Detection of Pancreatic Adenocarcinoma in vivo with S100A9 Liposomes

Tess V. Dupre1, Christopher England1, Justin Huang2, Lacey R. McNally1,2

1Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY
2Department of Medicine, University of Louisville, Louisville, KY

ABSTRACT

**Purpose:** Delivering effective drugs at effective concentrations to all cells in solid tumors, especially pancreatic adenocarcinoma, remains challenging due to low drug accumulation within tumors and high levels of systemic toxicity. To overcome these problems, S100A9 liposomes can be utilized to enhance drug delivery. We hypothesize that S100A9 liposomes will exhibit enhanced accumulation in pancreatic orthotopic tumors due to S100A9 specificity for the EMMPRIN receptor.

**Methods:** Pancreatic cell lines, S2VP10, MiaPaCa2, S2CP9, and Panc-1, were evaluated for EMMPRIN expression along with ES2 (positive control) and MCF7 (negative control) using western blot. Liposomes were bioconjugated with S100A9 and CF750 NIR dye. Specificity and activity of the S100A9 liposomes were studied using flow-cytometry. SCID mice were orthotopically implanted with S2VP10 cells. After tumors reached 3mm, 200 μl of 5 OD S100A9 liposomes was IV injected into mice. Mice were imaged using 2D AMI imaging and 3D MSOT.

**Results:** EMMPRIN expression was seen at 1.2X and 0.9X relative abundance in S2VP10 and MiaPaCa2 cells respectively; however, MCF7 expressed 0.1X relative abundance EMMPRIN. Flow-cytometry showed cellular uptake of S100A9 liposomes in S2VP10 and MiaPaCa2 cells at ~92% and ~84% respectively. 3D MSOT imaging and 2D AMI imaging showed accumulation of the S100A9 liposomes at the tumor.

**Conclusion:** The S100A9 liposome selectively targeted pancreatic orthotopic tumors in vivo, thus establishing that the stealth liposomes can be used as a drug delivery system for an alternative pancreatic cancer treatment.

RESULTS

Figure 1: (top) Western blot image of EMMPRIN Expression. (bottom) Dose symmetry of EMMPRIN Expression in pancreatic cell lines and positive and negative controls with respect to actin. EMMPRIN expression was seen at 1.2X and 0.9X relative abundance in S2VP10 and MiaPaCa2 cells respectively; however, MCF7 expressed 0.1X relative abundance EMMPRIN.

Figure 4: Accumulation of the S100A9 liposomes was observed at the 6hr time point using 2D AMI imaging. S2VP10 cells were orthotopically injected into mice. After tumors were palpable, S100A9 probe was injected to identify pancreatic tumors. Probe accumulated within tumors at 6hr post injection. A representative mouse (Right) demonstrates preferential binding. Probe accumulation in pancreas was confirmed ex vivo (Left).

CONCLUSION

EMMPRIN was found to be overexpressed in pancreatic cancer cell lines, making it an prime candidate for active targeting. The evaluation of Pancreatic. S100A9 liposomes showed an increased affinity for pancreatic cancer cell lines S2VP10 and MiaPaCa2. Accumulation of the liposomes observed via AMI imaging elucidates that S100A9 liposomes selectively targeted the pancreatic orthotopic tumor and has potential to be used as a delivery system for alternative pancreatic cancer treatment.

FUTURE DIRECTIONS

Pancreatic cancer cells are addicted to autophagy, thus using the metabolic process as a survival mechanism. We believe that utilizing the S100A9 liposomes as a drug delivery system for chloroquine, an autophagy inhibitor, will decrease systemic drug toxicity and increase pharmacological drug efficacy and potency.

ACKNOWLEDGEMENTS

This work was supported by CA139050 and R25-CA134283 from the National Cancer Institute

MATERIALS & METHODS

Pancreatic cancer cell lines MiaPaca2 and S2VP10 were utilized in this study along with MCF-7 and ES2 cell lines, a breast cancer and ovarian cancer cell line, respectively. MiaPaca-2 and MCF7 cells were grown in DMEM with 10% FBS and 1% L-Glutamine while S2VP10 and ES2 cells were grown in RPMI 1640 with 10% FBS and 1% L-Glutamine at 37°C. Western Blot analysis of EMMPRIN expression was used on S2VP10, MiaPaCa-2, Panc-1, S2CP9, ES2, and MCF-7 cell lines. MCF7, ES2, S2VP10, MiaPaCa2 were used for flow cytometry to ensure active binding of receptor. Immunocytochemistry was performed on S2VP10, ES2, and MCF 7. Cells were incubated with CF-750 dye alone, bioconjugated S100A9 probe, naked liposome, and S100A9 targeted liposome for 1-h; scraped and collected for flow cytometry. SCID mice were orthotopically implanted with S2VP10 cells. Tumor growth was monitored using 2D AMI imaging. After tumors reached 3mm, 200 μl of 5 OD S100A9 liposomes was IV injected into mice. Accumulation and biodistribution of the probe was monitored with AMI imaging.
Differential Expression of Chemokine Receptors in Lung Cancer Metastasis

Taylor N. Hermann, Rajesh K. Sharma, Zinal S. Chheda, and Bodduluri Haribabu.

