Mitochondrial Dynamics in Renal Cell with Burn Serum Stimulation - An Observational Study

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Background

Myoblasts and myocardiocytes have increased cell death with burn serum stimulation, which is associated with mitochondrial fission and function impairment. Acute kidney injury is a significant issue in burn patients; however, the role of the mitochondrial response in renal cells (RCs) has not been explored. The purpose of this summer project was to establish a method that observes mitochondrial dynamics in renal epithelial cells upon exposure to burn serum. We hypothesized that the mitochondrial fission/fusion cycle in human RCs would be disrupted following exposure to burn serum.

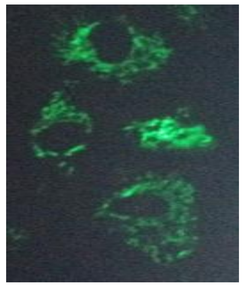
Methods

Human primary renal proximal tubule epithelial cells were cultured with Renal Epithelial Cell Growth Media in an incubator at 37°C with 5% CO_2. Upon reaching 65-70% confluency, RCs were treated with growth media containing 10% rat serum (RS) either from control rats or 40% total body surface area scald burn rats. 3nM of MitoTracker Green FM dye was added to all cell treatments to stain the mitochondria (MT). Live cell images were taken under a Nikon Ti Eclipse Confocal microscope at 6, 24, 48, 72, and 96 hour time points; subsequently, images were analyzed with Nikon software to identify fluorescent intensity, mitochondrial elongation and volume. T-tests were then applied to analyze the significance with Bonferroni correction. Significance after correction was determined if $p < 0.01$.

Results

Figure I: Human Renal Cells Exhibit Different Morphology in Normal Rat Serum (A) and Burn Rat Serum (B) at 24 Hours

A



B

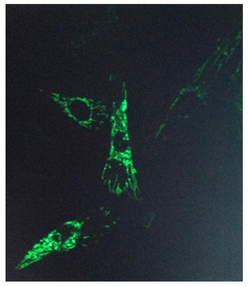


Table I: Fluorescent Intensity of Mitochondria in Renal Cells Exposed to Normal Rat Serum and Burn Rat Serum

Time	Intensity		P-value
	Normal Mean±SD	Burn Mean±SD	
6	814.9 ± 160.2	752.1 ± 121.9	0.1606
24	707.8 ± 154.2	723.1 ± 94.7	0.6737
48	674.1 ± 75.8	869.1 ± 374.5	0.0316
72	949.3 ± 434.6	639.7 ± 98.9	0.0097
96	1080.5 ± 418.0	772.5 ± 332.3	0.0007

Table II: Mitochondrial Elongation in Renal Cells Exposed to Normal Rat Serum and Burn Rat Serum

Time	Elongation		P-value
	Normal Mean±SD	Burn Mean±SD	
6	2.1 ± 0.7	1.9 ± 0.5	0.2139
24	1.9 ± 0.4	2.1 ± 0.6	0.2683
48	1.8 ± 0.5	1.9 ± 0.6	0.8123
72	1.5 ± 0.3	1.7 ± 0.4	0.2111
96	1.5 ± 0.4	1.8 ± 0.6	0.0359

Discussion

RC morphology was cuboidal and refractile in the culture media. During serum stimulation experiments, the MT of RCs gradually increased in fluorescent intensity and maintained a rod-like shape in both groups. Specifically, the MT exposed to normal RS had significantly higher intensity at the 72 and 96 hour marks than those in burn RS. Despite these results, cell viability was determined to be 48 hours, since 60% or more of cells had detached at the 72 and 96 hour marks, indicating cell death. There were no significant differences in elongation and volume (*data not shown*) between burn and normal serum stimulation at each time point.

Conclusion

The cell culture protocol for human primary renal epithelial cells was established this summer. Although rats and humans share evolutionarily conserved traits, the RS did not significantly influence the more evolved human RC. Future experiments will be performed with human serum.

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