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RESEARCH ABSTRACT FORM

TITLE: Regulation of Apoptosis by Caspase-3**AUTHOR:** Beasley, L., Malavez, Y, Arasu, M. and Doseff, A.I.**MENTOR(S):** Andrea I. Doseff, Ph.D.**INSTITUTION:** Dorothy M. Davis Heart and Lung Research Institute, Ohio State University
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Apoptosis or programmed cell death is an evolutionary conserved mechanism determining during development the proper number of cells. It also acts as a defense mechanisms destroying infected or mutated cells. Impaired cell death leads to diseases such as rheumatoid arthritis and cancer. The overabundance of apoptosis has been documented in neurodegenerative diseases as Parkinson's and Alzheimer's diseases. Caspases are cysteine proteases responsible for triggering apoptosis. Caspase-3 is a central caspase as several apoptotic stimuli activate this enzyme to induce cell death. Thus, understanding the mechanisms that regulate caspases is fundamental to modulate cell fate at will. Previous studies have shown that caspase-3 is regulated by PKC ζ (protein kinase C) phosphorylation. PKC ζ has been identified as the only protein kinase which phosphorylates Caspase-3 and thus provides a novel approach to studying apoptosis. Here, we used site-directed mutagenesis to introduce mutations in the predicted amino acids that can be phosphorylated by PKC ζ . Aspartic or alanine substitutions in one or multiple sites were generated. The mutants were cloned using a pENTR-D/TOPO vector, purified, screened with restriction enzymes and investigated further using kinase assays. Positive clones were sent for sequencing and will later be transfected into mammalian cells as well as MCF-7 breast cancer cells and forced to undergo apoptosis. Using these methods, we are able to manipulate cell apoptosis using these caspase-3 mutations.