Potential Treatments for Danon Disease

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Background

Danon disease is a congenital X-linked hypertrophic cardiomyopathy that results from mutations in LAMP2, a transmembrane lysosomal protein necessary for autophagy. The disease presents in childhood or adolescence, and life expectancy is 19 for men and 34 for women. Although Danon disease is rare, no specific therapy based on its biology is currently known. Recent in vitro studies using mouse by the Cherqui Lab have shown evidence supporting hematopoetic stem cell transplant as a novel treatment option for cystinosis [4] [6] [7]. Cystinosis is a metabolic disease that arises from mutations in a transmembrane lysosomal protein - cystinosin (CTNS gene). Detailed in vitro and in vivo studies have shown that rescue of the phenotype occurs because wildtype macrophages derived from the transplanted wildtype hematopoietic stem cells can transfer lysosomes containing wildtype cystinosin directly to Ctns-deficient cells in multiple organs of the host Ctns -/- mice. Because Danon disease also arises from mutations in a transmembrane lysosomal protein, LAMP2, a similar approach could be effective in rescuing the phenotype of LAMP2 mutant mice. The Adler Lab has recently developed an in vitro cell model for Danon disease using skin fibroblasts expanded from skin biopsies from two identified patients diagnosed with Danon disease, confirmed by RT-PCR [3]. These in vitro and in vivo models can be used show efficacy of hematopoietic stem cell transplant for Danon disease.

Materials & methods

In vitro studies

Lamp2 KO skin fibroblasts were used a an in vitro model for Danon disease. The loss and rescue of autophagy was measured by the use of a CAG-RFP-GFP-LC3B reporter (**Figure 1**).

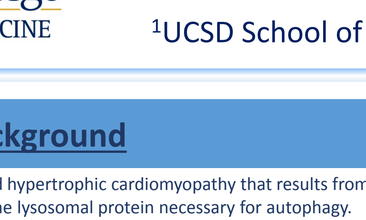

Figure 1: CAG-RFP-GFP-LC3B autophagy reporter system

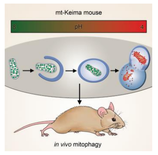

Figure 2: mt-Kiema mitophagy reporter system

Additionally, the mt-Kiema system was used as a reporter to measure mitophagy in vitro. Mt-Kiema is a mitochondrially expressed coral-derived molecule which fluoresces red in acidic pH and green at neutral pH [7] (**Figure 2**). Cardiomyocytes and skin fibroblasts were isolated from Mt-kiema Lamp2 KO and WT mice. Based on the fluorescence signal readout, the level of mitophagy could be quantified. Mt-kiema Lamp2 KO skin fibroblasts were also co-cultured with WT macrophages to study rescue of mitophagy.

In vivo studies

There were 4 experimental groups of mice: 1) WT 2) Lamp2 KO 3) Lamp2 KO transplanted with WT HSCs 4) Lamp2 KO transplanted with Lamp2 KO HSCs. HSCT was done at 8.5 weeks of age from 6 week old donors; the donor HSCs have a GFP reporter to observe tissue delivery post transplant. They were followed up 6 months post-transplant with physiological, hemodynamic, and echocardiographic studies.

Histological/biochemical studies

Hearts were harvested from Lamp2 KO mice 6 months post-WT transplant and cardiomyocytes were studied via confocal microscopy for the presence of macrophages in their tissue as well as Lamp2 transfer.

Results

In vitro studies

The CAG-RFP-GFP-LC3B reporter system was used to show a lack of autophagosome-autolysosome fusion in Lamp2 KO fibroblasts (**Figure 3A-D**). Upon co-culture with WT macrophages autophagosome-autolysosome appears to be rescued (**Figure 3K**) as well as expression of Lamp2 in these autophagy complexes.

The Mt-keima system shows that there is less mitophagy (indicated by red fluorescence) in the Lamp2 KO cardiomyocytes compared to the WT (**Figure 4**). When Mt-keima Lamp2 KO skin fibroblasts were co-cultured with WT macrophages, the level of mitophagy couldn't be imaged perhaps because of varying Mt-kiema expression in different cell types.

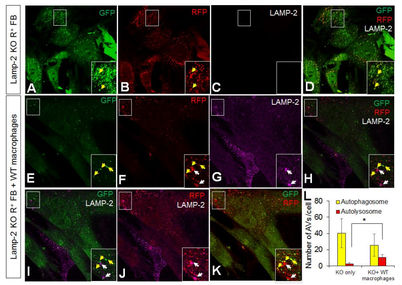

Figure 3: Measuring autophagosome-autolysosome fusion using the CAG-RFP-GFP-LC3B reporter system in A-D) Lamp2 KO fibroblasts; (E-K) Lamp2 KO fibroblasts co-cultured with WT macrophages. Yellow arrows indicate autophagosomes co-fluorescing green + red; white arrows indicate autolysosomes only fluorescing red.

