Turning the TCA Cycle Backwards To Repair Damaged DNA

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Genome instability, an enabling hallmark of cancer, drives carcinogenesis. Genomic stability depends upon the ability of the cell to exercise the DNA Damage Response and to repair its damaged DNA. Inhibitors of DNA repair, like histone H1 (Hho1 in yeast), must be removed before damaged DNA can be repaired. It was previously found that Hho1 can be removed via double-succinylation at K181 and K230. This is done by converting malate to succinate by fumarase (Fum1) and fumarate reductase (Frd1). However, the preceding process to obtain malate is unclear. Pyruvate carboxylase (Pyc1/2) and malate dehydrogenase (Mdh1/2/3) could be involved in generating malate. Our project aims to investigate if these two enzymes are involved in the same pathway as the tumour suppressor fumarase to aid damaged DNA repair. Protein-protein interaction indicates the involvement of proteins in the same pathway. Split-Ubiquitin Assays performed indicated interactions between Pyc1, Mdh1/2/3, Fum1, Frd1 and Hho1. Direct protein interaction was observed between Mdh2 and Fum1 through Co-Precipitation Assay. Chromatin Immunoprecipitation was done to show that Mdh2 is recruited to the site of damaged DNA. PYC1/2 gene knockouts were performed to investigate the essentiality of Pyc1/2 for DNA repair. It was shown that the double knockout strain is unable to repair damaged DNA. Western blot analysis was performed to demonstrate that the protein levels of Pyc1, but not of Pyc2, increase upon DNA damage. These findings show that Pyc and Mdh are involved in repairing damaged DNA, suggesting that metabolic enzymes can be reprogrammed to aid DNA repair.

Awards Won:
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