James Graham Brown Cancer Center, School of Medicine, University of Louisville, Louisville, KY 40202

Abstract

Lung cancer is the leading cause of cancer related deaths in the United States. Lack of early diagnosis, metastasis, and resistance to treatments are major causes of the poor survival rate of lung cancer patients. Metastasis, the spread of cancer cells from the primary tumor to other non-adjacent organs, accounts for most of the cancer related deaths. The commonly used metastasis models utilize intravenous injection of tumor cells, thus bypassing the expression of malignant cells an obligatory step in metastasis of spontaneous cancers. We developed a unique metastasis model by in vivo passaging the Lewis lung carcinoma (3LL) cells and maintaining them in vivo with 7-11 days of intermittent culture in between passages. When injected subcutaneously (i.e., parental 3LL cells or 3LL cell line) show robust metastasis. Chemokine receptors have been found to play an important role in the migration of the cancer cells. Chemokine receptors on cancer enable the cancer cells to metastasize to other areas in the body. Given the paramount importance of chemokine signaling and receptors in cancer metastasis, we decided to study the expression profile of every chemokine receptor. This was done in order to study the chemokine receptors which may play the most important roles in the metastasis of lung cancer in mice. Real-time PCR was used to examine the transcript levels of every chemokine receptor in 3LL and p-3LL cells and tumors. CCR6 and CXCR7 were found to be up-regulated in the passage tumors compared to the parental tumors, possibly indicating these receptors play a pro-metastatic role in cancer mice. CCR1 and CXCR3 were down-regulated in the tumors, possibly indicating these receptors either play an anti-metastatic role in play no role whatsoever. The chemokine receptors CCR1, CCR4, CCR6, and CXCR4 were up-regulated in the p-3LL cells as compared to the 3LL cells, indicating that these receptors may play an important role in promoting lung cancer metastasis. The chemokine receptors CCR1, CCR4, CCR6, CXCR3, CXCR5, and BLT1 all showed a significant reduction in the p-3LL cells. This down-regulation may mean these chemokine receptors do not play a role in the increase in metastasis of the p-3LL cells or may play an anti-metastatic role. Each of the other chemokine receptors demonstrated no significant transcript level change in the passage cells compared to the parental cells, suggesting these receptors do not play a role in metastasis.

Models of Experimental and Spontaneous Metastasis

Our work was to determine if the chemokine receptors are upregulated during spontaneous or metastatic growth, as these receptors play a significant role in tumor cell migration. Our work was to determine if the chemokine receptors are upregulated during spontaneous or metastatic growth, as these receptors play a significant role in tumor cell migration. Metastasis is the most common cause of cancer related deaths. The commonly used metastasis models utilize intravenous injection of tumor cells, thus bypassing the expression of malignant cells an obligatory step in metastasis of spontaneous cancers. We developed a unique metastasis model by in vivo passaging the Lewis lung carcinoma (3LL) cells and maintaining them in vivo with 7-11 days of intermittent culture in between passages. When injected subcutaneously (i.e., parental 3LL cells or 3LL cell line) show robust metastasis. Chemokine receptors have been found to play an important role in the migration of the cancer cells. Chemokine receptors on cancer enable the cancer cells to metastasize to other areas in the body. Given the paramount importance of chemokine signaling and receptors in cancer metastasis, we decided to study the expression profile of every chemokine receptor. This was done in order to study the chemokine receptors which may play the most important roles in the metastasis of lung cancer in mice. Real-time PCR was used to examine the transcript levels of every chemokine receptor in 3LL and p-3LL cells and tumors. CCR6 and CXCR7 were found to be up-regulated in the passage tumors compared to the parental tumors, possibly indicating these receptors play a pro-metastatic role in cancer mice. CCR1 and CXCR3 were down-regulated in the tumors, possibly indicating these receptors either play an anti-metastatic role in play no role whatsoever. The chemokine receptors CCR1, CCR4, CCR6, and CXCR4 were up-regulated in the p-3LL cells as compared to the 3LL cells, indicating that these receptors may play an important role in promoting lung cancer metastasis. The chemokine receptors CCR1, CCR4, CCR6, CXCR3, CXCR5, and BLT1 all showed a significant reduction in the p-3LL cells. This down-regulation may mean these chemokine receptors do not play a role in the increase in metastasis of the p-3LL cells or may play an anti-metastatic role. Each of the other chemokine receptors demonstrated no significant transcript level change in the passage cells compared to the parental cells, suggesting these receptors do not play a role in metastasis.

