You Are What You Eat: Harnessing Endothelial Cell Autophagy as a Potential Donor Organ Pre-treatment Strategy

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INTRODUCTION

- 4,545 deceased donor cardiac transplants were performed in the United States in 2023[1].
- The donor hearts are inevitably subjected to ischemia-reperfusion injury (IRI) that triggers a sequelae of adverse effects.
- IRI is first encountered by **microvascular endothelial cells**[2,3] (ECs), causing endothelial injury and predisposing **the donor heart to higher immunogenicity[2].**
- **Endothelial autophagy**, or "self-eating," the process of disposing of and recycling cellular machinery, has been implicated as a response to cardiac IRI[3].

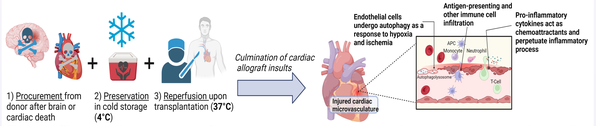

Figure 1:The cumulative impact of procurement, preservation, and transplantation on the cardiac microvascular endothelial cell (EC) barrier of the allograft. Warm ischemia during donor death and organ recovery, followed by cold ischemia during preservation and transport, and finally, reperfusion upon implantation results in a summative microvascular EC injury. EC injury then triggers a cascade of innate and adaptive immunological signaling, pre-disposing the donor hearts to higher immunogenicity.

RESEARCH OBJECTIVES

1)Describe changes in cardiac microvascular EC autophagy during CS and IRI.

2)Understand whether the impact of EC autophagy is protective or detrimental as related to IRI and cellular immunogenicity.

METHODS

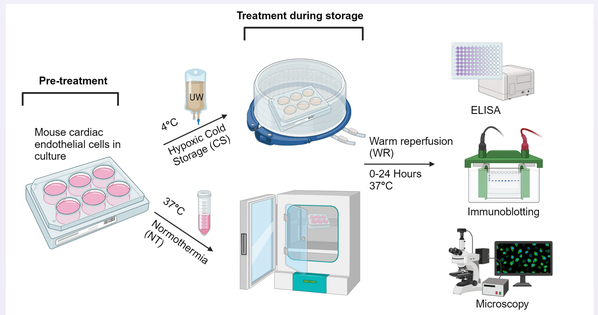

Figure 2: Modeling ischemia reperfusion injury (IRI) in vitro using mouse cardiac endothelial cells (MCECs). To mimic conditions of the donor heart during cold preservation and warm reperfusion, we subjected a monolayer of MCECs to either 6 hours in a hypoxic chamber at 4°C with University of Wisconsin (UW) solution or incubation in media at 37°C. Then, warm reperfusion was simulated by exchanging the UW solution for warm medium. ECs were processed for ELISA, immunoblotting, or imaging at times 0-24 hours post-reperfusion. For pharmacological modification of autophagy, ECs were either pre-treated in culture before storage and reperfusion or were treated during the storage period.

RESULTS

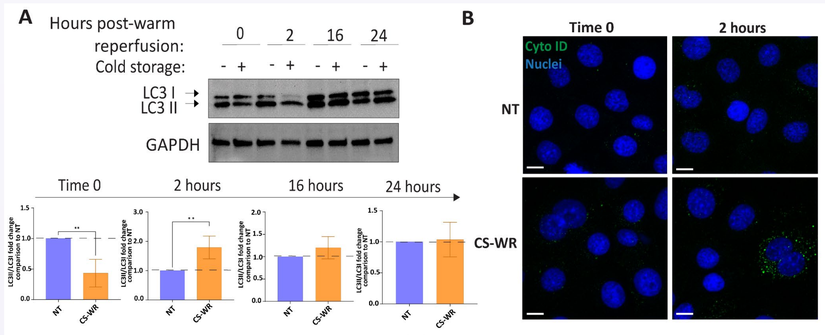

Figure 3: Early reperfusion injury in mouse cardiac endothelial cells (MCECs) enhances autophagy. (A) Immunoblots from MCEC lysates were probed for the autophagic marker LC3B. Quantification of the LC3B II/ LC3B I ratio demonstrated no change between normothermic (NT) and cold storage (CS) at 0-, 16-, and 24- hours post warm reperfusion (p=0.65, p=0.22, p=0.91). A statistically significant increase between NT and 2 hours post-reperfusion was observed (p=0.01). **(B)** Representative confocal images showing similar autophagosome formation in MCECs immediately following reperfusion and increased autophagosome formation in MCECs following CS and 2 hours post-reperfusion in comparison to NT conditions (green: autophagosomes, blue: nuclei, scale bar: 10 µm).

