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Non-Canonical Functions of TSC2 Protein in Mitotic Division

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Introduction: Tuberous Sclerosis Complex (TSC) is a debilitating developmental disorder characterized by a variety of clinical manifestations. While benign tumors in the heart, eyes, lungs, kidney, skin, and brain are a hallmark of the disease, often the most severe symptoms of TSC are neurological, including seizures, autism, psychiatric disorders, and intellectual disabilities. TSC is caused by a loss of function mutation in the TSC1 or TSC2 genes, which encode the hamartin/tuberin proteins respectively. These proteins function as a heterodimer that negatively regulates mechanistic Target of Rapamycin Complex 1 (mTORC1). The majority of work on TSC has been focused on the effects of mTORC1, a critical signaling hub, on regulation of diverse cell processes including metabolism, cell growth, translation, and neurogenesis. However, work focused on potential non-canonical functions of TSC2 is rare and the potential cell biological mechanisms involved are not well understood. Understanding whether mTORC1-independent functions of TSC2 contribute to patient phenotypes will be essential to tailoring treatment, as many patients are currently treated with mTOR-targeting agents.

Methods: To examine neurodevelopmental phenotypes in a cell-based model of TSC, we utilized TSC patient-derived induced pluripotent stem cells (iPSCs) that harbor a disease-causing heterozygous microdeletion mutation in the TSC2 gene. Patients are heterozygous for mutations, but the most widely accepted model is that second hit mutations in TSC1/2 occur in tumorigenesis. To model this state, CRISPR was used to create a similar deletion mutation in the other TSC2 allele, producing a homozygous mutant line. TALENs was also used to correct the heterozygous mutant to wild type, creating a set of isogenic lines.

Results: We observed aberrant multipolar mitotic division, a novel phenotype, in the TSC2 mutant iPSCs. Multipolar mitotic division occurs when there are more than the typical two spindle poles during mitosis. The multipolar phenotype is not significantly affected by treatment with rapamycin, an mTORC1 inhibitor, in the TSC2 mutant iPSCs, indicating that multipolar division is an mTORC1-independent phenotype. Interestingly, the multipolar phenotype in the TSC2 mutant cells does not continue through differentiation into mixed glutamatergic and GABAergic cortical neurons.

Discussion: Further analyses are in progress, but our findings show a non-canonical function for TSC2 protein that can provide crucial insight into TSC2 effects on normal development, ultimately identifying additional potential avenues for therapeutic intervention in patients with TSC.

Keywords:
Tuberous Sclerosis, Multipolar division, Development