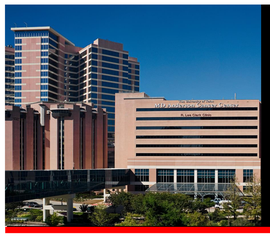

# Creation and characterization of patient derived xenograft animal model of hereditary leiomyomatosis associated renal cell carcinoma

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## 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 are also retrospectively determining 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

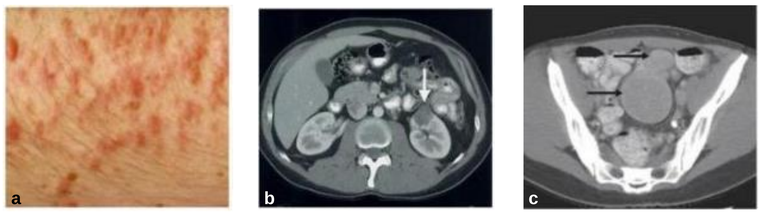


**Figure 1. Clinical Manifestation of HLRCC. (a)** Cutaneous leiomyomas resulting from HLRCC. **(b, c)** CT imaging of type 2 papillary RCC.

### *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 carboxylation 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 $mm^3$ 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

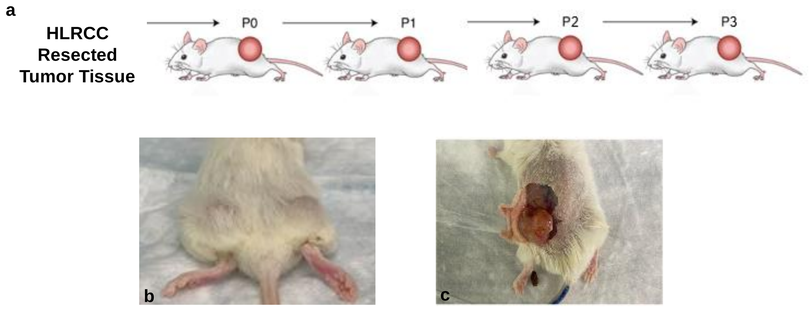


**Figure 2. HLRCC PDX Animal Model. (a)** HLRCC was implanted into NOD scid mice and passaged 3 times before being harvested. **(b)** Cutaneous HLRCC PDX tumors growth in mice during passage process. **(c)** Harvesting of cutaneous HLRCC PDX tumor tissue.

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## Results

### *HLRCC PDX and normal kidney tissue have varying levels of HIF 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 B-actin and Histone H-3 (Figure 3a, 3b), loading controls, remained constant for HLRCC PDX and normal human kidney tissue.

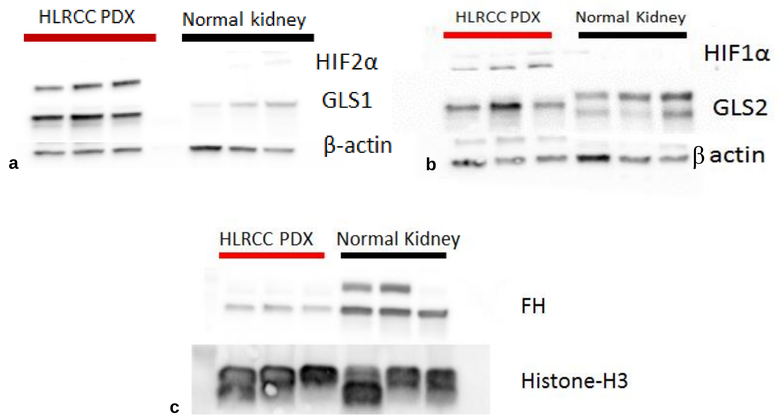


**Figure 3. Final Western Blots of lysates from 3 unique HLRCC PDX tumors and lysates from normal human kidney tissue. (a)** HLRCC PDX tissue displayed increased expression of HIF2α and GLS1. **(b)** HLRCC PDX tissue displayed increased expression of HIF1α. **(c)** FH had lower expression in HLRCC PDX tissue.

### *Histology of HLRCC PDX tissue and HLRCC patient tissue reveal morphological similarity.*

We next sought to determine the histological and genetic similarity between HLRCC PDX tissue and the original HLRCC patient tissue. Analysis of tissue histology revealed that HLRCC PDX tissue and HLRCC patient tissue are morphologically similar per MD Anderson pathologist, Dr. Rao.

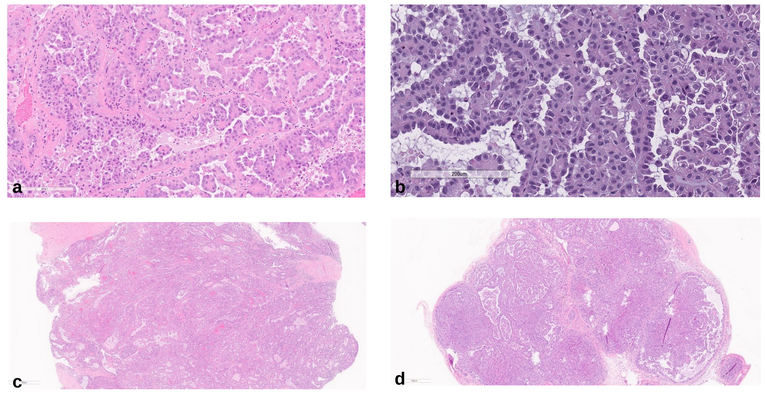


**Figure 4. Histology of HLRCC PDX tissue and HLRCC patient tissue. (a, c)** HLRCC patient tissue histology. **(b, d)** HLRCC PDX tissue histology.

### *STR Fingerprinting of HLRCC PDX tissue and HLRCC patient tissue reveal genetic similarity.*

To further confirm the genetic similarity of the HLRCC PDX model and the HLRCC patient tissue, short tandem repeat (STR) analysis was utilized to compare alleles at different loci between the two samples. Tandem repeats confirmed that HLRCC PDX tissue was genetically similar to the original HLRCC patient tissue (Table 1).

**Table 1. STR Fingerprinting of HLRCC PDX tissue and HLRCC patient tissue.**

| Sample | RCC84M1 | RCC84M2 | RCC84pat |
|---|---|---|---|
| AMEL | X | X | X |
| CSF1PO | 10,11 | 10,11 | 10,11 |
| D13S317 | 10,11 | 10,11 | 10,11 |
| D16S539 | 12 | 12 | 12 |
| D18S51 | 12 | 12 | 12 |
| D21S11 | 28,29 | 28,29 | 28,29 |
| D3S1358 | 16,18 | 16,18 | 16,18 |
| D5S818 | 9,11 | 9,11 | 9,11 |
| D7SD20 | 11 | 11 | 11 |
| D8S1179 | 13,14 | 13,14 | 13,14 |
| FGA | 24 | 24 | 24 |
| TH01 | 8 | 8 | 8 |
| TPOX | 8,11 | 8,11 | 8,11 |
| vWA | 16,17 | 16,17 | 16,17 |

HLRCC PDX tissue

HLRCC patient 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 increase results in increased expression of GLS1. **2)** FH-deficient 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 due to FH-deficient HLRCC will lead to the activation of specific proteins associated with oncogenesis. These proteins are inclusive of VEGF (angiogenesis), PDGF (tumor growth), and TGFα (tumor growth).

## Future Research

- Look toward treating HLRCC PDX 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.

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