Background: Breast cancer (BCA) affects one in eight women in the US. Doxorubicin (Dox) and Trastuzumab (Tz) remain prevalent chemotherapies for breast cancer, but cause cardiotoxicity with significant morbidity and mortality in a subset of patients. The current study is a sub-study of an ongoing prospective observational study investigating if specific cardiaco factors, growth factors, genetic polymorphisms and self-reported physical activity can predict which women will develop cardiac dysfunction from chemotherapy. The sub-study of PACE was aimed at characterizing the self-reported physical activity during first three months of chemotherapy.

Methods: In a prospective, longitudinal study, 132 newly diagnosed breast cancer women receiving either AC or Tz were enrolled over a 4-year period. Baseline data on age, BMI, personal history of hypertension, hyperlipidemia, diabetes mellitus, tobacco use, and coronary artery disease, family history of cardiomyopathy and self-reported physical activity at enrollment and at 4 time-points during first three-months of chemotherapy were ascertained. Enrolled participants were given baseline physical activity questionnaire and 4 additional CHAMPS questionnaire during chemotherapy (validated International Physical Activity Questionnaire – IPAQ and CHAMPS questionnaire respectively). CHAMPS questionnaire reported the various forms of physical activity in metabolic equivalent for task per hour per week (MET-hr/wk). Complete questionnaires from 86 patients were analyzed.

Results: The mean age of this cohort was 50 years old with a Caucasian predominance. Women average stage of breast cancer was Stage II. Physical activity significantly decreased in Dox treatment in compared to TzS. However looking at the combined group of Dox and TzS there were a statically significant trend showing a overall decrease in self-reported physical activity.

Conclusions: Women enrolled in PACE have a high number of cardiovascular risk factors (hypertension, hyperlipidemia, and overweight). Our study demonstrates that most women describe a decrease in their physical activity during chemotherapy. It remains unknown if an exercise prescription decreases the likelihood of developing cardiotoxicity. Further work is needed with ongoing prospective studies to investigate the effect of exercise on cardiac function during chemotherapy treatment.

INTRODUCTION

Adaptive exercise for patients undergoing chemotherapy for cancer treatment is critical. Many studies have revealed that patients undergoing cancer treatment who exercise more than their counterparts report decreases in depression and fatigue with concurrent increases in muscle strength, aerobic capacity, mental health, and overall immune function13. Some longitudinal studies have also found overall decreases in long-term mortality rates in patients who exercise during chemotherapy14,15. The long-term effects of exercise on the cardiovascular system have been well documented and include prevention of systolic and diastolic dysfunction and mitigation of elevated blood pressures16. The purpose of this study was to characterize the natural trend of self-reported physical activity during breast cancer treatment.

METHODS – QUESTIONNAIRE SCHEME

Fig. 1: Flow diagram for patient enrollment

Fig. 2: IPAC and CHAMPS Questionnaires were given at these corresponding times.

REFERENCES

Introduction

- Ovarian cancer has a 65% mortality rate due to lack of early screening and diagnostic technologies. 1
- Ovarian cancer symptoms are non-specific—bloating, dysphagia, change in bowel movements, general discomfort.
- Current diagnostic approaches are MRI, CT, PET, CA-125 levels, and highly invasive biopsy.
- Calorimetry has proven a useful non-invasive screening tool in Lyme Disease, Lupus, Rheumatoid Arthritis, and cervical cancer. 2,3
- Calorimetry is performed using a differential scanning calorimeter (DSC), which compares the difference in excess specific heat capacity between sample and reference solutions as a function of temperature—this plot is referred to as a “thermogram.”
- Plasma samples from five women with stage IIC, epithelial ovarian cancers, with various levels of CA-125 were chosen for this study. The ovarian cancer of patient OC6 was of mesenchymal origin.
- One pooled healthy plasma control and one case-specific plasma control (OC5) were used for reference.
- The techniques used to examine the plasma samples were HPLC-SEC, BCA assay, SDS gel electrophoresis, and DSC.
- The goal of this project was to observe thermogram changes between healthy and diseased plasma samples and further investigate these alterations using HPLC-SEC, DSC, and mass spectrometry.

