Abstract

Prolonged exposure to crystalline silica (CS) leads to silicosis due to chronic inflammation and fibrosis of the lungs. Individuals with silicosis are twice as likely to develop lung cancer. A key aspect of this CS-induced inflammation is the migration of neutrophils to the lungs. The process of neutrophil recruitment to the lungs begins with the chemotactic agent Leukotriene B4 (LTB4) binding to BLT1 and BLT2 receptors. The main producers of LTB4 are macrophages and mast cells. Once this process has begun other mediators such as IL-1β and neutrophil active chemokines also play a role promoting the inflammation. When inhaled CS travels to the alveoli of the lungs where it enters mast cells, macrophages, and epithelial cells through phagocytosis. As the phagosomes progress, lipid bodies begin to appear in the cytosol. After fusion of the lysosome and phagosome the inflammasome protein complex appears and produces IL-1β. By inhibiting the formation of the phagolysosome, IL-1β production is stunted while LTB4 production is heightened. This shows that the production of LTB4 and IL-1β are triggered independently of one another. The pathway through which inflammasomes are constructed and produce IL-1β is understood, while the pathway through which LTB4 is produced is not yet clear. The first objective of this study is to stain different cellular compartments using microscopy to determine the connection between phagocytosis and LTB4 production in macrophages. While mast cells are known to play an important role in inflammation and produce even more LTB4, little is known about the process through which this occurs. Rat basophilic leukemia (RBL) cells share many properties with mast cells. The second objective of this study is to determine if RBL cells make LTB4 in response to CS.

Background

In 2015, lung cancer was responsible for approximately 27% of all cancer deaths, making it the number one cancer killer of both men and women (1). Factors such as smoking, genetic susceptibility and various environmental hazards are known to increase the risk of lung cancer. One of these environmental hazards is crystalline silica, the second most abundant element on the earth. Millions of workers in the US alone are exposed to CS every year. When in crystalline form silica is inhaled into the lungs where the particles become trapped and damage lung tissue. The damaged tissue eventually becomes scar tissue that forms granulomas. The damage done by CS causes the incurable, but preventable, lung disease silicosis. Individuals with silicosis are at an increased risk of developing lung cancer, making CS a human carcinogen.

Results

This cycle attracts neutrophils and causes chronic inflammation

LTB4 Production by RBLs in Response to CS

Serum Free Media

* * * * ***

LTB4 pg/mL

No priming

100ng IgE

10ng PMA

10ng LPS

100ng IgE + 10ng LPS

10ng PMA

10ng IgE + 1µM PMA + 1µM Ionomycin

Confluent microscopy of nucleus (blue), CS (white), lipid bodies (green), and membrane (red) in a macrophage

Future Directions

- Refine the conditions in which CS induces LTB4 production in RBL cells.
- Use RBL cells without priming in serum free media to study LTB4 production in mast cells.
- Continue with confocal microscopy to determine the connection between phagocytosis and LTB4 production.

Methods

RBL-2H3 cells were plated in a 24-well cell culture plate with a cell density of 1x10⁴ cells/well. Cells adhered to the cell plate overnight in 500 µl of 10% FBS MEM. A variety of priming agents were used. Cells were primed overnight with 10 ng PMA, 100 ng IgE, 10 ng LPS, 10 ng PMA + 1 µM Ionomycin. Before CS stimulation the cells were switched to either 200 µl of 1% FBS media or serum free media. 1 µM PMA + 1 µM Ionomycin was used as a positive control. After the cells were stimulated with 100 pg/µl of CS for 5 hours, the supernatant was removed and a LTB4 measurement was performed. Experiments were done in triplicate cultures.

Conclusions

- In 10% FBS MEM, with and without priming, CS does not induce LTB4 production in RBL cells.
- In serum free media, with and without priming, CS induces LTB4 production in RBL cells for all conditions except PMA (10ng)+Iono (1µM).
- CS induces the most LTB4 production in RBL cells without priming in serum free media. This was expected because CS also induces the most LTB4 production in bone marrow derived mast cells without priming in serum free media.
- Using RBL cells in place of mast cells will allow for studies to be completed using a more durable and accessible cell line.

Acknowledgements

This research was supported by National Cancer Institute grant R25-CA134283 and Kentucky Lung Cancer Research Program.