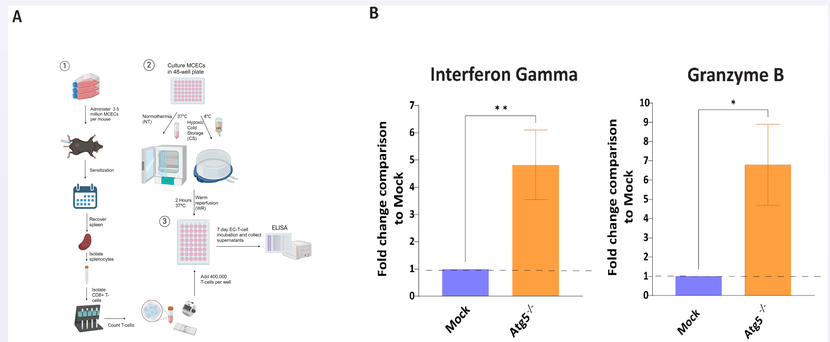

Figure 5: **Modeling CD8+ T-cell response following ischemia-reperfusion injury in vitro using mouse cardiac endothelial cells (MCECs) demonstrated increased T-cell activation in Atg5 knockout cells. (A)** (1). Wildtype (WT) MCECs were injected into WT BL/6 mice for sensitization, and spleens were procured for CD8+ T-cell isolation following a 3-week period (2). WT, Atg5$^{-/-}$, or mock-transfected control MCECs were cultured in 48-well plates and subjected to injury or control conditions (3). CD8+ T-cells were added to plates in co-culture and incubated at basal conditions for 7 days, after which cell supernatants were collected for interferon-gamma and granzyme B ELISA analysis. **(B)** Interferon-gamma and granzyme B levels were significantly increased in supernatants of Atg5$^{-/-}$ MCECs following EC-T-cell co-culture with Mock control or Atg5$^{-/-}$ MCECs following cold storage and warm reperfusion (p=0.018, p=0.015).

