Mechanical Load and Hormonal Effects on Angiomyolipoma Cells

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Tuberous Sclerosis Complex (TSC) is a disease characterized by a genetic predisposition to tumor formation in many organ systems including the skin, kidneys, lungs, and central nervous system. This tumorogenesis is presumed to be due to the constitutive activation of mammalian target of rapamycin complex 1 (mTORC1) cellular signaling. TSC patients develop both renal angiomyolipomas and the lung cystic disease lymphangioleiomyomatosis (LAM). Despite a unifying genetic basis (*TSC1* or *TSC2* mutation) LAM has an almost complete female predominance, contrasting markedly with other TSC manifestations, which have no gender disparity. This suggests that additional factors drive the pathogenesis of TSC. We propose that a combination of mechanical strain and 17-β estradiol may be responsible for this unique disease distribution in the female lung.

We examined the influence of cyclic mechanical stretch, 17-β estradiol, and RAD001 (an mTORC1 inhibitor) on the growth of a *TSC2* deficient cell line as compared to a *TSC2* re-expressing cell line.

Immortalized *TSC2* deficient cells derived from a human renal angiomyolipoma were compared against a culture of similar cells in which *TSC2* expression was induced via retroviral transfection. Cells were grown in media containing 17-β estradiol, RAD001, and no treatment; both with and without cyclical mechanical stretch stimulation (20% elongation @ 1Hz, 48 hrs). Following treatment, cells were harvested and analyzed for protein expression by western blot and morphology with immunocytochemistry.

We observed that together, 17-β estradiol and mechanical stretch produced a consistent synergistic upregulation of cyclin B1, cdc2 p34, and focal adhesion kinase (FAK) in *TSC2* deficient cells that was not observed in *TSC2* re-expressing cells. RAD001 treatment had a neutral or negative effect on the expression of these proteins. Using Alexa 594-conjugated phalloidin to fluorescently label the actin cytoskeleton, a distinct actin phenotype was observed in cells exposed to both cyclic mechanical stretch and 17-β estradiol, in which filaments appeared relatively disorganized, and somewhat "frayed."

The observed changes in expression and cytoskeletal organization may be indicative of a uniquely proliferative and migratory phenotype induced by the dual stimuli of 17-β estradiol and cyclic mechanical stretch. While the described results are preliminary, additional studies are being carried out to clarify the significance of these results. In addition, microarray analysis of RNA samples collected during these experiments will be performed. Ultimately, these phenotypic changes may offer an explanation for the unique disease manifestation of LAM.

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