References

1. Lung Cancer Fact Sheet. Lung Health & Diseases.
2. Crystalline Silica Exposure Health Hazard Information. OSHA Fact Sheet

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Nutritive Intake Relates to Activated Cytotoxic T Cells in Lung Cancer Patients

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Abstract

Knowledge of chemotherapeutic nutrition can outweighs our familiarity with post-diagnosis nutrition and the implication of nutritional intake on factors that may influence lung cancer progression. We predict percent energy from fat would increase triglyceride (TG) and LDL levels as well as dysregulated immune responses (increased NK and CTL cell counts, cytotoxic T lymphocytes (CD8+)) and stimulated TNF-α and IL-6. Additionally we postulated that an increase in fruit and vegetable servings would decrease serum TG and LDL levels while decreasing activation of these inflammatory and immune responses. Lung cancer patients (n = 62, 34 female) were recruited from the Brown Cancer Center. Patients were within 5 years of diagnosis and had primarily non-small-cell lung cancer, with disease stage ranging from early to advanced. Self-report questionnaires assessing fruit and vegetable intake and percent energy from fat were collected and blood samples were drawn. Lipid panels provided triglyceride (TG) and LDL levels. Whole blood samples were subjected to flow cytometry for measurement of CD3/CD8 Cytotoxic T lymphocytes (CD8+)), and CD56 natural killer (NK), (CD56+) cell count. PHA-stimulated peripheral blood mononuclear cells were assayed to quantify TNF-α and IL-6 levels. Preliminary bivariate Spearman correlations were used to determine potential control variables by assessing relationships between demographic, clinical, social, and medical variables with outcome variables. Hierarchical linear regressions adjusted for age at diagnosis and cancer stage. Nutrition indicators were entered as independent variables with lipid indicators and factors of activated immunity as dependent variables.

Methods

Lung cancer patients (n = 62, 34 female) were recruited from the Brown Cancer Center. Patients were within 5 years of diagnosis and had primarily non-small-cell lung cancer, with disease stage ranging from early to advanced. Self-report questionnaires assessing fruit and vegetable intake and percent energy from fat were collected and blood samples were drawn. Lipid panels provided triglyceride (TG) and LDL levels. Whole blood samples were subjected to flow cytometry for measurement of CD3/CD8 Cytotoxic T lymphocytes (CD8+) and CD56 natural killer (NK), (CD56+) cell count. PHA-stimulated peripheral blood mononuclear cells were assayed to quantify TNF-α and IL-6 levels. Preliminary bivariate Spearman correlations were used to determine potential control variables by assessing relationships between demographic, clinical, social, and medical variables with outcome variables. Hierarchical linear regressions adjusted for age at diagnosis and cancer stage. Nutrition indicators were entered as independent variables with lipid indicators and factors of activated immunity as dependent variables.

Results

Preliminary bivariate Spearman correlations were used to determine potential control variables by assessing relationships between cytokines of interest and demographic, social, clinical, and medical variables. All were unrelated to significant. Empirical control variables of age at diagnosis, and stage of disease, were entered in all tests of hypotheses.

Conclusions

• There is reason to further investigate nutrition as a mediator of immune and inflammatory response due to the association of fruit and vegetable intake and CTL count.
• A diet consisting of greater fruit and vegetable intake may decrease the functionality of CTL response in lung cancer patients which could be disadvantageous to survival and tumor clearance.
• Nutritive intake of fruit, vegetable and fat had no significant relationship to lipid indicators. Our results were inconclusive regarding TNF-α, and no significant relationship was found between nutrition and IL6.
• Possible additional mediators not identified through this study may contribute as factors of causation for dysregulated immune responses.
• Use of a larger sample size and a more detailed nutrition questionnaire outlining the specificity of immune intake would lend greater understanding of the role nutrition takes in lung cancer.

References


Acknowledgements

Research supported by a grant (R25-CA134283) from National Cancer Institute and the School of Medicine Summer Research Scholar Program.
The effect of reactivation of pRb on the metabolism of cancer cells

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Background

The retinoblastoma protein, Rb, is a tumor suppressor that is commonly found to be inactivated in cancer cells. The anti-proliferative activity of RB is mediated by its ability to inhibit the transcription of genes that are required for cell-cycle progression, like Cyclin A, which is targeted and repressed by active pRb. Phosphorylation of pRb allows E2F-DP transcription factors to dissociate from pRb and become active. When E2F is free it activates target genes like cyclin A, which pushes the cell through the cell cycle. Expression of constitutively active pRb, which is a truncated/mutated form of pRb that cannot be phosphorylated, results in decreased cell number and division.

