Department of Surgery, Division of Organ Transplant and Comprehensive Transplant Center

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

Meredith E. Taylor[1,2], Dinesh Jaishankar[1,2], Ashley P. Strouse[2,5], Yu Min Lee[1,2], Satish N. Nadig[1,2,3,4]

[1]Department of Surgery, Feinberg School of Medicine, [2]Division of Organ Transplant and Comprehensive Transplant Center, [3]Department of Microbiology, Immunology, and Pediatrics , [4]Simpson Querrey Institute, [5]Northwestern University, Weinberg College of Arts and Sciences

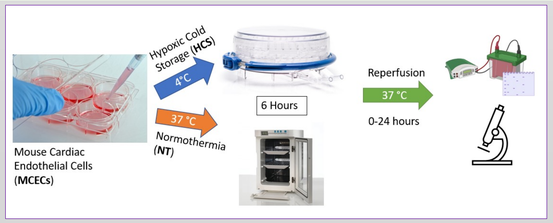
Background

- 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[1]
- **Autophagy**, the process of cellular machinery disposal and recycling[2], is altered during physiological stressors, and has been implicated in the mechanism of IRI in transplantation[3]

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

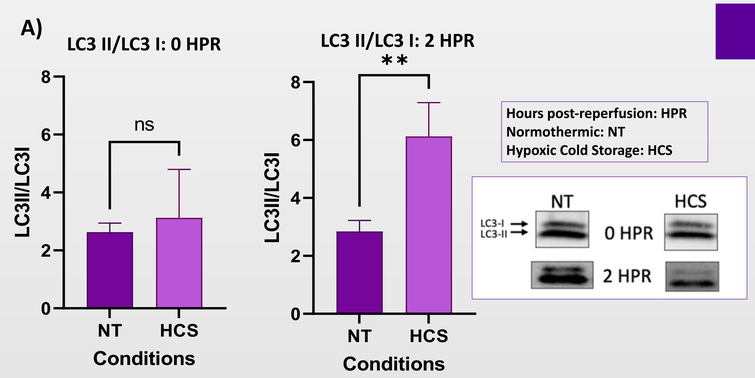


Figure 1: Endothelial cell autophagy levels are increased two hours post-reperfusion following HCS and are minimally changed immediately following HCS. (A) LC3 II/ LC3I quantified from immunoblotting of whole cell lysates demonstrated a significant increase between NT and HCS exposed MCECs two hours post-reperfusion and minimal change between the two conditions immediately following HCS (p=0.0098 and ns). (B) Representative confocal images showing increased autophagosome formation in MCECs following HCS and two hours of reperfusion with warm media in comparison to those only exposed to HCS (green: autophagosomes, blue: nuclei).

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

Acknowledgments

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References

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Methods schematic created using BioRender

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