Changes in Endothelial Cell Autophagy Following Hypoxic Cold Storage and Reperfusion: A Potential Therapeutic Target for Pre-Treatment in the Donor Organ

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Background

• The donor organ experiences ischemia-reperfusion injury (IRI) upon transplantation, first encountered by microvascular endothelial cells
• Additive effects of hypoxic cold storage (HCS) followed by reperfusion are known to cause endothelial injury and pre-dispose the donor organ to higher immunogenicity
• Autophagy, the process of cellular machinery disposal and recycling, is altered during physiological stressors, and has been implicated in the mechanism of IRI in transplantation

Research Objectives

• Understand how HCS alone and reperfusion injury affects endothelial cell autophagy using an in vitro model with mouse cardiac endothelial cells (MCECs)
• Hypothesis: Endothelial cell autophagy is upregulated during IRI, which could be protective, as it is a quality control mechanism

Methods

Assays and Quantification

• Immediately following HCS or normothermic (NT) conditions and at two-hours post-reperfusion, cell lysates were collected for immunoblotting of microtubule-associated protein 1 light chain 3 (LC3B)
• Confocal imaging performed using Cyto-ID Autophagy Kit (Enzo Life Sciences) for autophagosome visualization

Results

• After six hours of HCS or NT conditions, LC3B-II/LC3B-I demonstrated no change between the experimental groups, indicating similar levels of autophagosome formation
• A significant increase in LC3B-II/LC3B-I between NT and two hours post-reperfusion was observed (P<0.01), demonstrating increased autophagosome formation
• Confocal microscopy confirmed no change in autophagosome formation immediately following cold storage with an observed increase at two hours post reperfusion

Conclusions

• MCECs have increased autophagosome formation after two hours of reperfusion injury
• There is likely an association between HCS, IRI, and autophagy
• Modulating autophagy through pre-treatment could be a viable strategy to protect the endothelium of the donor organ

Future Directions

• Impact of endothelial autophagic flux on cellular function and immunogenicity during HCS and IRI is unknown
• Genetic modification and pharmacologic manipulation for induction and suppression of autophagy during HCS and IRI

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References