STRONG CHILDREN'S RESEARCH CENTER

Summer 2016 Research Scholar

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ABSTRACT

Title: *Effects of Altered Cyclophilin D expression, acetylation and activity on myocyte differentiation.*

Background:

Mitochondrial permeability transition (MPT) is involved in regulated cell death and mediated by the opening of the mitochondrial permeability transition pore (mPTP). Research studies have found that the opening of the mPTP, located in the inner mitochondrial membrane, is non-pathologic in the early heart and does not cause the release of apoptotic proteins. Instead, closure of the mPTP leads to maturation of mitochondrial structure and function and cardiomyocyte differentiation. Cyclophilin D (CypD) is a mitochondrial matrix peptidyl-prolyl isomerase known to regulate the opening of the mPTP. Increasing evidence suggest that acetylation of CyPD at lysine 166 increases its activity and the likelihood of mPTP opening.

Objective:

Determine the effect of altered CypD expression, on cardiomyocyte differentiation using modified RNA of CypD. We hypothesize that cardiomyocytes from CypD knockout hearts that are transfected with inactive forms of CypD will differentiate faster than those transfected with active forms of CypD.

Results:

We analyzed the pattern of Z and I bands of the developing contractile filaments in cultured cardiomyocytes, which were transfected with modified RNA of CypD. Our results show that using .4µl of RNAiMax loaded with 400 ng of modified RNA led to a high percentage of transfected cells. The Z- and I-bands and CypD were visualized by immunocytochemistry with specific antibodies. We found that active mutant forms of CypD (WT CypD and K166Q) had less than 50% Z and I bands near the plasma membrane or in the center, while those transfected with inactive forms of CypD (R96G, K166R) had more than 50% Z or I bands throughout the cell cytoplasm. (n= 2 experiments, per transfection 30 transfected and 30 untransfected cells analyzed).

Conclusion:

Cardiomyocytes transfected with inactive forms of CyPD were more differentiated than cardiomyocytes transfected with active forms of CyPD.