Role of chemokines and chemokine receptors

Metastasis is a process causing cell death. Many causes such as changes in the expression of inflammatory cytokines from the host can promote cancer metastasis. Chemokines are a type of cytokine which are expressed by chemokine receptors on the surface of cancer cells. Chemokine receptors are expressed on different types of cancer. Some are always expressed but others are only activated when induced. Some chemokines have been found to inhibit tumor growth and angiogenesis while others have been found to promote cancer spreading processes. These roles of chemokines are still being studied.

Chemokine receptors are expressed on different types of leukocytes. Some are always expressed but others are expressed only when induced. Some chemokines have been found to inhibit tumor growth and angiogenesis while others have been found to promote cancer spreading processes. These roles of chemokines are still being studied.

Some chemokines have been found to inhibit tumor growth and angiogenesis while others have been found to promote cancer spreading processes. These roles of chemokines are still being studied.

Chemokine receptors are expressed on different types of leukocytes. Some are always expressed but others are expressed only when induced. Some chemokines have been found to inhibit tumor growth and angiogenesis while others have been found to promote cancer spreading processes. These roles of chemokines are still being studied.
Stable Isotope Resolved Metabolomics Reveals Functional Biochemistry in Primary Non Small Cell Lung Cancer Cell Line

Connor J. Kinslow,1 Teresa W.M. Fan, Ph.D.,2 Jin Lian Tan, M.S.,1 Pawel K Lorkiewicz, Ph.D.,2 Ramya Balasubramaniam, M.S.,2 Andrew N. Lane, Ph.D.1
James Graham Brown Cancer Center1 and Department of Biochemistry2
University of Louisville School of Medicine

1. Introduction
Lung cancer is the second leading cause of death nationwide. 5-year survival rate for localized cancer (52.5%) is much higher than for advanced stages (3.5%). However, only 15% of patients are diagnosed at this early stage. Development of early stage biomarkers will drastically decrease death rates. Stable isotope-resolved metabolomics (SIRM) provides a functional readout of cellular activity and is therefore a useful tool in biomarker discovery and fundamental cancer biology. We have established a primary lung squamous cell carcinoma line (PSLC1) in collaboration with Dr. Jun Yan, and studied it in situ and in mouse xenografts using SIRM. Here we report the metabolic activities of the cell line in culture using two different tracers to central metabolism.

2. Methods
PSLC1 cells were grown in either [U-13C]-glucose or [U-13C,15N]-glutamine labeled medium. Cell extracts and medium were prepared and analyzed by 1H-NMR, HSQC, TOCSY, GC-MS, and FTICR-MS.3,4

3. Results

4. Metabolic Profiles of PSLC1 cells

5. Lactate Derives from Glucose

6. Glucose-Dependent TCA Cycling from Glutamine

7. Cells Consume Glutamine and Excrete Glutamate

8. Pyruvate:Carbohydrate Antiport is High

9. Glucose Consumption and Lactate Production Indicate That the Cells Are Highly Glycolytic

10. Glucose and Glutamine in De Novo Nucleotide Biosynthesis

11. Glutamine Is the Major Precursor in Proline Biosynthesis

12. 13C-Metabolomics Analysis Confirms C-MYC Overexpression

13. Discussion
• C-MYC overexpression was determined by Western Blot analysis combined with SIRM via Pro-META analysis
• Cells exhibited linear glucose consumption that persisted even as consumption of other nutrients diminished
• Glutamine represents a major contributor for biosynthetic and bioenergetic cellular needs
• Glutaminase and enzymes involved in proline metabolism may be up-regulated by C-MYC overexpression
• SIRM indicates that pyruvate carboxylase may be up-regulated

The ability of SIRM to generate hypotheses about protein expression demonstrates its potential use as a first line biomarker detection method. Comparison of cell culture data with upstream in situ and xenograft data will allow us to assess model accuracy and the effects of tumor microenvironment, which will help us interpret human in situ data more reliably.

14. References

15. Acknowledgements
This work was supported by grants R25-CA134283-01A2 (Hein), P01CA163232-01A1 (Lane), P2GM103482 (Miller), NSF EPSCoR EPS-0447974 (Fan) and EPS-0132295 (Wittebrodt), and the National Cancer Institute R25 Cancer Education Program.
Cancers are among the most common and life-threatening diseases in the world. Developing effective therapeutic strategies for cancer remains a major challenge. SOX9 is a transcription factor that plays a critical role in development, differentiation, and proliferation of various cell types. Understanding the mechanisms of SOX9 activation and regulation could provide new therapeutic targets for cancer treatment.

Methods:
- SOX9 homology model was created from the human SOX9 sequence.
- Selected compounds were screened for their ability to bind to the SOX9 DNA binding domain.
- MTT assays were performed to determine the antiproliferative activity of selected compounds.
- Western blot analysis was used to investigate the protein expression levels of SOX9.