Objective

The aim of our study is to test how the reactivation of the Rb protein will affect the metabolism of non-small cell lung cancer cells. We inserted a constitutively active form of Rb (PSM-Rb) into NSCLC cells and measured the metabolism in cancer cells compared to the empty plasmid PCDNA3. The metabolism was measured through glucose uptake and glycolysis. Our study’s aim was whether activation of the Rb protein disrupts the metabolism of A549 NSCLC cells.

Methods

We cloned PSM-Rb into PCDNA3 mammalian expression vector and ran western blots to ensure the expression of PSM-Rb. PSM and the empty plasmid, PCDNA3 were transfected into A549 NSCLC for 24 or 48 hours. We measured the effect of changing the concentrations of transfected plasmid, 1 µg or 2 µg on the cell count, and measured the metabolism of PSM vs. PCDNA3 cells. To measure glucose uptake, 14C radioactive 2-deoxy-glucose was added to the cells in glucose-free media. Cells were incubated for one hour, washed 3X with ice cold RPMI glucose-free media, and lysed using 0.1% SDS. The lysates were then collected, and scintillation fluid was added to measure the radioactive activity of C14 into the cells. To measure glycolysis, media was changed to 500 µl of regular media in each well and 2 µl of 3H-glucose were added to each well and incubated for an hour. The medium was collected and spun at 8000 rpm in the centrifuge to pellet any cells that may be in the media. 150 µl of the medium was added to a 500 µl tube within a scintillation vial containing 1ml of H2O. The vials were incubated for 48 hours to allow for the evaporation of 3H2O into the surrounding H2O, and then 5ml of scintillation fluid were added and radioactivity was measured. The radioactivity indicates the amount of 3H2O produced by enolase, an enzyme within glycolysis. The cells in the well are lysed with 0.1% SDS for 5 minutes, and were collected to measure the protein concentration.

Results

The cloning of PSM was successful since the results of the western blots showed Rb specific protein bands that were present in PSM-Rb.

The lower cell count for PSM compared to PCDNA3 confirms the active effect pRb has on attenuating the cell-cycle progression and division which is evidence for its role as a tumor suppressor in cancer cells. Our experiments also suggest that 24-hour transfections were not effective in showing a significant difference between the metabolism of PSM and the PCDNA3 control. However, the 48-hour transfection showed significant results for the glucose uptake in PSM vs PCDNA3 cells. Hence, the cells need 48 hours for the Rb protein from the PSM plasmid to be effective. Our data also suggest that the metabolism is lower in cells that express the active Rb protein. Our conclusion is that the activation of the Rb protein disrupts the growth and glucose metabolism of non-small cancer cells in the A549 cell line.

Conclusions

The cloning of PSM was successful since the results of the western blots showed Rb specific protein bands that were present in PSM-Rb, but were absent on PCDNA3 samples. Cell counts of PSM and PCDNA3 showed significant decrease in cell numbers for PSM compared to that of PCDNA3. Increasing the transplanted plasmid concentration from 1 µg to 2 µg decreased the cell count for both PSM and PCDNA3. Our results for 24 hour transfections show no significant difference between PSM and PCDNA3 on glucose uptake nor in glucose flux (glycolysis). However, 48 hour transfections show substantial decreases between PSM and PCDNA3 for the glucose uptake and the glycolysis measurements.

Acknowledgments

NCI R25 grant support University of Louisville Cancer Education Program NLM/NIH/NCI (R25 CA134238)
Brown Graham Cancer Center, University of Louisville, Clem lab: Romayas Biyi, Lindsey Reynolds, Stephanie Metcalf, and Miriam Reynolds

References


Figure 1. Hyperphosphorylation of pRb allows E2F transcription factors to dissociate from pRb and become active, and hence allows cell-cycle progression.

Figure 2. Comparison of WT Rb to the constitutively active PSM-Rb. PSM-Rb lacks the N-terminal domain and contains phosphorylation site mutations (alanine) within the C-terminal, which allows for PSM-Rb to be always on.

Figure 3. Cloning of PSM into the PCDNA3 mammalian expression vector was done at the BamH1 restriction site.

Figure 4. Flowchart.

Figure 5. The cloning of PSM was successful since the western blots showed Rb specific protein bands that were present in PSM-Rb.

