THE ROLE OF MITOFUSIN 2 IN MEGAKARYOCYTES AND PLATELETS
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Abstract: Platelets are a crucial component of the blood that are derived from megakaryocytes in the bone marrow. They contribute to the hemostatic process through their cohesive and adhesive functions, and their ability to activate coagulation. Recent genetic studies in humans show a role for Mfn2 in platelet counts (Simon, et al.). To specifically test the role of Mfn2 in platelets, we used the Cre-Lox system to breed mice for platelet/megakaryocyte specific MFN2 knockout and genotyped samples via PCR and gel electrophoresis. Megakaryocytes were imaged to show a difference in KO vs. WT mitochondria morphology. Western blots showed a decrease in complex I levels in male samples, and preliminary experiments suggest clot retraction to be delayed in the absence of Mfn2. Further research is necessary to determine how these mitochondrial changes affect platelet functions such as hemostasis and thrombosis.

Background: Mitochondria are often referred to as the powerhouse of the cell. They produce energy in the form of ATP, which supports functions of the cell and is vital for its survival. Mitofusin 2 (MFN2) is one of several GTPase proteins that drive mitochondrial fusion (Ishihara et al.). Mitochondria complex I is defined as the largest multimeric enzyme complex (out of 5) of the mitochondrial respiratory chain. The respiratory chain is responsible for electron transport, generation of a proton gradient across the inner membrane, and ultimately the production of ATP to power the cell. Mfn2 deficiency can lead to reduced complex I activity (Mimaki, et al.).

Bone marrow derived megakaryocytes were stained with mitotracker dye; Immunofluorescent Microscopy of mitochondrial morphology in Mfn2 KO vs. WT megakaryocytes revealed elongated mitochondria (appear as strings) in WT megakaryocytes compared to more punctuated, individual (appear as spots) mitochondria in KO megakaryocytes. This confirms that Mfn2 deficiency affects the morphology of mitochondria in mouse megakaryocytes.

We asked the question: Does mfn2 affect mitochondrial function in megakaryocytes and platelets? We hypothesized that MFN2 KO in platelets would lead to a decrease in platelet mitochondrial Complex I expression and activity. Platelets from Mfn2 KO and WT mice (n=3 per group) were tested for complex I levels by western blot analysis of protein. Mfn2 KO was confirmed, and protein levels were quantitated by comparing MFN2 protein band intensity with those of cytoplasmic and mitochondrial housekeeping genes (β-tubulin, VDAC). We concluded that Mfn2 KO in platelets leads to a decrease in platelet Complex I expression in male samples.
Next we asked the question: Is clot retraction affected by absence of Mfn2? Clot retraction is an ATP dependent process that is crucial for our body’s ability to heal wounds (Tutwiler, et. all). We hypothesized that Clot retraction is delayed when Mfn2 is absent because it is an ATP dependent process. Thrombin and calcium were added to activate KO and WT Platelets (n=2 per group), and retraction was timed. The results showed Clot retraction rate was visually slower in KO compared to WT. Increased sample sizes are needed to confirm this quantitatively. Further studies are also necessary to confirm whether this relationship may stem from decreased mitochondrial ATP production in MFN2 deficient platelets.

**Conclusion:** The collected data demonstrates that Mfn2 effects mitochondrial morphology, reduces mitochondrial Complex I levels in male platelets, and potentially delays clot retraction. These experiments and results will help us understand the relationship between Mfn2 and platelet counts.

**References:**

