound databases. All existing metabolites will be analyzed and their structure resolved.

- MOL files are generated by registering the database structural information to a common file format.

- During our research, we have identified a variety of open-source, low-cost, and free tools that can be used to perform this task.

- For example, we have identified a tool called Checkmol that can be used to process MOL files from different databases.

- The MOL file parser will generate a new file format that can be used to efficiently store and analyze metabolite fingerprints.

- This will allow for the rapid comparison of metabolite structures and the identification of potential functional groups.

- This phase will be completed by the end of the project.

Results

The MOL file parser has been successfully developed and is now in use.

- The results have been incorporated into the project plan.

- The new file format has been validated and is now being used.

- The tool has been made available to the public.

- The project is currently in its final stages.

- The final report will be submitted soon.

- The project will be presented at several conferences.

- The project will be published in a peer-reviewed journal.

Discussion

The MOL file parser has been successfully developed and is now in use.

- The results have been incorporated into the project plan.

- The new file format has been validated and is now being used.

- The tool has been made available to the public.

- The project is currently in its final stages.

- The final report will be submitted soon.

- The project will be presented at several conferences.

- The project will be published in a peer-reviewed journal.

- Future Directions

- We will continue to develop and refine our computational tools for molecular comparison and metabolic placement.

- We will continue to evaluate and compare our methods to other existing methods.

- We will continue to explore the potential applications of our methods in the field of metabolomics.

- We will continue to collaborate with other researchers and organizations to further develop and refine our methods.

- We will continue to seek funding to support our research.

References


Enhancement of Oncolytic Adenovirus Therapeutic Efficacy by Combination with Temozolomide

Jonathan Nitz\(^1\), Sam H. Zhou\(^1, 2\), Kelly M McMasters\(^1, 2\), Jorge G. Gomez-Gutierrez\(^1\)

\(^1\)Departments of Surgery, \(^2\)James Graham Brown Cancer Center

School of Medicine, University of Louisville

**Introduction**

Adenoviral therapy is especially promising for lung tumors because adenoviruses have a natural predilection for the lung, making inhalational therapy feasible. Adenoviruses with deletion of the E1b gene have been used in clinical trials to treat cancers that are resistant to conventional therapies. The efficacy of viral replication within cancer cells determines the results of oncolytic therapy. Oncolytic adenovirus lacking the E1b gene induces autophagy in lung cancer cells. Inhibition of autophagy with 3-methyladenine (3MA) resulted in a decreased synthesis of adenovirus structural proteins, and thereby a poor viral replication; promotion of autophagy with rapamycin increased adenovirus yield. This indicates that adenovirus-induced autophagy correlates positively with virus replication and oncolytic cell death. These results further suggest that the chemotherapeutic agent, temozolomide (TMZ), which increases cancer cell autophagy, may improve the efficacy of oncolytic virotherapy. In this study an oncolytic adenovirus lacking E1B gene (Adhz60) was combined with TMZ and the lung cancer killing effect was assessed. It was found that TMZ increased adenovirus early proteins which resulted in increased virus yield, and combination therapy induced synergistic lung cancer killing effect in comparison with cells treated with only Adhz60 or TMZ, there was a close association between increased E1A expression and increased accumulation of autophagy marker LC3-II in cells infected with Adhz60 and treated with TMZ. These results suggest that combination therapy of oncolytic adenovirus and TMZ is a promising approach for lung cancer therapy.

**Results**

![Fig. 1](Fig. 1.png)

**Fig. 1** Autophagy inhibited and promoted

![Fig. 2](Fig. 2.png)

**Fig. 2** A) Cytopathic effect. B) Adenovirus yield. C) MTT assay. D) Expression to E1A, autophagy marker LC3-I and II and actin.

![Fig. 3](Fig. 3.png)

**Fig. 3**

A) MTT Assay
B) Apoptosis markers

![Fig. 4](Fig. 4.png)

**Fig. 4**

(-) TMZ WI-38 (+) TMZ

**Conclusions**

In summary, Our preliminary findings indicate that TMZ enhances oncolytic adenovirus replication which resulted in a more efficient virotherapy. In addition, combination of Adhz60 with TMZ induced a synergistic cancer cell killing effect in non-permissive lung cancer cells. The mechanism by which TMZ enhanced virotherapy efficiency suggest an association with TMZ-induced autophagy. Thus combination therapy of oncolytic adenovirus and TMZ is a promising approach for lung cancer therapy.

**Acknowledgements**

This work was supported by Award Numbers R01CA129975 (HSZ), R01CA80784 (KMM), and grant R25- CA-134283 from the National Cancer Institute and GMB081410 (KMM & HSZ) from the Kentucky Lung Cancer Research Program.
GERD-Related Symptom Assessment in Subjects with Malignant Dysphagia Receiving Esophageal Stents

D. Alan North, Melissa Schlegel, Robert CG Martin, II, M.D., Ph.D
Division of Surgical Oncology, Department of Surgery, University of Louisville School of Medicine

Introduction
- The use esophageal stents at the GE junction in patients with malignant dysphagia has been widely challenged as to the impact it has on reflux related QOL symptoms.
- Confounding factors include patient awareness of reflux in light of dysphagia relief post-stent, and the ability to distinguish true reflux vs regurgitation of obstructed food contents.
- The purpose of our study is to assess reflux related and overall QOL symptoms, while for the first time incorporating a prospective, validated questionnaire in patients undergoing GE junction stenting for adenocarcinoma.

Methods
- IRB approved prospective clinical trial using a validated GERD assessment (GERD-HRQL) and dysphagia assessment
- The main goal of the questionnaire was to evaluate pre and post-stent heartburn related quality of life symptoms.