Figure 6. The graphs show the radioactivity in (a), (b) °C glucose uptake, and (c), (d) °H2O release of PSM and PCDNA3 after 24 or 48 hour transfections. The 48-hour transfections are needed for PSM-Rb to be effective and to show some difference compared to PCDNA3 with regards to °C glucose uptake. The °H2O release results were not significantly different for PSM and PCDNA3 at neither 24 nor 48-hour transfections. Figure 7. Increasing the concentration of the transplanted plasmids at 72 hours decreased the number of cells alive in both PSM and PCDNA3. PSM showed a lower number of cells than PCDNA3 at both 1 µg and 2 µg plasmid concentrations.

Figure 7. Release of Rb from PSM and PCDNA3 at different plasmid concentrations.
Sensitizing Pancreatic Cancer Cells to Chemotherapeutics by Modulating Intracellular Iron Homeostasis

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Abstract

Currently, the only successful treatment for pancreatic cancer is invasive surgery. Previous studies to develop chemotherapeutic agents targeting pancreatic tumors have not led to any identifiable success. Due to the extreme vascularization of pancreatic tumors, we hypothesized that the overexpression of heme oxygenase-1 (HO-1) is responsible for the resistance of pancreatic cancer cells to chemotherapeutic drugs. Coinciding with elevated HO-1 expression, pancreatic cancer cells have increased expression of the heavy chain subunit of the intracellular iron-storage protein ferritin (FTH-1). To test our hypothesis, a series of experiments were designed to modulate the expression levels of HO-1 and FTH-1 in vivo. The results indicated that Mia Paca-2 cells, which are used as a model for pancreatic cancer cells, can be used to test the effects of various agents on the expression levels of HO-1 and FTH-1. The results showed that reducing HO-1 expression in Mia Paca-2 cells led to a decrease in cell viability, while increasing FTH-1 expression led to an increase in cell viability. These results suggest that modulating the expression levels of HO-1 and FTH-1 in pancreatic cancer cells can improve the therapeutic effects of chemotherapeutic agents.

Objectives

• Study whether reducing the expression of HO-1 in pancreatic tumor cells improves the therapeutic effects of oxaliplatin.
• Investigate whether expression levels of FTH-1 are involved in the pancreatic tumor cells’ responses to oxaliplatin.
• Examine whether inhibiting HO-1 enzymatic activities by zinc protoporphyrin-9 promotes pancreatic cancer cell sensitivity to oxaliplatin.

Results

Conclusions

• Decreasing HO-1 expression in pancreatic tumor cells sensitizes pancreatic cancer cells to oxaliplatin.
• Reducing the expression of FTH-1 transiently and stably enhances cytotoxic activities of oxaliplatin in pancreatic tumor cells.
• The HO-1 enzymatic activity inhibitor zinc protoporphyrin-9 promotes therapeutic effects of oxaliplatin.

Acknowledgments

Funding for this project was provided by R25-CA134283 grant from National Cancer Institute. This research was also supported by a Undergraduate Research Grant from Executive Vice President For Research and Innovation Internal Grant Program at University of Louisville.
INTRODUCTION

- Prostate cancer (PCa) affects over one million men each year worldwide.
- There is a 28% 5-year survival rate for metastatic PCAs.
- PCa risk factors include age, lifestyle, environmental factors, and chromosomal abnormalities.
- African-American men have higher incidence and mortality rates compared to any other races.
- The role of toll-like receptors (TLRs) in the innate immune response is to induce the expression of immune response and pro-inflammatory cytokines, chemokines, and interleukins.
- Dysregulation of TLR signaling pathways is proposed to lead to inflammatory-induced cancers, including PCa.
- Our lab has revealed significant interactions among TLRs rs3318319, TLR10 rs11696957, and IRF7 rs33424406 as the best 3-factor predictor of PCa risk among U.S. and Jamaican men.

RESEARCH GAP

The impact of two or more sequence variants in TLR, cytokine, and immune response associated sequence variants in relation to PCa risk among men of African descent remains understudied.

RESEARCH OBJECTIVE

Evaluate the individual and joint modifying effects of inflammatory and immune response associated sequence variants in relation to PCa risk among men of African descent.

HYPOTHESIS

Inheritance of one or more sequence variants (linked with cell survival, pro-inflammatory response, immune/tumor cell migration, chemotaxis, invasion, angiogenesis, and lymph node metastasis) will have increased PCa risk relative to men who inherit the wildtype genotype.

STUDY DESIGN

814 men were recruited from cancer screening programs, hospitals, or cancer centers located in Washington, D.C., South Carolina, and Kingston, Jamaica.

