Hepatic Steatosis Induced By Bioactive Lipids Regulated by AKT Isoform Specific Signals

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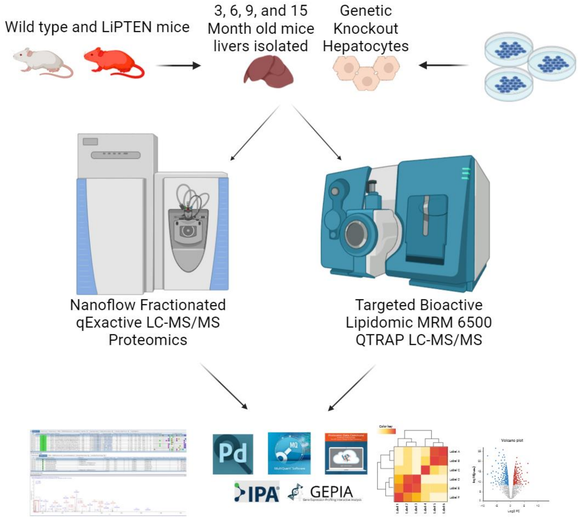
Introduction

Hepatocellular carcinoma (HCC) is the second leading cause of cancer deaths in the world. Patients diagnosed with HCC have a median survival time of just 11 months and 5-year survival rate of less than 20%. Unfortunately, the majority of HCC patients are diagnosed late with disease stages, precluding them from surgical treatment options. As such, it is paramount to discover novel molecular targets for treatment of HCC. Leveraging proteomics, metabolomics, and transcriptomics we utilize a PTEN-null animal model that recapitulates human disease progression to determine molecular signatures that could be used for patient prognosis and treatment in heterogenous liver cancer progression. We then further analyze the potential cellular sources of the metabolic dysregulation occurring in HCC by looking at hepatocyte metabolism.

Methods

Liver Isolation, Extraction, and Mass Spectrometry Analysis

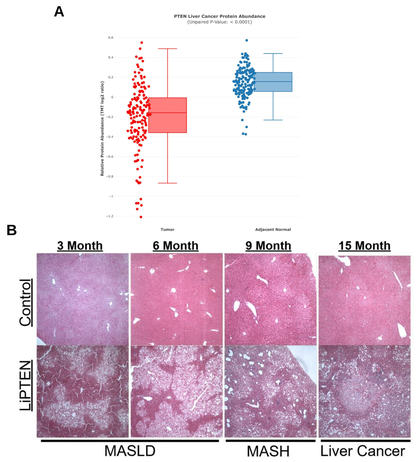
Fig 1. Overall Workflow Summary.



Results

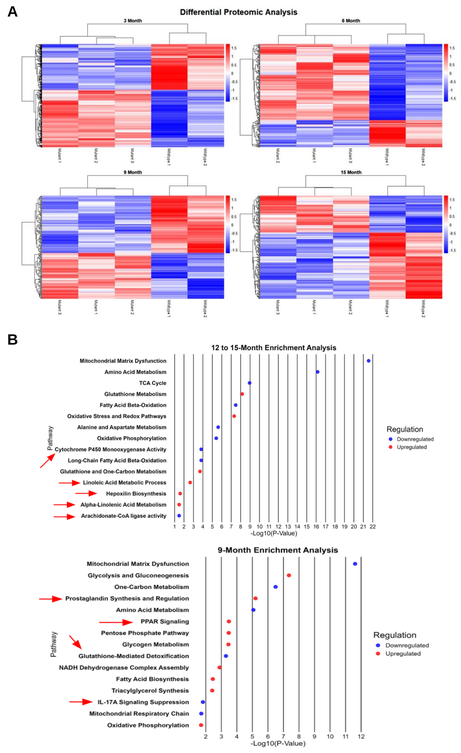
Proteomics Profiling

Fig 2: Validation of PTEN-null Model. **A.** cProSite analysis comparing tumor versus adjacent uninvolved (normal) tissues derived from HCC patients shows significant decreased expression of PTEN. **B.** Histological validation of liver specific deletion of PTEN (LiPTEN) murine model recapitulated human liver disease progression



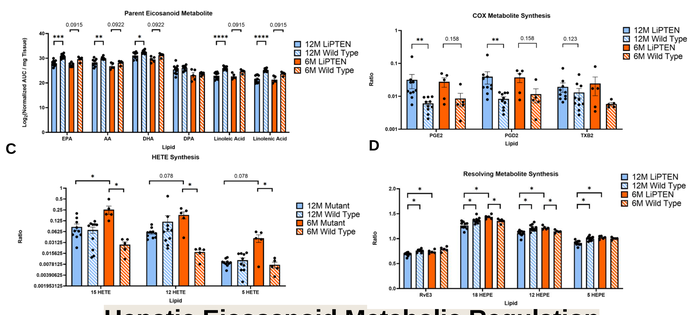
Differential Proteomic Analysis

Fig 3: Differential Proteomic Analysis. **A.** Differential analysis reveals unique proteomes. **B.** Enrichr analysis using differentially expressed proteins shows eicosanoid dysregulation as key node in the steatosis to tumorigenesis transition.



