Characterization of PGAM5

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Abstract

Programmed cell death (PCD) is a naturally occurring event that is highly regulated and required for normal development. The protein phosphoglycerate mutase 5 (PGAM5) has been implicated in apoptosis, necrosis, and autophagy of mitochondria, known as mitophagy. However, the mechanisms by which PGAM5 contributes to cell death are poorly understood. In this dissertation, we provide new insight into the function and regulation of PGAM5 and how PGAM5 may be contributing to cell death. Current models suggest that PGAM5 promotes cell death via necrosis and apoptosis. Our data, however, suggests that PGAM5 is necessary for development and protects cells from apoptosis. Our studies in mice demonstrate that disruption of the PGAM5 gene is embryonic lethal. We derived PGAM5-deficient mouse embryonic fibroblasts (MEFs) from a heterozygous mouse containing one disrupted PGAM5 allele. PGAM5-deficient MEFs were more prone to staurosporine-induced cell death, which was accompanied by a two-fold increase in caspase activity when compared to wild-type MEFs. Reconstitution of PGAM5 in PGAM5-deficient MEFs increased their resistance to cell death. These data provide evidence that PGAM5 is protecting the cells from apoptosis. Furthermore, we discovered a highly conserved WDxNWD motif across various species of PGAM5 protein, which we demonstrate is necessary for both the phosphatase activity of PGAM5 and normal mitochondrial morphology. Mutation of the WDxNWD motif abolished PGAM5's phosphatase activity, while overexpression in cells of PGAM5 with mutations in the WDxNWD motif caused fragmentation of mitochondria. We show that PGAM5 forms a multimeric complex, which is necessary for phosphatase activity. Multimerization of PGAM5 is disrupted when mutations are made within the WDxNWD motif. Taken together, we suggest that multimerization of PGAM5 is required for PGAM5's phosphatase activity and maintaining normal mitochondrial

morphology, while disruption of the PGAM5 complex inactivates PGAM5's phosphatase activity, leading to fragmentation of mitochondria, and ultimately cell death by apoptosis.