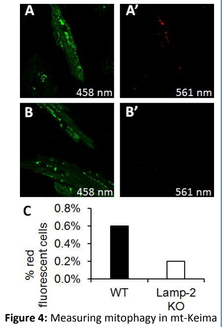

Figure 4: Measuring mitophagy in mt-Keima cardiomyocytes A) WT, B) Lamp2 KO

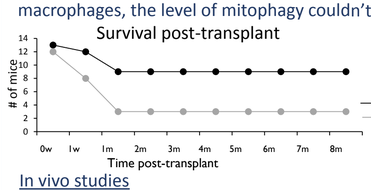

Figure 5: Survival of mice post-transplant
Lamp2 KO transplanted w/ WT
Lamp2 KO transplanted w/KO

In vivo studies

Survival post-transplant is shown in **Figure 5.** Physiologic studies included measuring weight and muscle grip. Although there was no statistically significant recovery of weight in Lamp2 KO mice with WT transplant, there was statistically significant recovery of muscle grip in these mice (**Figure 6A-B**).

Echocardiographic studies showed an 18% increase in % fractional shortening in Lamp2 KO mice post WT transplant vs. Lamp2 KO mice as well as a 18% decrease of left ventricular mass in Lamp2 KO mice post WT transplant vs. Lamp2 KO mice as shown in (**Figure 6C-D).**

Histological/biochemical studies

Confocal microscopy on heart tissue of mice Lamp2 KO mice 6 months post-WT transplant showed evidence of Lamp-2 expression as well as presence of WT macrophages (GFP reporter) (**Figure 7**).

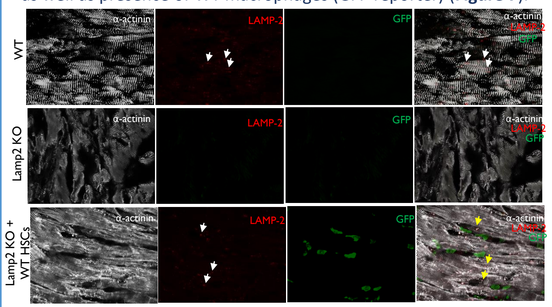

Figure 7: Confocal microscopy of heart tissue harvested from WT, Lamp2 KO, Lamp2 KO post-WT transplant 8 month old mice (6 months post-transplant)

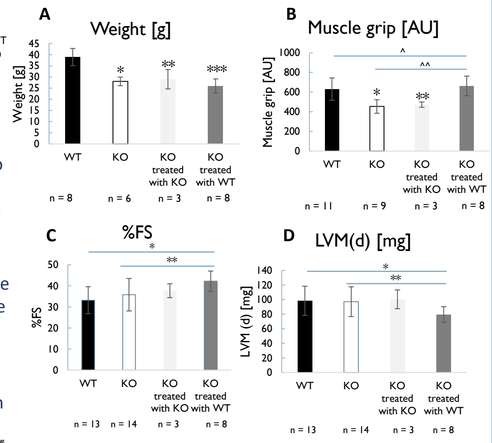

Figure 6: (A-B) Weight and muscle grip measurements in mice 6 months post-transplant (C-D) Echocardiographic data measuring % fractional shortening and left-ventricular mass in mice 6 months post-transplant

Terminal study

Finally, a preliminary comparison of heart mass amongst mice showed a 11% increase in LAMP2 KO mice compared to WT and a 17% decrease in Lamp2 KO mice post-WT transplant compared to Lamp2 KO mice (**Figure 8**).

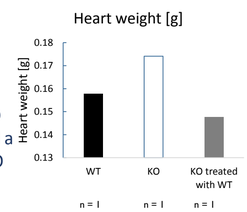

Figure 8: Weight measurements of hearts harvested from WT, Lamp2 KO, Lamp2 KO post-WT transplant 8 month old mice (6 months post-transplant)

Conclusions

In vitro studies using the CAG-RFP-GFP-LC3B and Mt-keima system show evidence of a defect in autophagy as well as mitophagy in Lamp2 KO fibroblasts and cardiomyocytes respectively compared to WT cells. Autophagy studies do show a rescue of autophagosome-autolysosome fusion upon co-culturing Lamp2 KO fibroblasts with WT macrophages. Rescue of mitophagy in cardiomyocytes remains to be shown. These results support the biology of autophagy rescue through WT macrophage transfer via hematopoietic stem cell transplant in potential Danon disease patients.

Further, physiological studies could show that muscle grip, not so much weight, can be an indicator of rescue of the Danon phenotype in in vivo Lamp2 KO mouse models post-WT transplant. Additionally, even though there appears to be some rescue of the hypertrophic cardiomyopathy in Danon mouse models based on echocardiography and a preliminary terminal study measuring heart mass, more precise analysis needs to be done with MRI and/or hemodynamic studies to confirm rescue of this cardiac phenotype.

Finally, confocal microscopy shows evidence of WT macrophages homing to cardiac tissue and expressing Lamp2 in Lamp2 KO Danon mouse models many months post WT transplant. Further studies of cardiac and muscle tissues studying autophagy rescue, protein expression of Lamp2 in cardiac and muscle tissue, and more need to be done to confirm the complete rescue of the Danon phenotype in mouse models post-transplant.

Overall, these studies provide evidence in support of hematopoietic stem cell transplant as a potential treatment option for Danon disease, but further experiments still need to be done to confirm rescue of all aspects of the Danon phenotype in vitro and in vivo.

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