Methods

- SDS Gel Electrophoresis of Whole Plasma
- SDS Gel Electrophoresis of Fraction 27
- SDS Gel Electrophoresis of Fraction 20
- SDS Gel Electrophoresis of Fraction 11
- Thermograms of Individual Proteins in Plasma
- DSC Thermograms of Whole Plasma
- Pure Protein and Ovarian Cancer Elution Profiles
- DSC Thermograms of Fraction 27
- DSC Thermograms of Fraction 20
- DSC Thermograms of Fraction 11

Conclusions

- DSC is a non-invasive technique that can screen for disease in blood plasma.
- HPLC-SEC allows identification of proteins causing shifts in the whole plasma thermograms.
- Changes in thermal profiles of these proteins infer alterations to the abundant proteome in ovarian cancer. These changes could result from alterations in protein interactions in the presence of tumor specific antigens or from post-translational modifications to abundant plasma proteins.

Future Work

- Increase the number of samples for statistical significance.
- Mass spectrometry will be applied to identify the presence of disease-specific markers and to investigate the nature of changes in the ovarian cancer proteome.

Acknowledgements

- Future research will be conducted at the University of Louisville School of Medicine and the James Graham Brown Cancer Center.

References

Inhibition of melanoma metastases by targeting regulator of G protein signaling 2 (RGS2)

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Abstract

While new targeted therapies for melanoma have been developed recently, the prognosis for metastatic melanoma remains dismal. To that end, the investigation of mechanisms for melanoma progression is an important translation research area that can bridge basic science with clinical practice. In cutaneous melanoma, the mitogen-activated protein kinase (MAPK) is constitutively activated. Activation of this pathway leads to the induction of epithelial-to-mesenchymal transition (EMT), a process that resembles the genesis of cancer stem-like cells resulting in tumor invasion, aggressiveness, and metastasis. The regulator of G protein signaling (RGS) protein plays an important role in the development of vasculature. RGS family members are regulatory molecules that act as GTPase activating proteins (GAPs) for G alpha subunits of heterotrimeric G proteins. However, none of the current research has entailed the connection of RGS with melanoma progression. This project used an established melanoma progression model to show that one of the RGS family members, RGS2, was upregulated in highly metastatic melanoma cell lines. Upregulation of RGS2 in metastatic cell lines was also associated with hyperactivated MAPK pathway and induction of EMT. Manipulated expression of RGS caused increased migration and invasion abilities in less-metastatic melanoma cell lines by RGS2 siRNA knockdown in highly-metastatic melanoma cell lines. These results confirm that over expression of RGS2 can promote melanoma metastasis by inducing EMT through activation of the MAPK pathway. It provides a starting point for mechanism-based evidence in support of RGS2 as a therapeutic target for inhibiting melanoma progression.

Introduction

- RGS2 is a GTPase regulatory protein that is overexpressed in aggressive metastatic melanoma cell lines
- Epithelial-mesenchymal transition (EMT) is a molecular program whereby epithelial cells undergo reprogramming so that they are now able to migrate into vasculature and lymph thus initiating metastatic progression. EMT is activated through the MAPK pathway.
- Analyzing the role of RGS in promoting melanoma progression through EMT could lead to new therapeutic targets

Methods

An A375 established melanoma progression model was used with three generations having increasing metastatic potential (A375P, A375MA1, A375MA2). 1)RGS2, EMT markers, and MAPK expression were checked using Real-time PCR and Western Blot.
2)Migration abilities of (A375MA2:A375MA1 vs A375P) were checked using an assay
3)RGS2 was knocked down using siRNA transfection. Migration and invasion assays were conducted to determine metastatic potential. RGS2 levels were confirmed using Real-time PCR and Western Blot.
4)RGS2 was upregulated using plasmid transfection. Migration and invasion assays were conducted to determine metastatic potential. RGS2 levels were confirmed using Real-time PCR and Western Blot.

