Tuberous Sclerosis Renal Disease: From Cell Biology Quirks to Possible Cures

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Introduction: Tuberous Sclerosis Complex (TSC) is an autosomal dominant genetic disorder due to a mutation in *TSC1* (hamartin) or *TSC2* (tuberin). Renal angiomyolipomas occur in approximately 80% of patients. TSC proteins regulate mammalian target of rapamycin complex 1 (mTORC1) mediated protein translation, cell growth, autophagy and survival. Loss of TSC protein in angiomyolipoma cells produces elevated mTORC1 activity and protein translation, leading to endoplasmic reticulum (ER) stress and activation of the unfolded protein response. Angiomyolipoma cells may be closer to their threshold capacity to compensate for additional proteomic stress. TSC2-deficient human renal angiomyolipoma cells (TRI102) display increased sensitivity to ER stress caused by proteasome inhibition compared to TSC2-rescued cells (TRI103). Increased temperature may cause additional ER stress.

Aims/Hypothesis: We hypothesized that TRI102 (TSC2-deficient) cells will have increased vulnerability to thermal stress compared with TRI103 (TSC2-rescued), and this effect will be augmented by pharmacological agents targeting mTORC1 and the 26S proteasome.

Methods: Mutant TRI102 and genetically rescued TRI103 cells were incubated at 37^{o}C compared with 45 or 50^{o}C for 1, 6, and 24 hours with and without mTOR inhibitor, RAD001, and proteasome inhibitor, MLN2238. Cell viability was determined using crystal violet DNA dye binding or propidium iodide dye exclusion assays. Western blotting was used to assess changes in levels of phosphorylated SAPK/JNK, heat shock proteins and proteasome function.

Results: Initial cell viability studies suggest loss of TSC protein function may increase cell sensitivity to thermal stress. Additionally, combination of thermal stress and proteasome inhibition (based on western blot analysis of ubiquitin) appears to enhance stress-induced cytotoxicity based on viability studies and western blot analysis of the stress-sensitive SAPK/JNK.

Conclusion: These pilot experiments offer mechanistic insight into possible targeted therapy for patients with TSC-associated renal angiomyolipomata. These studies support angiomyolipoma ablation using magnetic resonance imaging guided high intensity focused ultrasound to induce thermal stress. Our long term aim is to change the ablative process from thermally induced necrosis to apoptosis. The clinical benefit of this approach will be absence of renal scarring and post-procedural sequelae like fever and pain.

Acknowledgements: This study was supported by NIH grant T35 DK 60444.