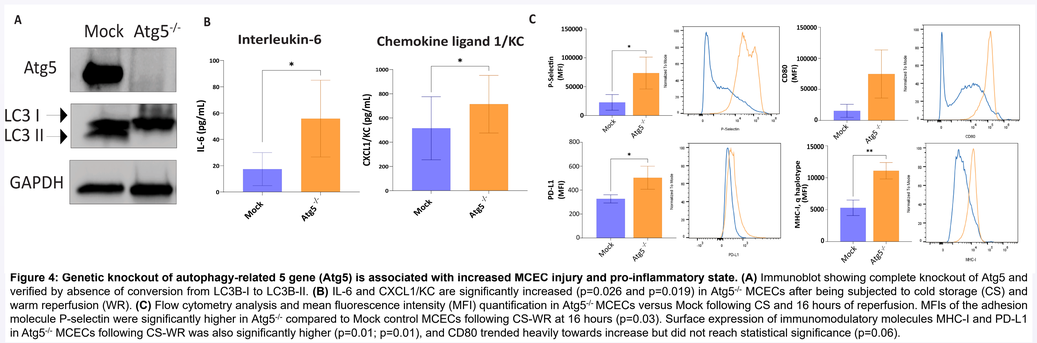

Figure 4: Genetic knockout of autophagy-related 5 gene (Atg5) is associated with increased MCEC injury and pro-inflammatory state. (A) Immunoblot showing complete knockout of Atg5 and verified by absence of conversion from LC3B-I to LC3B-II. **(B)** IL-6 and CXCL1/KC are significantly increased (p=0.026 and p=0.019) in Atg5$^{-/-}$ MCECs after being subjected to cold storage (CS) and warm reperfusion (WR). **(C)** Flow cytometry analysis and mean fluorescence intensity (MFI) quantification in Atg5$^{-/-}$ MCECs versus Mock following CS and 16 hours of reperfusion. MFIs of the adhesion molecule P-selectin were significantly higher in Atg5$^{-/-}$ compared to Mock control MCECs following CS-WR at 16 hours (p=0.03). Surface expression of immunomodulatory molecules MHC-I and PD-L1 in Atg5$^{-/-}$ MCECs following CS-WR was also significantly higher (p=0.01; p=0.01), and CD80 trended heavily towards increase but did not reach statistical significance (p=0.06).

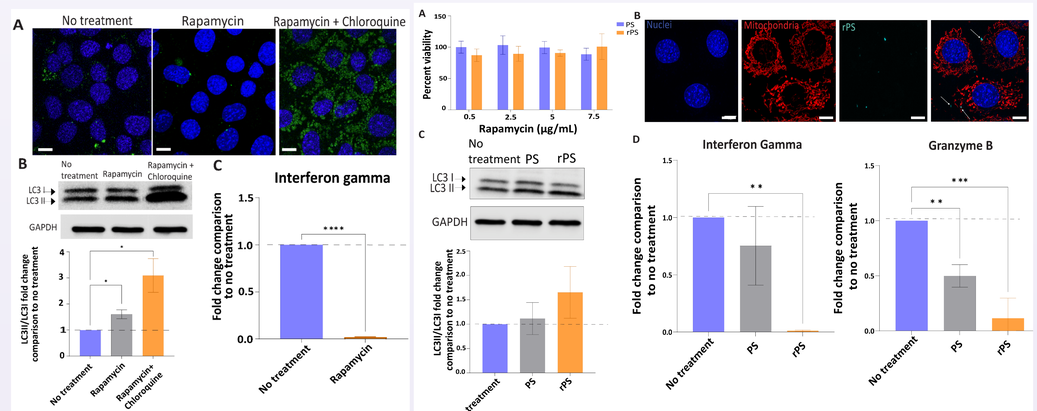

Figure 6: Pharmacologic autophagy induction in ECs prior to cold storage decreased EC immunogenicity. (A) Confocal imaging of autophagosomes (green) and nuclei (blue) comparing untreated MCECs to rapamycin or rapamycin plus chloroquine pre-treatment. **(B)** LC3B immunoblotting showed LC3 II/ LC3 I was significantly increased in rapamycin or rapamycin-chloroquine combination-treated MCECs (p= 0.036, p=0.016). **(C)** There was a significant decrease in interferon gamma between untreated and rapamycin pre-treated MCECs following co-culture of MCECs and CD8^{+} T-cells (p<0.001).

Figure 7: Rapamycin loaded polymersomes (rPS) are taken up by mouse cardiac endothelial cells (MCECs) during cold storage, bolster autophagy, and decrease EC immunogenicity (A) Uptake of rPS in MCECs. Mitochondrial staining (red) was used to provide intracellular context, with nuclei (blue) and rPS (aqua blue with white arrows). **(B)** MCEC viability was found to be similar amongst titrations of rPS and PS. **(C)** Immunoblotting of rPS-treated MCEC lysates under cold storage-warm reperfusion showed increased autophagy via measurement of LC3 II/ LC3 I. **(D)** Interferon-gamma and granzyme B levels were significantly decreased in the rPS-treated cells following EC-T-cell co-culture (p=0.002p=0.001). Interferon-gamma and granzyme B decreased when comparing untreated cells to those treated with PS (p=0.074, p=0.005).

CONCLUSIONS

- MCEC **autophagy increases during early post-reperfusion** time points, likely as a natural adaptation to stress.
- EC autophagy may be protective during IRI by **mitigating EC immunogenicity,** as deletion was deleterious to cell health.
- Bolstering microvascular EC autophagy in donor hearts during CS prior to transplantation may mitigate insults incurred during IRI.
- Delivery of autophagy-inducing **rapamycin via nanoparticles** to cardiac ECs has the potential to increase the longevity of cardiac allografts and reduce the burden of systemic immunosuppression.

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