**Specific Aims**

Tuberous sclerosis complex (TSC) is a genetic disorder that results in the formation of benign tumors throughout the body, including the kidneys[1]. TSC is caused by inactivating mutations in either the hamartin or tuberin tumor suppressor genes. These genes code for proteins that form a heterodimer with GTPase-activating protein activity toward Rheb, a negative regulator of the kinase mTORC1[2]. Loss of this regulatory activity 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 in malignant renal cell carcinoma (RCC) in comparison to benign renal angiomyolipoma (AML) is unknown*[3].

My **primary goal** is to find differences in the role of tuberin in RCC in comparison to AML. I will use *Rattus norvegicus* as a model organism because it has been shown to manifest both TSC-associated RCC and AML and shows high homology with human tuberin (91%)[4]. I **hypothesize** that differential manifestations of tuberin in RCC and AML will elucidate why some tumors become invasive while others do not. My **long-term goal** is to better understand the differences between malignant and benign models of TSC for further insight into carcinogenesis and potential future treatments.

Aim 1: Identify conserved sequences in intergenic areas flanking the tuberin gene and introns that may be important for gene regulation and protein structure.

**Approach**: I will use BLAST to identify tuberin homologs in humans and homologous species. I will then build phylogenetic trees to identify highly conserved sequences over evolutionary time and compare these areas with motifs on MEME. On the best candidates, I will use CRISPR/Cas9 to create knockout mice and screen for growths in the kidneys.

**Rationale**: While tuberin exons are well-characterized, approximately 10% of patients with TSC have no mutation identified. Alternate mechanisms of TSC pathogenesis that may provide information about why specific malignancies occur.

**Hypothesis**: I hypothesize that mutations in conserved regions will identify important intronic and intergenic sequences that cause TSC in patients who are currently no mutation identified by altering gene regulation and protein structure.

Aim 2: Identify differential expression between benign versus cancerous growths.

**Approach:** I will isolate whole blood and whole-tissue samples from growths in TSC patients with either RCC or AML, either, or both. I will then use RNA-seq to determine differential gene expression both for a single patient and against the cohort. Then I will use gene ontology to identify gene function patterns for identified differences. Finally, I will use CRISPR/Cas9 to create mice with deletions in differentially expressed genes and screen for RCC and AML histology.

**Rationale**: Differential gene expression between people and/or systems may result in differential manifestations of TSC.

**Hypothesis**: I hypothesize that differential gene expression may result in the difference between malignant versus benign growths in TSC.

**References**

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