Effects of FASTKD1 Overexpression on Cardiac Mitochondrial Architecture

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The mitochondrion possesses its own small 16.6kb circular genome, complete with all the necessary machinery to need to replicate and transcribe the mtDNA, and then translate the resultant mtRNAs into protein [1]. However, how mitochondrial RNA (mtRNA) is regulated is poorly understood. Consequently, the purpose of our ongoing study is to better understand how this occurs, and, hence, modulates mitochondrial function and the cellular and organ response to pathological stress.

We are examining on a newly identified family of 5 mitochondrial proteins, the Fast Kinase Domain-containing proteins (FASTKDs), which specifically regulate mtRNA levels [2]. For example, deletion of FASTKD1 in cancer cells specifically decrease the mRNA for ND3, a Complex-I protein, whereas deletion of FASTKD3 has the opposite effect [2]. We are currently focusing on FASTKD1 as it is highly expressed in the myocardium, our main test system, and our in vitro and in vivo data show that FASTKD1 can alter mitochondrial function and stress responsiveness.

Manipulation of mitochondrial proteins, especially those that regulate mtDNA and mtRNA expression, can alter mitochondrial ultrastructure. Thus, we wanted to assess whether increased levels of FASTKD1 altered mitochondrial architecture in both cultured myocytes and intact hearts. We chose EM for these studies, as it possesses the fidelity to ascertain precisely what is happening to mitochondrial ultrastructure including cristae architecture and junctions and network formation.

For the cell culture studies, neonatal rat ventricular myocytes (NRVMs) were plated on sapphire coverslips and infected with either a β-galactosidase (βGal, control) or FASTKD1-encoding adenovirus. The NRVMs were then fixed, subjected to high-pressure freezing and processing, and imaged by TEM. Initially, the myocytes did not plate well on the sapphire discs with a large number of dead and dying cells. Doubling the density of NRVMs improved the viability and high quality TEM images were obtained. Mitochondria in the βGal-infected NRVMs exhibited the classical densely packed cristae arranged in close series (Fig. 1). However, in the FASTKD1-infected cells, there was often “delamination” between the cristae (Fig. 1). Such structural changes could certainly explain the modest but significant alterations in mitochondrial function that we have seen in FASTKD1-overexpressing cardiac myocytes. However, the unusual delamination was not expected and is very intriguing as it begs the question of how this occurs. There is no real information as to whether the cristae adhere to one occur in a densely packed cardiac mitochondrion. This is genuinely exciting and will provide significant impetus for future research directions.

For intact heart studies, we used transgenic (TG) mice that express ~3-fold levels in FASTKD1 protein in the heart compared to their non-transgenic (NTG) littermates. Anesthetized 3-month old male NTG and FASTKD1 TG mice had their hearts perfused with fixative. The hearts were then excised, the left ventricle isolated and minced, and further processed for TEM. Excellent TEM images were obtained of the ventricular mitochondria (Fig. 2). Compared to the cultured NRVMs there does not appear to be any obvious changes between the genotypes. This is most likely due to a number of factors. The in vivo model is modest chronic overexpression throughout the animal’s life. Thus, compensatory mechanisms can kick in. We do not see any changes in baseline function in
mitochondria from the TG mice, which would fit with no major alterations in mitochondrial architecture. That being said, aging or stressing the mice (e.g., heart attack, diabetes, etc.), may reveal a difference between NTG and TG animals. Indeed, we already know that the TG mice do better following myocardial infraction, which causes severe mitochondrial disruption.

In summary, acute overexpression of FASTKD1 in cultured myocytes induced a “delamination” phenotype, whereas chronic overexpression in myocytes in the intact heart did not exert a significant effect on mitochondrial architecture, at least at baseline. Future studies will be aimed at deciphering these differences and further examining the mechanism behind the changes in the cultured cells.

References:

Fig 1. Mitochondrial Architecture in FASTKD1-Overexpressing Cardiac Myocytes. Electron Micrographs of βGal- and FASTKD1-infected NRVMs. The 4000x images are higher magnifications of the regions inside the red box.

Fig 2. Mitochondrial Architecture in FASTKD1-Overexpressing Mouse Hearts. Electron Micrographs of left-ventricular sections from non-transgenic (NTG) and FASTKD1 transgenic (TG) mice.