- Total population: 603 U.S. men and 211 Jamaican men
- U.S. population: 170 cases, 433 controls
- Jamaican population: 109 cases, 102 controls

METHODS

To test the hypothesis, we evaluated individual and joint modifying effects of Toll-like response genes (n = 32), cytokines (n = 36), and chemokines (n = 5) associated sequence variants among men of African descent.

All analyses were performed using statistical analysis software (SAS 9.4) and entropy-based multi-dimensionality reduction 2.0 beta 8.4

CLINICAL RELEVANCE

The discovery of significant interactions between inflammatory and immune response associated SNP's in relation to PCa risk may help:
- Improve PCa detection strategies;
- Discern between lethal and non-lethal disease based on one's genetic composition;
- Identify new immune/inflammatory response related genes targets needed for the effective treatment of aggressive prostate cancer.

RESULTS

Table 1. Role of TLR Sequence Variants on PCa risk among men of African descent.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls n (%)</th>
<th>Cases n (%)</th>
<th>Age Adjusted OR (95% CI)</th>
<th>P-value</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR10 exonic SNP (rs2231149)</td>
<td>AA</td>
<td>302 (39.79)</td>
<td>305 (39.19)</td>
<td>1</td>
<td>0.284</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>161 (21.33)</td>
<td>172 (22.18)</td>
<td>0.94 (0.66, 1.34)</td>
<td>0.727</td>
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<tr>
<td></td>
<td>GG</td>
<td>239 (30.88)</td>
<td>267 (33.63)</td>
<td>1.27 (0.95, 1.69)</td>
<td>0.110</td>
</tr>
</tbody>
</table>

Table 2. Validation of a Cytokine-TLR Complex Interaction along IRAK4-TOLLIP Axis and PCa among Men of African Descent using MDR.

<table>
<thead>
<tr>
<th>Best Model (dbSNPID#)</th>
<th>Interactions</th>
<th>Cross Validation Consensus Coefs</th>
<th>Average Testing Consensus Coefs</th>
<th>Permutation Testing Consensus Coefs</th>
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</thead>
<tbody>
<tr>
<td>TOLLIP_rs5743899</td>
<td>49</td>
<td>0.571</td>
<td>0.073</td>
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<tr>
<td>IRAK4_rs4251520</td>
<td>2346</td>
<td>0.606</td>
<td>0.001</td>
<td>0.001</td>
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</tbody>
</table>

Table 3. Role of Cytokine Sequence Variants on PCa risk among men of African descent.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Controls n (%)</th>
<th>Cases n (%)</th>
<th>Age Adjusted OR (95% CI)</th>
<th>P-value</th>
<th>P-trend</th>
</tr>
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<tr>
<td>IL1A</td>
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<td>367 (42.01)</td>
<td>378 (45.98)</td>
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<tr>
<td></td>
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<td>192 (23.59)</td>
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<tr>
<td></td>
<td>GG</td>
<td>237 (29.15)</td>
<td>245 (30.14)</td>
<td>1.24 (0.95, 1.62)</td>
<td>0.106</td>
</tr>
</tbody>
</table>

Table 4. Cytokine Complex Interaction along IL1B-IL10-IL1RN Axis and PCa among U.S. Men Validated using MDR.

<table>
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<tr>
<th>Best Model (dbSNPID#)</th>
<th>Interactions</th>
<th>Cross Validation Consensus Coefs</th>
<th>Average Testing Consensus Coefs</th>
<th>Permutation Testing Consensus Coefs</th>
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<tr>
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<td>0.606</td>
<td>0.001</td>
<td>0.001</td>
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</tbody>
</table>

Table 5. Role of Chemokine Sequence Variants on PCa risk among men of African descent.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Controls n (%)</th>
<th>Cases n (%)</th>
<th>Age Adjusted OR (95% CI)</th>
<th>P-value</th>
<th>P-trend</th>
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</thead>
<tbody>
<tr>
<td>CCL5</td>
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<td>311 (37.19)</td>
<td>316 (38.77)</td>
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<td></td>
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<td>146 (18.16)</td>
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<tr>
<td></td>
<td>GG</td>
<td>237 (29.15)</td>
<td>245 (30.14)</td>
<td>1.24 (0.95, 1.62)</td>
<td>0.106</td>
</tr>
</tbody>
</table>

Table 6. Validation of a Cytokine-TLR Complex Interaction along CCL5-CCR9 Axis and PCa among Men of African Descent using MDR.

<table>
<thead>
<tr>
<th>Best Model (dbSNPID#)</th>
<th>Interactions</th>
<th>Cross Validation Consensus Coefs</th>
<th>Average Testing Consensus Coefs</th>
<th>Permutation Testing Consensus Coefs</th>
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</thead>
<tbody>
<tr>
<td>TOLLIP_rs5743899</td>
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<td>0.571</td>
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Figure 1. TLR SIGNALING PATHWAY

Figure 2. Synergistic 3-way Cytokine Interaction along IL1B-IL10-IL1RN Axis and PCa among Men of African Descent using MDR.

Figure 3. Synergistic 3-way Cytokine-TLR Interaction along IL1B-IL10-IL1RN, IRAK4-TOLLIP, and CCL5-CCR9 axes were identified as the significant PCa predictors among cytokine, cytokine and TLR, and chemokine and TLR SNPs, respectively.

Figure 4. A Complex 3-way Chemokine-TLR Interaction was identified as the best PCa predictor among Men of African Descent. This interaction is driven a minor CCL5 and CCR9-TLR4 interaction.

Figure 5. Synergistic 3-way Cytokine-TLR Interaction among IL1B, IL10 and IL1RN targets needed for the effective treatment of aggressive PCa.

Figure 6. Performance of individual and combined inhibition of IL1B, IL10, IRAK4, and TOLLIP expression in PCa cell lines and monitor effects on tumor behavior using preclinical models.

SUMMARY

- Identify new inflammatory and immune response genes that jointly modify PCa risk, disease progression, and disease recurrence among large ethnically diverse sub-groups using novel bioinformatics tools.
- Perform individual and combined inhibition of IL1B, IL10, IRAK4, and TOLLIP expression in PCa cell lines and monitor effects on tumor behavior using preclinical models.

FUTURE DIRECTIONS

- We thank Rick Kittles, Mary Jackson and others for contributing study participants from Washington D.C., South Carolina, and Jamaica.
- Acknowledgements: National Cancer Institute grant R25-CA-134283, Clinical Translational Science Pilot Grant to LRK, “Our Highest Potential” in Cancer Research Endowment to LRK, and P20-MD00175 NH MCHD to KS.

ACKNOWLEDGEMENTS
Effect of Pharmacological Inhibition of Phosphoserine Aminotransferase (PSAT1) on Metastatic Breast Cancer Motility

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Departments of Biochemistry & Molecular Genetics¹ and the James Graham Brown Cancer Center²
University of Louisville³, Louisville, KY

ABSTRACT

Prior studies have shown that the serine biosynthesis pathway is upregulated in breast cancer cell lines, specifically the enzyme phosphoserine aminotransferase (PSAT1). These data suggest that PSAT1 is in some way connected to breast cancer development and progression. Interestingly, additional studies have shown that silencing PSAT1 resulted in no changes in cellular proliferation; therefore, raising the question of whether PSAT1 plays some role in cellular motility and the metastasis of breast cancer cells. Based on this fact, we hypothesized that pharmacological inhibition of PSAT1 with small molecule antagonists may decrease the overall motility and metastatic character of breast cancer cells (MDA-MB-231).

To determine if PSAT1 has a functional role in cellular motility, two different assays were conducted: a wound healing assay (or scratch assay) and a motility assay. In the scratch assay, MDA-MB-231 triple-negative breast cancer cells were plated at 100k cells per well in a 24-well culture plate. The adherent monolayer of cells was subsequently “scratched” with a pipet tip and then treated with vehicle or different concentrations of PSAT1 inhibitors. The healing of the scratch was monitored, using an EVOS imaging microscope, until the scratch completely healed.

The motility assay utilized 24-well Milipore hanging cell inserts that contained a porous membrane. In this assay, IMEM media was placed directly into each well of a 24-well plate. Then, the inserts were placed into each well and 25k cells were plated in serum-free IMEM media directly into this insert. All inhibitors were added in equal concentrations to the serum-free and regular IMEM media solutions to prevent dilution during the assay. After 24 hours, the membrane was fixed with methanol and stained using crystal violet. Images of each membrane were then taken and compared to control inserts.

Overall, both cellular motility assays provided consistent data. However, of the eight PSAT1 inhibitors tested, none showed a significant decrease in cellular motility when compared to controls. Taken together, the results are inconclusive and neither support nor refute the proposed hypothesis that using small molecule inhibitors of PSAT1 may decrease the overall motility and metastatic nature of MDA-MB-231 breast cancer cells. Fortunately, initial testing using genetic PSAT1 suppression has shown the effects of PSAT1 inhibition in MDA-MB-231 cells. The eight inhibitors tested were only a small fraction of the molecules identified as possible antagonists of PSAT1. Therefore, looking forward, many more potential PSAT1 inhibitors await similar motility testing. Ultimately, if one of these inhibitors proves to show success in vitro, it could have future clinical relevance for the treatment of metastatic breast cancer.

INTRODUCTION

It is currently the metastatic (stage IV) breast cancer 5-year survival rate is 25% compared to 90% and 72% for stages I and II, respectively.

Therefore, copious amounts of research are focused on breast cancer metastasis and understanding the underlying mechanisms which enable breast cancer cells to metastasize throughout the body.

Of the many studies conducted, several have shown that the serine biosynthesis pathway is highly upregulated in breast cancer cell lines, specifically the enzyme phosphoserine aminotransferase (PSAT1).

Interestingly, additional studies have shown that silencing PSAT1 resulted in no changes in cellular proliferation.

This raises the important question: does PSAT1 play some role in cellular motility and metastasis of breast cancer cells?

RATIONALE

Previous studies within the lab demonstrate that PSAT1 knockdown conditions decreases overall cellular motility (MDA-MB-231 cells). The results of motility and wound healing assays (shown in Figure 1) illustrate this phenomenon and suggest PSAT1 has some role in motility and metastatic character. This decrease in cellular motility makes PSAT1 a good candidate for potential therapeutic targeting via pharmacological inhibition in metastatic breast cancer cells.

METHODOLOGY

Pharmacological inhibition of PSAT1 with small molecule antagonists may decrease the overall motility and metastatic character of breast cancer cells (MDA-MB-231).

RESULTS

DISCUSSIONS

ACKNOWLEDGEMENTS

Research funded by the National Cancer Institute R25 CA134283-04 Cancer Education Program Grant at the University of Louisville.
Detection of pancreatic cancer using a modified gelatin nanocontrasting agent

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ABSTRACT

Purpose: Modern diagnostic methods of pancreatic cancer have not presented themselves as an effective option for patients due to the inherent difficulties that are associated with pancreatic cancer. These difficulties arise from the inability for most modern imaging modalities to accurately target/imaging the location of tumor regions and apply effective treatment. Due to these difficulties, nanotechnology has gained much interest as a possible method of tumor targeting as well as a vehicle for drug delivery. We hypothesized that the gelatin-coated mesoporous silica nanoparticles conjugated with a targeting ligand, Syndecan-1, will provide increased detection and treatment of pancreatic cancer.

Methods: Mesoporous silica nanoparticles coated with gelatin (MSN-G) were synthesized from a colloidal mixture of octyl trimethoxyoxylsilane (CTAB) along with tetraethoxysilane (TEOS). Desired pores were formed through a series of dialysis processes. A gelatin-coat was synthesized and formed onto the MSN. The MSN-G were modified to contain a fluorescent dye and incorporate a targeting ligand protein for the IGF receptor. Several methods were utilized to characterize the MSN-G including Energy-dispersive x-ray spectroscopy (EDX), UV-Vis Spectroscopy, and Transmission Electron Microscopy (TEM). Pancreatic cancer cells (Panc1, SVP10L, and PANC) were grown and covalently conjugated with Matrix Metalloproteinase-2 (MMP2) and Prolose Inhibitor and then treated with the conjugated MSN-G. Odyssey infrared imaging was utilized to determine the binding affinity of the Syndecan-1 ligand and the degradation interaction between the MMP2 and gelatin coat. Furthermore, SVP10L cells, containing the IR-780 dye encapsulated by the MSN-G were transformed into tissue phantoms and imaged using Multiphoton/Confocal/Optical Tomography (MSOT).

Results: TEM images along with Dynamic Light Scattering (DLS) results demonstrated that the MSN-G were approximately 35 nm in size as detected by the two techniques, indicating the successful encapsulation of the MSN with the gelatin coat. Odyssey infrared imaging displayed increased signal at higher concentrations of MMP2. This increased signal demonstrated the higher level of gelatin degradation from the MSN-G, compared to lower amount of degradation found in the presence of the Prolose Inhibitor. Imaging at MMP2 displayed approximately 24 times and 16 times greater binding intensity compared to the MMP Inhibitor for SVP10L and PANC respectively. MSN-G were placed into tissue phantoms and imaged via MSOT. A spectrum for the MSN-G encapsulated with IR-780 dye was obtained.

Conclusion: IGF1 receptor targeted, gelatin-coated MSNs were found to possess substantial tumor cell binding against multiple cell lines and demonstrated potential as a therapeutic nanocontrast agent for pancreatic cancer.

RESULTS

Figure 1: Transmission electron microscopy (TEM) and Scanning transmission Electron Microscopy (STEM) imaging of gelatin-coated MSNs (MSN-G). MSN-G were synthesized and conjugated with Matrix Metalloproteinases (MMP2) and Prolose Inhibitor and then treated with the conjugated MSN-G. Odyssey infrared imaging was utilized to determine the binding affinity of the Syndecan-1 ligand and the degradation interaction between the MMP2 and gelatin coat. Furthermore, SVP10L cells, containing the IR-780 dye encapsulated by the MSN-G were transformed into tissue phantoms and imaged using Multiphoton/Confocal/Optical Tomography (MSOT).

Figure 2: Dynamic Light Scattering (DLS) results demonstrated that the MSN-G were approximately 35 nm in size as detected by the two techniques, indicating the successful encapsulation of the MSN with the gelatin coat. Odyssey infrared imaging displayed increased signal at higher concentrations of MMP2. This increased signal demonstrated the higher level of gelatin degradation from the MSN-G, compared to lower amount of degradation found in the presence of the Prolose Inhibitor. Imaging at MMP2 displayed approximately 24 times and 16 times greater binding intensity compared to the MMP Inhibitor for SVP10L and PANC respectively. MSN-G were placed into tissue phantoms and imaged via MSOT. A spectrum for the MSN-G encapsulated with IR-780 dye was obtained.

Figure 3: Evaluation of gelatin degradation and IR-780 dye release from MSN-G. A 24L sample of Syndecan-1 conjugated MSN-G were placed into dialysis tubing and dialyzed in a solution of PBS pH 7.4. A 10 µg/mL solution of MMP2 was introduced into the particle solution and absorbance was measured by UV-Vis Spectroscopy over a period of 8 hrs. Optimal absorbance intensity of the IR-780 dye was found at ~2.5 hours. The MSN-G exhibited ~1.5 times greater dye release in the MMP2 that compared to MMP2. This relative release illustrates the selective gelatin degradation found between MMP2 and the MSN-G.

Figure 4: UV-Vis spectrum of IR-780 loaded MSN-G. The graph demonstrates an absorbance peak of ~780 nm. The identification of the 780 nm peak indicates the successful loading of IR-780 dye into the MSN-G and confirms the fluorescent capability of this particle.

Figure 5: Energy-dispersive x-ray spectroscopy (EDX) of the MSN-G confirmed the presence of bioabsorbable and more importantly non-cytotoxic elements found in the MSN-G. The structure of the MSN-G consist of mainly silica, carbon, and oxygen.

CONCLUSION

Theranostic nanoparticles provide the benefits of enhanced tumor detection along with serving as a vehicle to transport chemotherapeutics

A biological marker found in aggressive cancer lines is the elevated levels of matrix metalloproteinase 2, a gelatinsase, and their corresponding enzymatic activity of degrading gelatin compounds

This study demonstrated the successful synthesis of gelatin-coated mesoporous silica nanoparticles (MSN-G) conjugated with a Syndecan-1 ligand for therapeutic imaging of pancreatic cancer

The MSN-G displayed selectively enhanced gelatin degradation in the presence of MMP2 for multiple cell lines in in vitro studies for both Odyssey infrared imaging and tissue phantoms

Further studies involve in-depth characterizations studies of the MSN-G and continuation into in vivo studies

ACKNOWLEDGEMENTS

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Synthesis of Syndecan-1 Conjugated MSN-G Schematic

Figure 6: Odyssey infrared imaging displayed significant difference in the level of signal intensity between varied concentrations of MMP2 for IR-780 dye conjugated MSN-G. Approximately 48 times greater for SVP10L and 16 times greater for Panc1

Figure 7: Visualization of targeted MSN binding to IGF1 positive cells using MSOT and cells incubated with 50 µL of MSN-G for 1 hour. Cells were washed and insert第四届 into tissue phantoms and signal intensity was determined using MSOT. The resulting graphs display greater signal intensity present in the MMP2 well compared to the cells alone and MMP inhibitor.

20 nm