The Role of APE1 in DNA Damage Response to Oxidative Stress

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BACKGROUND: Cells are continuously being exposed to various insults that can damage the genome of an organism. The most frequently occurring DNA damage is reactive oxygen species (ROS)-induced oxidative stress which can lead to the prevention of DNA replication and transcription. However, a built-in response to the oxidative damage takes place called the Base Excision Repair (BER) pathway that includes crucial enzymes called AP Endonucleases. One of these enzymes, known as AP Endonuclease 1 (APE1), is a DNA damage repair enzyme that is known to make a nick in the phosphodiester backbone of DNA. The project is to determine the mechanism behind APE1 and understand its response to oxidative stress. So far it has been determined that APE1 is involved early in the BER pathway, but whether it plays a role in the DNA Damage Response (DDR) pathway remains a mystery unknown. The findings of this ongoing project will help to better understand neuropathological disorders and cancer, and lead to new opportunities for possible cures.

METHODS: Our major hypothesis in this project is that APE1 plays a previously uncharacterized, but essential role in the DDR pathway activation. To achieve this hypothesis, we utilize the cell-free extracts derived from eggs of Xenopus laevis (African Clawed Frog), allowing for the study of in vivo biochemistry without the need for cell cultures are utilized along with biochemical and molecular approaches including site-directed mutagenesis and immunoblotting analyses are used to test our hypothesis.

RESULTS AND CONCLUSIONS: Our results demonstrated that hydrogen peroxide-induced Chk1 phosphorylation was compromised in APE1-depleted egg extracts, indicating APE1 is important for the DDR activation. Moreover, protein-protein interaction assays demonstrated that APE1 associated with several critical DDR proteins. Further investigations are under way to understand the mechanism behind APE1 and elucidate its role in both the DDR and BER pathways. Understanding the molecular mechanism(s) of APE1’s distinct role in the DDR pathway will lead to new methods/approaches to use using APE1 as a chemo-preventive target in cancer therapy.