**Specific Aims**

Tuberous sclerosis complex (TSC) is a disorder that results in the formation of benign tumors throughout the body, including in the kidneys[1]. TSC is caused by inactivating mutations in either the hamartin or tuberin tumor suppressor genes. Hamartin and tuberin form a heterodimer that has GTPase-activating protein activity toward Rheb, a negative regulator of the kinase mTORC1[2]. Loss of this regulatory activity affects multiple pathways including AKT, MAPK, AMPK, b-catenin, calmodulin, MTORC1, CDK, and results in dysregulation of cell growth and proliferation, thus resulting in tumor formation. These tumors are typically benign, with the exception of renal cell carcinoma. *The differential role of tuberin (TSC2) in metastatic renal cell carcinoma as compared to typical benign hamartomas is unknown*[3].

My **primary goal** is to find differences in the role of tuberin in renal cell carcinoma as compared to other benign manifestations of TSC. I will use the mouse *Mus musculus* because it is able to represent the various manifestations of TSC and shows high homology with human tuberin at 91%[4]. I **hypothesize** that differential manifestations of tuberin in renal cell carcinoma and hamaratomas will eludicate why renal cell tumors become invasive while others do not. My **long-term goal** is to better understand the differences between metastatic and benign models of TSC for further insight in carcinogenesis and future treatment.

**Aim 1**: Identify conserved sequences in intronic TSC2 that may be important for transcription.

Approach: I will use BLAST to identify TSC2 homologs in a wide variety of organisms and humans. I will then build phylogenetic trees using neighbor-joining and the BLOSUM62 matrix. I will identify highly conserved intronic sequences over evolutionary time then use CRISPR/Cas9 to create mice with deletions of these sequences. I will then screen for any pathogenic effects.

Rationale: Patients with no mutation identified may have intronic variants that affect TSC2 function.

Hypothesis: I hypothesize that mutations in conserved regions will identify important intronic sequences for TSC2 transcription.

**References**

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