Bioactive Lipid Mediated Inflammation

Fig 4: Bioactive and Oxidized Lipid Enrichment in LiPTEN Liver Disease. **A.** Dysregulation of unsaturated fatty acid metabolism **B.** Increased COX metabolism driving inflammation. **C.** Early enhancement of lipoxygenase activity, collapsing late stage. **D.** Dysregulated resolving biosynthesis



Hepatic Eicosanoid Metabolic Regulation

Fig 5: Hepatic Eicosanoid Metabolism is AKT Isoform Specific. **A.** Proteomics reveals distinct isoform specificity for inflammatory response, TNF-α, NF-kβ, and prostaglandin synthesis pathways in AKT-KO hepatocytes. **B.** Enzymatic activity analysis shows AKT isoform dependence in COX-mediated hepatic eicosanoid metabolism after IGF stimulation. **C.** Hepatic lipoxygenase enzymatic activity also is AKT isoform dependent after IGF stimulation.

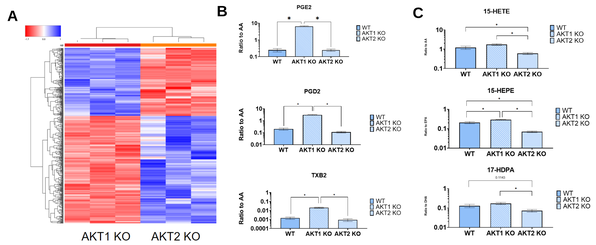
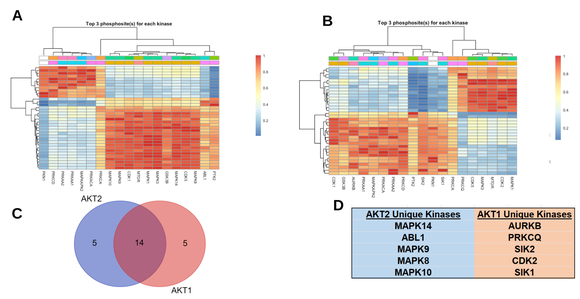


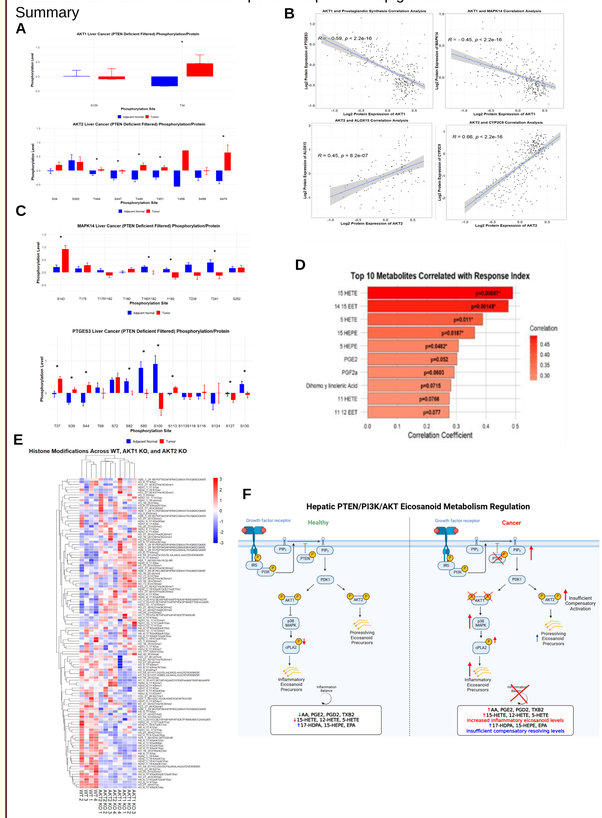
Fig 6: Phosphoproteomics Reveals Isoform Specificity in Kinase Metabolic Regulation. **A.** AKT1KO phosphosite enrichment. **B.** AKT2KO phosphosite analysis. **C.** Unique enriched kinase identification analysis. **D.** Table of unique identified enriched kinases from phosphosite analysis. **E.** Validation of kinase specificity.



Results Cont.

Clinical Translation and Future Directions

Fig 7: Eicosanoid Dysregulation and AKT Isoform Specificity in Clinical Samples. **A.** Clinical HCC data reveals isoform-specific AKT regulation. **B.** Correlational analysis confirms that AKT1 suppresses COX-driven prostaglandin biosynthesis, while AKT2 promotes LOX and CYP450 lipid mediator production. **C.** Tumor tissues show elevated PTGES3 phosphorylation at S133, enhancing PGE2 synthesis, and increased MAPK14 phosphorylation at S143, activating p38 MAPK and cPLA2-driven AA release. **D.** Lipid mediators predict better immunotherapy response, as 15-HETE, 14,15-EET, and 5-HETE positively associate with treatment outcomes. **E.** Histone modification point to a potential epigenetic link **F.** Mechanistic Summary



Conclusions

AKT1 and AKT2 have distinct yet coordinated roles in hepatic eicosanoid metabolism. AKT1 suppresses p38 MAPK, cPLA2, and COX activity to limit pro-inflammatory prostaglandin production—a regulation lost in AKT1-deficient models and clinical HCC. AKT2 supports LOX and CYP450-driven resolution-phase lipid synthesis but loses effectiveness under chronic stress, despite sustained phosphorylation. This suggests AKT2 requires additional regulatory inputs that become disrupted in disease. Together, these findings highlight AKT isoform-specific contributions to lipid balance and support targeted modulation of AKT1 and AKT2 to mitigate inflammation in liver disease.

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