Abstract
In order to better understand the development of hereditary leiomyomatosis associated renal cell carcinoma (HLRCC), a patient derived xenograft (PDX) animal model was generated and characterized in our lab from a 24-year-old patient’s resected HLRCC tumor. In addition to the animal model, we also retrospectively determined the number of type 2 papillary RCC patients at MD Anderson that had reduced fumarate hydratase (FH) expression.

Background
Hereditary Leiomyomatosis

• Autosomal dominant
• Characterized by multiple cutaneous leiomyomas, early onset uterine leiomyomas, and type 2 papillary RCC
• Mutations in FH, an essential enzyme in the citric acid cycle

Fumarate hydratase (FH) mutation
• Mutations in FH increase patient risk for the development of type 2 papillary renal cell carcinoma (RCC), a rare and aggressive type of renal cancer
• Results in a decrease in FH expression and a buildup of fumarate → leads to the stabilization of hypoxia inducible factor (HIF)
• FH-deficient RCC → increased glutamine-dependent carbonylation for energy

Methods
HLRCC PDX Animal Model
• Implanted HLRCC resected tumor tissue from a 24-year-old woman into NOD scid gamma (NSG) mice subcutaneously
• Tumors harvested when they reached ~1500 mm² and resected tumor tissue was passaged into another set of NSG mice
• At the conclusion of the 3rd passage, tumor tissue from PDX model was subjected to a series of analytical studies

Results
HLRCC PDX and normal kidney tissue have varying levels of HIF1α and GLS expression. We carried out gel electrophoresis and Western Blot analysis to measure the expression of lysates from 3 unique tumors from our HLRCC PDX model as well as lysates of normal human kidney tissue. This analysis revealed the HLRCC PDX model had higher expression of HIF1α and HIF2α (Figure 3a, 3b). HLRCC PDX model also displayed higher expression of GLS1 but had similar GLS2 expression levels when compared to normal human kidney tissue. FH displayed lower levels of expression in the HLRCC PDX model when compared to the normal human kidney tissue. (Figure 3c). Expression of β-actin and Histone H3 (Figure 3a, 3b), loading controls, remained constant for HLRCC PDX and normal human kidney tissue.

Conclusions
1) HLRCC PDX animal model displayed
• Increased expression of HIF1α and HIF2α
• Increased expression of GLS1
• Decreased expression of FH compared to normal human kidney tissue
2) We generated an HLRCC PDX animal model that was morphologically and genetically similar to the original HLRCC patient tissue

Our findings illustrate 1) FH-deficient HLRCC leads to an increase in HIF1α and HIF2α. This increased expression in HIF1α and HIF2α increased expression of HIF1α and HIF2α. This increase in expression of HIF1α and HIF2α leads to increased expression of GLS1. 2) HLRCC’s reliance on glutamine-dependent carboxylation in place of oxidative phosphorylation also leads to an increase GLS1.

Discussion
We hypothesize that HLRCC is glutamine addicted and requires glutamine for sufficient metabolism. We also hypothesize that the accumulation of fumarate may lead to FH-deficient HLRCC and will lead to the activation of specific proteins associated with oncogenesis. These proteins are inclusive of VEGF (angiogenesis), PDGF (tumor growth), and TGFr (tumor growth).

Future Research
• Look toward treating HLRCC mice with drugs that inhibit the activity of specific enzymes and transcription factors such as HIF or VEGF
• Create a database for MD Anderson patients with FH-deficient type 2 papillary RCC in order to draw relationships for future research projects

References.