Results

Conclusions

- RGS2, EMT markers, and MAPK are upregulated in highly metastatic cell lines (A375MA1/MA2) vs poorly metastatic cell line (A375P).
- RGS2 siRNA transfection knockdown cell lines showed decreased migration and invasion potential (MA1/MA2 vs P)
- RGS2 plasmid transfection knock-in cell lines showed increased migration and invasion potential (MA1/MA2 vs P)
- Overexpression of RGS2 promotes melanoma metastasis by inducing EMT through activation of MAPK
- These findings could lead to new therapeutic targets for inhibiting melanoma progression

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Abstract

MicroRNAs (miRNAs) act as oncomiRs, tumor suppressors, and as post-transcriptional regulators of target mRNAs by binding to sites in the mRNA 3′UTR in the RNA-induced silencing complex (RISC) containing Argonaute (Ago) proteins. Published expression studies of miRNA in lung cancer provide a global level of expression and may not be indicative of miRNA functionality, which is important for the identification of candidate therapeutic targets. This study hypothesized that miRNAs and mRNA interact with one another in complex reaction networks that differentiate lung adenocarcinomas, a type of non-small cell lung cancer (NSCLC) that is increasing globally in men and women and in smokers and non-smokers, from normal adjacent lung tissue. A newly developed protocol, high-throughput sequencing together with UV-crosslinking and immunoprecipitation (HITS-CLIP), was performed on A549 cells and flash frozen lung adenocarcinomas and normal adjacent tissue. This study uniquely isolated mRNA-miRNA complexes by immunoprecipitation with the Ago2 protein using radiolabeled linkers to follow the complex during purification. Further RNA isolation was performed by protease digestion and phenol-chloroform extraction. Then, RT-PCR and subsequent gel extraction was utilized to amplify bound miRNA-mRNA complexes to which linkers were ligated and the tagged RNA has been sent to RNA sequencing. This study successfully isolated and prepared for sequencing stable mRNA-miRNA complexes bound to Ago2 in lung adenocarcinomas and normal adjacent tissues. Once the samples are sequenced, this study will enter the bioinformatics stage to identify the differentially expressed miRNA-mRNA complexes in lung adenocarcinoma versus normal adjacent tissue. 

Material & Methods

Results

Conclusions

Stable miRNA-mRNA complexes were successfully isolated and prepared for RNA sequencing from flash frozen human lung adenocarcinoma tumors.

Once the samples are sequenced, this study will enter the bioinformatics stage to identify specific miRNA-mRNA complexes differentially expressed in lung adenocarcinoma and normal adjacent tissue.
Development of Theranostic Mesoporous Silica Nanoparticles for Pancreatic Cancer

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ABSTRACT

Introduction: Modern methods of pancreatic cancer diagnosis and treatment are severely lacking and have failed to provide effective treatment options for patients. The root cause of this inadequacy stems from the hypovascularized nature of pancreatic cancer, making traditional chemotherapeutics and cancer detecting contrast agents nearly useless. A potential solution for tumor targeting difficulties is through the implementation of nanotechnology, specifically targeting ligands capped, theranostic nanoparticles. We hypothesize that pH-responsive chitosan-capped mesoporous silica nanoparticles (MSNs) with the targeting ligand, urokinase plasminogen activator (UPA) will serve as theranostic agents for treatment and diagnosis of pancreatic cancer.

Methods: MSNs were synthesized by employing tetraethyl orthosilicate (TEOS), tetramethylammonium bromide (CTAB), tetrathyl orthosilicate (TEOs) and chitosan through the sol-gel method. The synthesized MSNs were characterized by transmission electron microscopy (TEM) and zeta-potential measurements. Afterwards, gemzar chemotherapy drug was encapsulated into these nanoparticles to observe the pH-dependent release profiles in vitro. Furthermore, MSNs were tagged with UPA to increase the binding efficiency of these nanoparticles towards the pancreatic tumor cells (2SCP and 2SP10). The binding efficiency of both tagged and non-tagged MSNs was observed at various pHs (7.4 to 6.5) by employing fluorescence microscopy, Odyssey infrared imaging and tissue phantoms. For that, various types of dyes were used, such as, rhodamine B and indocyanine green (ICG). Finally, UPA-tagged MSNs with ICG were injected into mice infected with 2SCP tumors cells to observe the distribution of these nanoparticles in vivo by multiphoton optical spectroscopic tomography system (MSOT).