Results:
- The selected compounds inhibited the proliferation of cancer cells.
- Western blot analysis showed a reduction in SOX9 protein levels.

Conclusion:
- The selected compounds demonstrate potential antiproliferative activity against cancer cells.
- Further studies are needed to confirm the results and to develop these compounds as potential therapeutic agents.

Future Directions:
Future studies will attempt to demonstrate that these compounds work by directly inhibiting SOX9. Techniques include: SOX9-driven luciferase reporter assay, electrophoretic mobility shift assay (EMSA), immunohistochemistry, and western blots.

Acknowledgments:
Research support was granted from R25-CA138493 from The National Cancer Institute (NCI) Cancer Education Program, the University of Louisville School of Medicine, & the James Graham Brown Cancer Center.

References:
The Anti-glycolytic Small Molecule Inhibitor PFK158 Cooperates with Temozolomide to Induce Cell Death in Melanoma Cells

Adam Morrison1,2, Julie O’Neal3, Jason Chesney3
Departments of Surgery1, Biochemistry2, and Department of Medicine3
University of Louisville School of Medicine

INTRODUCTION

From 1979 to 2009 there has been an 800 percent increase in melanoma among young women and a 400 percent increase among young men. It is estimated that 45,060 new cases of invasive melanoma in men and 31,630 in women will be diagnosed in the US in 2013. Temozolomide (TMZ) is an alkylating agent commonly used in the treatment of melanoma. Response rates to TMZ for metastatic melanoma remain poor, suggesting the need for alternative therapies. The mechanism that imparts TMZ with its therapeutic activity is its ability to methylate or alkylate DNA, which triggers apoptotic cell death. Glutathione (GSH) is involved in prevention and repair of DNA damage. Since we believe that the 6-phospho-2-kinase (PFK158) inhibitor decreases GSH, we hypothesized that the addition of PFK158 to cells treated with TMZ would increase cellular sensitivity to TMZ. Additionally, since melanoma cells are glycolytic, effects of PFK158 may also cooperate with TMZ to promote cell death. PFK158 and TMZ were used in combination on the A375 melanoma cell line. The efficacy of the combination therapy was evaluated using flow cytometry to assess the death of the melanoma cells.

METHODS

Cell Culture- A375 cells were cultivated in DMEM with 10% FBS and gentamicin.

Treatment- Cells were grown 2,000 cells/well were plated the following day and were treated with TMZ for 5 days. PFK158 was then added and the cells allowed to incubate for an additional day.

Measuring Cell Death- Cells were trypsinized and collected in FACS tubes. Propidium Iodine and Annexin V were added and incubated for 15 minutes. The cells were analyzed by flow cytometer. 10,000 events were recorded on each sample. Dead cells (PI) and pre-apoptotic cells (Annexin V) were stained by probes allowing the counts to be made.

RESULTS

The addition of PFK158 to Temozolomide treated cells produces more cell death via apoptosis than TMZ or PFK 158 alone.

Future experiments will address the mechanism of additive cell death.

ACKNOWLEDGEMENTS

Supported by grant R25-CA-134283 from the National Cancer Institute
Sinusoidal endothelial cell-derived extracellular matrix regulates basal and stimulated macrophage activation

Lauren G. Poole, Jenny D. Jokinen, Veronica L. Massey, Juliane I. Beier, and Gavin E. Arteel
Department of Pharmacology and Toxicology, University Research Center, Laboratory of Molecular Toxicology, University of Hertfordshire, UK.

ABSTRACT

Background: Sinusoidal lining prevents the entry of circulating leukocytes, and has protective roles in immune surveillance. Macrophages are the primary cell type involved in the response to liver injury, via direct and indirect actions. Tumor necrosis factor (TNF)-α, a pro-inflammatory cytokine, is released in response to liver injury, and stimulates liver sinusoidal endothelial cells (LSECs) to produce extracellular matrix (ECM) that retains resident macrophages in situ.

Aim: To explore the role of TNF-α in LSECs and the interaction between LSECs and resident macrophages in the liver.

Methods: LSECs were harvested from the livers of mice, and cultured with or without TNF-α. TNF-α expression and production were measured by Real-Time PCR and ELISA, respectively. The interaction between LSECs and resident macrophages was assessed using co-culture experiments.

Results: TNF-α expression was increased in LSECs cultured with TNF-α. This expression was dependent on the concentration of TNF-α. The interaction between LSECs and resident macrophages was enhanced by TNF-α, as indicated by increased ECM production.

Conclusion: TNF-α plays a role in LSECs and the interaction between LSECs and resident macrophages. The results suggest that TNF-α may be a key regulator of LSECs and the response to liver injury.

References:

