Loss of Tumor Suppressor PTEN Induces Leptin Mediated Leptin Gene Expression: A Feed-Forward Loop Operating in the Lung

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Resulting Publication:

Loss of Phosphatase and Tensin Homolog (PTEN) Induces Leptin-mediated Leptin Gene Expression feed-forward loop operating in the lung

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Technical Summary of Work:

Elevated levels of systemic and pulmonary leptin are associated with diseases related to lung injury and lung cancer. However, the role of leptin in lung biology and pathology, including the mechanism of leptin gene expression in the pathogenesis of lung diseases, including lung cancer remains elusive. Herein, using conditional deletion of tumor suppressor gene Pten in the lung epithelium in vivo in transgenic mice and human PTEN-null lung epithelial cells, we identify LEP driven feed-forward signaling loop in the lung epithelial cells. Leptin mediated leptin/leptin-receptor gene expression, likely amplifying leptin signaling that may contribute to the pathogenesis and severity of lung diseases, resulting in poor clinical outcomes. Loss of Pten in the lung epithelial cells in vivo, activated adipocytokine signaling, and induced leptin synthesis as ascertained by genome-wide mRNA profiling and pathway analysis. Leptin gene transcription was mediated by binding of transcription factors NRF-1 and C/EBP-δ to the proximal and STAT3 to the distal promoter regions as revealed by leptin promoter-mutation, chromatin immunoprecipitation (ChIP) and gain and loss of function studies in lung epithelial cells.

Leptin treatment induced expression of leptin and leptin-receptor in the lung epithelial cells via activation of the MEK/ERK, PI3K/AKT/mTOR and JAK2/STAT3 signaling pathways. Expression of constitutively active MEK-1, AKT and STAT3 proteins increased, while treatment with MEK, PI3K, AKT and mTOR inhibitors decreased LEP expression, indicating that LEP via MAPK/ERK1/2, PI3K/AKT/mTOR and

JAK2/STAT3 pathways, in turn, further induces its own gene expression. Thus, targeted inhibition of the LEP mediated feed-forward loop provides a novel rationale for pharmacotherapy of disease associated with lung injury and remodeling, including lung cancer.

Plain Language Summary of Work:

Leptin (LEP) is a 16-kDa protein hormone that plays a key role in regulating energy intake and expenditure, including appetite and hunger, metabolism, and behavior. It is one of the most important adipose-derived hormones. Leptin functions by binding to the leptin receptor. Elevated levels of LEP in the lung and serum are associated with, and potentially exacerbate severity and progression of lung diseases including acute lung injury (ALI), acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD), airway remodeling associated with asthma and lung cancer. Despite advancements in understanding the pathophysiology of ALI, ARDS, COPD, asthma and lung cancer, how LEP is induced and contributes to severity and progression of these lung diseases remains poorly understood.

In the present work, I have demonstrated that loss of Pten, (a tumor suppressor gene) in the lung epithelium in vivo in transgenic mice and in PTEN-null human lung epithelial cells induces LEP signaling. LEP induced transcription of the LEP and LEPR gene is mediated by binding of certain proteins NRF-1 and C/EBP-δ to proximal regions and STAT3 to distal regions of the LEP gene promoter.. Increased LEP expression in Pten deficient respiratory epithelial cells elicits an autocrine feed-forward loop via up-regulation of LEPR on the lung epithelial cells. AS shown by my research the LEP/LEPR signaling loop is driven by activation of PI3K/AKT/mTOR, MEK/ERK and JAK/STAT3 pathways. These three signaling pathways induced/increased expression of both, LEP and LEPR gene, thereby setting up a positive feed-forward LEP/LEPR signaling loop in the lung epithelium. Taken together, aberrant amplification of the LEP mediated LEP signaling loop potentially deregulates modulatory role of LEP, likely exacerbating the severity of lung diseases, including cancer, leading to poor clinical outcomes. Identification of the signaling loop and association of leptin gene with Pten paves the way for novel drugs for lung diseases like cancer.

Summary of the Significance of the Work:

My research has demonstrated that leptin, which is an obesity associated gene can itself regulate its own expression in lung epithelial cells and likely in PTEN deficient lung tumors and other lung diseases. This finding opens up avenues to develop therapies that can silence leptin signaling in lung pathologies, thereby reducing disease associated morbidity. Also, this is the first association of obesity of and lung cancer, which has potential impact on health care management and treatment of patients who suffer from obesity and can be predisposed to developing lung cancers.

My Specific Contribution:

I conceptualized, designed and executed the complete project as the lead investigator. My detailed contribution is as follows:

1. Created and validated a transgenic mouse model with conditional PTEN knockout.
2. Performed microarray analyses by isolating RNA from mouse lungs with PTEN knockouts.
3. Carried out bioinformatics analysis of microarray data to identify PTEN regulated genes (includes pathway and functional clustering analysis).
4. Performed all cell culture with lung cancer cells and transfection experiments.
5. Performed all protein extraction and western blot experiments
6. Designed Real Time PCR primers and conducted Real Time PCR experiments.
7. Designed Chromatin Immunoprecipitation (ChIP) primers and performed ChIP assays.
8. Performed all immunohistochemistry experiments.
9. Analyzed data from the Electric Cell Substrate Impedance Sensing/Wounding (Migration) Assay.

Summary of the Implementation/Influence of the Work:

I was selected to deliver an oral presentation about this project at the prestigious American Thoracic Society conference in Philadelphia, 2013. The research paper has been recommended as being of high impact in F1000 prime.