Results: TEM pictures showed that the synthesized MSN had a size around 120 nm. Zeta-potential measurements revealed that charge density of MSN dependent on pH. The release experiments showed that these nanoparticles were pH-sensitive because the release of gemzar depended on the pH. Gemzar released ~2x the quantity from MSNs at pH 6.5 in comparison to pH 7.4. Fluorescence microscopy, Odyssey infrared imaging and tissue phantoms showed that uptake of MSNs by pancreatic tumor cells depended on the pH and tagging of UPA. Lowering a pH and tagging a ligand drastically increased the uptake of MSNs in pancreatic tumor cell in vitro. Specifically in tissue phantoms, ICG-loaded MSNs at pH 6.5 demonstrated 20x and 7x more cell signal than without ligand or at pH 7.4, respectively. Furthermore, UPA-ICG-loaded MSNs were successfully detected in orthotopic pancreatic tumor of mice within 4 hours of imaging time by MSOT.

Conclusion: UPA tagged, pH sensitive MSNs demonstrate potential as a theranostic nanoparticle for pancreatic cancer.

RESULTS

Figure 2: Transmission electron microscopy image of MSNs. After MSN particles were synthesized, particles were dried onto lacey-carbon coated grids and viewed. MSN particles contained visible mesopores, spherical structures with a diameter of ~120 nm and a pore size of ~3 nm.

Figure 3: Conjugation of the MSN with chitosan and APTES. Zeta Potential analysis validates the conjugation of chitosan-coupled MSN (C-MSN)(APTES) chitosan and (3-Aminopropyl)triethoxysilane (APTES) (not shown), dual conjugated C-MSN. The decreasing voltage with corresponding increases in pH results from increased protonation of the chitosan chains. The addition of APTES MSNs with pH change to the particles resulting in a more positive charge for the C-MSN-AP which compared to the C-MSN.

Figure 4: Gemzar-loaded MSNs were evaluated for pH specific drug release. The Gemzar MSN solution was incubated in phosphate buffer saline (PBS) solution of either 6.5, 6.8, or 7.4 pH. Release of Gemzar was based upon absorbance measured using UV–Vis spectrophotometry over a period of 10 hours. The amount of absorbance found in PBS solution directly correlated to the level of Gemzar drug that had been released from the MSNs. The MSNs at pH 6.5 were bound to release ~2x the amount of Gemzar as compared to the MSNs kept at pH 6.8 and 7.4. These results illustrate the effective pH sensitivity of the MSNs.

CONCLUSION

- Theranostic nanoparticles offer the benefit of facilitating both tumor detection as well as providing therapeutic agents
- One of the few universal characteristics of cancer is acidic extracellular tumor microenvironment making pH sensitive therapy agents do well
- This study illustrates the successful synthesis of pH sensitive, silica based nanoparticles (MSNs) with UPA targeting ligand for theranostic imaging of pancreatic cancer
- UPA tagged MSNs bind more effectively in vitro and in vivo compared to non-UPA tagged MSNs against multiple cell lines
- With further analysis and implementation of this technology, there is potential to revolutionize the detection and treatment of pancreatic cancer and other metastatic disease
- Future studies involve assessment of Gemzar release in vitro and further evaluation of the MSNs using additional in vivo models.

ACKNOWLEDGEMENTS

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Figure 5: Fluorescence microscopy images of UPA targeted MSN treated 2SCP cells. Fluorescent dye was encapsulated within MSN particles to measure bioavailability uptake of particles. A) FITC (fluorescein) and DAPI (nuclear staining) fluorescent staining. B) Negative control cells.

Figure 6: Tube only, MSN-UPA, MSN-Cont. A) 2SCP cells were treated with UPA targeted MSN. C) Blocking of UPA with UPA antibody diminished UPA targeted MSN particles in 2SCP cells.

Figure 7: Visualization of targeted MSN binding to UPB positive cells using phantoms with MSOT. Cells incubated with 50 µg/mL PBS for 2 hours. Cells were washed and transferred into tissue phantoms (light scattering agar + lidipid) and inserted into the MSOT.

Figure 8: UPA targeted MSNs encapsulated with ICG accumulated within the pancreatic tumor and spleen. The nanoparticles were injected at 1 µl by i.x. Particle accumulation was evaluated after 4 h using imaging with Multiphoton Optical Tomography.

Figure 9: 3D printed agarose-laden gel with a tumor plug and surrounding normal tissue. This phantom mimics the complex tumor architecture observed in vivo.