Acknowledgements
Supported by grant R-25-CA-134283 from the National Cancer Institute

RESULTS:
- Dysphagia: Demonstrated improvement, trending toward statistical significance (p=0.063) with 10 patients reporting absent to mild dysphagia and only 1 patient reporting moderate to severe dysphagia post-stent, compared with 6 patients reporting moderate/severe dysphagia pre-stent.
- Heartburn: All patients reported minimal to no symptoms post-stent compared with 2 patients suffering from moderate to severe heartburn pre-stent.
- Regurgitation: Improvement trending toward statistical significance (p=0.086) with 8 patients reporting no regurgitation, 2 minimal, and 1 reporting moderate regurgitation symptoms post-stent.
- Combined quality of life scores demonstrate a significant improvement in quality of life from pre-stent with an overall QOL score pre-stent of 168 ± 8.98 and an overall QOL score post-stent of 62 ± 6.48 (P=0.001) with a lower score representing a better quality of life.

Conclusions
Reflux related quality of life symptoms are minimal to nonexistent following esophageal stent placement and proton pump inhibitors for palliation of malignant dysphagia at the GE junction. Esophageal stenting should become the initial and primary mode of palliation in ALL patients who present with GE junction obstruction.
Abstract

Background and Objective: Therapeutic drugs for pancreatic adenocarcinoma are limited because of off-target toxicity. We hypothesize that preferential targeting by ligand-guided liposomes will allow delivery of therapeutic concentrations while limiting undesirable toxicity. This study evaluates the ability to target S2VP10 and PANC-1 pancreatic adenocarcinoma cells with Syndecan-1 ligand-targeted liposomes binding to the IGF-1R receptor.

Methods: S2VP10 and PANC-1 cells were evaluated for IGF-1R expression using Western blot. IGF-1R-targeted liposomes encapsulating a 750-NIR dye were constructed using Syndecan-1 ligand as the targeting ligand for IGF1-R. Binding of targeted liposomes was compared to non-targeted liposome using flow cytometry. S2VP10 or PANC-1 pancreatic adenocarcinoma cells were orthotopically implanted into SCID mice. When tumors reached 3mm diameter, 200 μL of 5 OD Syndecan-1 liposomes were injected by iv. Fluorescent imaging confirmed targeted liosome binding in vivo and of the pancreas and liver ex vivo.

Results: IGF-1R was expressed by S2VP10 cells at higher levels than PANC-1 cells as observed by western blot. Flow cytometry showed 86% and 80% uptake for S2VP10 and PANC-1 cells, respectively. Fluorescence imaging showed peak S2VP10 pancreas tumor liposome uptake at 24 hours and ex vivo uptake within the pancreas tumor but not in the liver.

Conclusions: These experiments suggest that Syndecan-1 ligand-targeted liposomes would improve tumor targeting and delivery of therapeutic concentrations of drugs to pancreatic adenocarcinoma tumors.

Introduction

Drugs capable of killing pancreatic adenocarcinoma cells are often only effective at dosages that are toxic to healthy cells. Liposomes are artificially prepared vesicles with a lipid bilayer that are typically only a few micrometers in diameter. They can be loaded with a drug or dye and prepared with surface ligands that can target the liposome to specific cell surface receptors. Once the liposome attaches to a target cell, the lipid membrane fuses with the cell membrane, delivering the internal payload to the cytoplasm directly, allowing therapeutic drug concentrations to be delivered without lethal toxicity to healthy cells. This experiment analyzes successful targeting of ligand-guided liposomes to pancreatic adenocarcinoma cells.

Results

Figure 1. Western blot demonstrates IGF-1R relative abundance in four pancreatic cancer cell lines. U251 (positive control) and ES-2 (negative control) were also evaluated. S2VP10 cells expressed IGF-1R at the highest levels of the pancreatic cell lines evaluated.

Figure 2. Liposome binding to tumor cells was measured using flow cytometry. Binding of control dye, non-targeted liposomes, and Syndecan-1 liposomes were evaluated in S2VP10, PANC-1, and control cancer cells. Binding of Syndecan-1 targeted liposomes was highest in S2VP10 cells (80%) while binding of non-targeted liposomes was relatively low.

Figure 3. Syndecan-1 targeted liposomes accumulation within pancreatic tumors was evaluated using NIR-fluorescence imaging. Mice were evaluated for liposome accumulation at 12 hours post injection. Pancreatic tumors were identified using bioluminescence imaging. Un targeted liposomes primarily accumulated in the bladder. Syndecan-1 targeted liposomes accumulated within the pancreatic tumor.

Conclusions

• IGF-1R is expressed at high levels in both S2VP10 and PANC-1 cells (higher in S2VP10).

• IGF-1R can be effectively targeted in vitro on S2VP10 and PANC-1 cells by liposomes using Syndecan-1 as a targeting ligand.

• S2VP10 and PANC-1 pancreatic tumors can be preferentially targeted by Syndecan-1 liposomes in vivo.

• Use of Syndecan-1-guided liposomes shows promise for improving the ability to deliver therapeutic drug concentrations to pancreatic adenocarcinoma tumors.

Future Directions

Syndecan-1 targeted liposomes accumulated within the tumor, thus indicating its potential as a drug delivery vehicle. Future goals will include encapsulation of chemotherapy agents within the Syndecan-1 targeted liposomes to evaluate treatment efficacy for pancreatic adenocarcinoma.

Acknowledgements

Research supported by grant CA139050 and R25- CA134283 from the National Cancer Institute as well as the School of Medicine Summer Research Scholar Program.