ERK1/2 do not phosphorylate proteins in mitochondria and myofilaments

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Introduction

Heart disease continues to be the most prevalent health issue in the United States. Previous studies have demonstrated the cardio-protective benefit of an increase in extracellular signal regulated kinase 1 and 2 (ERK1/2) activity in mice. In this study, genetically modified mice with knockouts of both the DUSP6 and DUSP8 genes (DKO) were used to study whether increased ERK1/2 phosphorylation in DKO mice changes the expression of these death-related proteins. We found out the increase in ERK1/2 phosphorylation mainly stayed in cytoplasm, which no obvious changes in mitochondria. Consequently, the expression levels of BCl-2 and Bax were not altered. We also investigated whether myofilament proteins could be the phosphorylation targets. Our data indicated that there was no significant difference of myofilament protein phosphorylation between wild type and DUSP6/8 DKO mouse hearts. In conclusion, our preliminary data suggest that the protective role of ERK1/2 in the heart is possibly through their unidentified cytosolic function without influencing the phosphorylation of either mitochondrial or myofilament proteins.

Materials and Methods

- Mice of 2 months of age were euthanized via cervical dislocation using isoflurane.
- The hearts were then homogenized into very small pieces and analyzed for their absorbance using a spectrophotometer at 595 nm.
- Standard Western Blot procedure was performed using PVDF membrane (Figure 2)
- Both primary and secondary antibodies were added to prevent unspecific binding of other proteins.
- Lastly the membranes were rinsed and analyzed using the licor-Oydssey machine in the Cook Health Science building downtown.

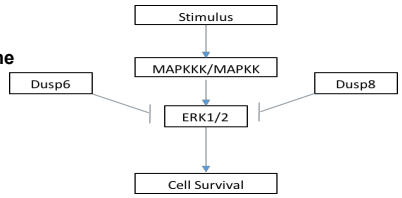

Figure 1. Visual representation of the basic relationship between Dusp6/8 and ERK1/2.

Results

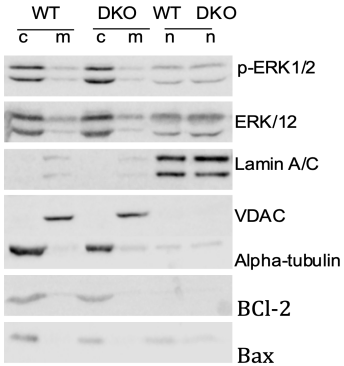

Fig. 2 Analysis of distribution of phospho-ERK1/2, ERK12/ in different cellular compartments.
C, cytoplasm,m, mitochondria; n, nucleus.
Alpha-tubulin, VADC, and Lamin A/C are markers for cytoplasm, mitochondria, and nucleus respectively. The levels of BCL-2 and Bax were not influenced by increased ERK1/2 phosphorylation in DKO mice which is consistent with the data showing the low levels of ERK1/2 in the Mitochondria.

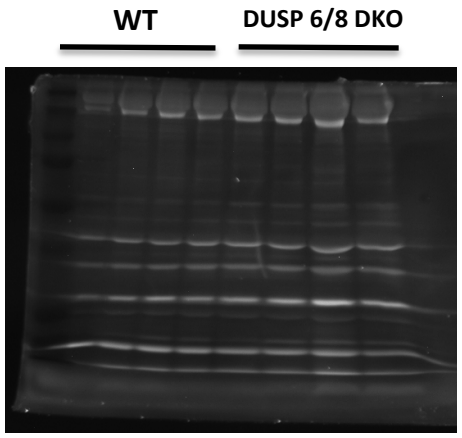

Fig. 3. Comparison of phosphorylation of myofilament proteins isolated from the hearts of wild type(WT) or DUSP6/8 KO Mice.
No obvious difference was observed between DKO and WT.

In the heart western blot analysis showed increased P-ERK1/2 and ERK1/2 in the cytoplasm, but not in the mitochondria. This was consistent for both WT and DKO mice.

Conclusion

- Our genetically modified mouse model demonstrates, that an increase in phosphorylation of ERK1/2 plays a protective role mainly through their unidentified cytosolic function without influencing mitochondria-mediated cell death pathway.
- An increase in ERK1/2 as well as P-ERK1/2 was shown in the cytoplasm.
- The alpha-tubulin marker is the cytoplasm marker and is consistent with the ERK1/2 levels in the cytoplasm so this tells us that our levels are relatively pure.
- ERK1/2 localized to the cytoplasm means that when the levels of Bax and BCL-2 increase upon cell death it is not due to the increased levels of ERK1/2 in the mitochondria, but for a reason that needs to be studied further in the future.

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