RCAN1 suppresses mitochondrial fission, protecting the heart from calpain-mediated damage from I/R.

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Dysregulation of calcium handling and mitochondrial function are hallmarks of heart disease and can influence susceptibility of the heart to ischemia reperfusion (I/R) injury. Regulator of Calcineurin 1 (RCAN1) is an endogenous feedback inhibitor of calcineurin, a calcium-activated phosphatase involved in pathological cardiac remodeling and physiological hypertrophy. Mice lacking RCAN1 are more susceptible to I/R damage. We used an in vitro model of simulated I/R (sim-I/R) and advanced imaging techniques to dissect RCAN1’s role in mitochondrial dynamics and cell survival. SiRNA was used to deplete the RCAN1.4 and RCAN1.1 protein isoforms from neonatal rat ventricular myocytes (NRVMs). Volumereconstituted confocal images and Western blot, showed that knock down of RCAN1 resulted in an increase in the number of mitochondria per cell, a decrease in the mean mitochondrial volume, and a decrease in phosphorylation of the fission protein DRP1. SiRCAN1-depleted NRVMs showed a decreased ATP production, mitochondrial membrane potential, oxygen consumption, and the capacity for mitochondrial calcium uptake, consistent with an increase in mitochondrial fission. Gene expression likewise reflected a shift toward glycolytic metabolism. EM studies of hearts from Rcan1 KO mice or NRVMs depleted of RCAN1 showed increased mitochondrial fragmentation and a loss of cristae structure. Myocyte death following sim-I/R was significantly greater in siRCAN1-depleted cultures than in siRNAcontrols. Resistance to sim-I/R could be restored in siRCAN1-depleted NRVMs by treatment with either the mitochondrial fission inhibitor Mdivi, or the calcineurin inhibitor FK506. Importantly, we found a greater increase in both cytosolic calcium levels and calpain activity in the SiRCAN1-depleted NRVMs subjected to sim-I/R when compared to controls. We hypothesize that RCAN1 helps preserve mitochondrial fusion by inhibiting calcineurin-independent translocation of DRP1 to mitochondria and that myocytes deficient for RCAN1 are more susceptible to calpain-mediated I/R damage because of a decrease in the capacity to buffer calcium.

Keywords: RCAN1, mitochondrial fission, DRP1