SMITH-KINGSMORE SYNDROME FOUNDATION RESEARCH UPDATE

TEAM: Andrew C. Liu (PI), Yang Shen (postdoc fellow), Destino Roman (research assistant)

DATE: 11/02/2020

BACKGROUND:

SKS is an autosomal dominant disorder caused by heterozygous de novo or inherited gain-of-function (GOF) mutations of the mTOR gene. mTOR is a master nutrient/energy sensor and plays central roles in protein synthesis, cellular homeostasis, cell growth and proliferation, synaptic plasticity, and cancer. The clinical features of SKS patients include neurological and cognitive symptoms, but also metabolic deregulation and non-cognitive manifestations. Metabolic deregulation caused by SKS may underlie the cognitive symptoms. The long-term objective of this research is to investigate the metabolic and molecular mechanisms of SKS pathogenesis and offer new strategies for treatment.

A major direction of our research is to investigate the SKS effects on sleep/wake homeostasis which is regulated by the hypothalamus, and more specifically, the suprachiasmatic nuclei (SCN). In our recent work, we discovered that mTOR also regulates circadian clock function. SKS patients generally have poor-quality sleep. More recently, our clinical team (Dr. Prada) observed that MTOR inhibitors (e.g. rapamycin) altered sleep/wake states. The sleep/wake deregulation caused by SKS and rapamycin likely impacts feeding behavior and metabolism. Although a primary concern for SKS is neuropathology, the sleep dysfunction demands attention because it affects every system in the body including, but not limited to, the brain, and impacts the wellbeing of both patients and caregivers.

More specifically, the SKS mutants, presumed to be hyperactive, have not been systematically characterized. Clinical and molecular evidence supports the notion that these mutations are not the same. In this research, we will develop cellular models and use them to determine the SKS activities under starving and nutrient-rich conditions and rapamycin dosage effects. Further, we will assess cell growth rate and circadian clock function. These results will inform more precise, personalized treatments strategies, i.e. optimize the benefits for individual patients and reduce adverse effects. In a separate line of research, we will generate animal models of select SKS alleles to recapitulate mutations that occur in patients. Given the essential role of mTOR in cell energy homeostasis, this research is expected to have broader implications in other pathologies including neurology, aging and cancer.

RESEARCH METHODS: (# ALLELES AND # MICE)

Methods and Experiments. 1) Recombinant DNA cloning to construct the SKS mutations in mammalian expression vectors. 2) Transfection or infection to introduce the mutants to mammalian cells. 3) Cell treatment: glucose, amino acid, or serum starvation; complete medium for activation; rapamycin. 4) Cell lysis and Western blot analysis to determine the mTOR activities. 5) Use the CRISPR knock-in method to generate the mutations in the endogenous MTOR gene loci. 5) The effects on cell growth/size and clock rhythms will be assayed by flow cytometry and real-time bioluminescence (Lumicycle) recording, respectively. 6) Generation of mouse models of select SKS mutations.

Summary of Recent Progresses.

- Spring and summer 2020: generated 46 mutations which include known SKS mutations and new ones identified by our SKS diagnosis team. Will include new alleles.
- Summer 2020: tested over 12 alleles under conditions deprived of glucose, amino acids and serum, namely total starvation. There was concern about cell health.
- August-October 2020: established the conditions to more accurately determine the SKS alleles under glucose, amino acid, or serum starvation, as well as rapamycin inhibition.
- August-October 2020: focused on 6 SKS alleles for their activity under glucose or amino acid starvation. Each variant responds differently to different condition.
- Fall 2020: In collaboration with Dr. Hogenesch at CCHMC, we obtained two mouse models (R1480-C1483 deletion and V2406M). We are breeding the founders. If breeding is successful, we will expand the mice for behavioral, neurological and molecular studies. The cell and mouse model studies will form synergy.

CONCLUSIONS:

The SKS mutants display different basal activity under glucose/serum starvation conditions. Among the six mutations tested, C1483F, Del (R1480-C1483), S2215Y and F1888C are more active (hyperactive) than wild type mTOR, whereas G2464V is slightly hyperactive and V2406M is not significantly hyperactive. We will use the established conditions to compare and contrast the different SKS alleles relative to wild type MTOR and to each other. In the next 2-3 months, we will assess these six mutants in all the starvation conditions and expand the alleles to 12 or more. These data will be combined with clinical data in a new manuscript for publication. In the next 1-2 years, we intend to expand this analysis to all the mutations.

KEY LEARNINGS AND SURPRISES:

Over just the past six months, we learned that these SKS mutations cause hyperactivity, but their activities differ. This holds true under different basal conditions, for example, in media deprived of serum, glucose, or amino acids. Further, our preliminary data suggested a feed forward mechanism for some alleles to regulate their own activity, which will be validated and further characterized.