Investigation of RhoA-Dependent Regulation of Phospholipase C ε in Cardiovascular Disease

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Cardiovascular diseases are the leading cause of death in the United States. Phospholipase Cε (PLCε) is required for normal cardiovascular function, as it regulates the intracellular Ca^{2+} concentration and activates protein kinase C (PKC) signaling pathways. PLCε itself is activated downstream of G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs) through direct interactions with small G proteins, including Rap1A and RhoA. Regulation of PLCε by Rap1A at the perinuclear membrane has been well-characterized, as this pathway contributes to cardiac hypertrophy and heart failure. In contrast, RhoA-dependent activation of PLCε is cardioprotective against ischemia and reperfusion injuries. This pathway is initiated by activation of $G_{12/13}$-coupled receptors, particularly the sphingosine-1-phosphate (S1P) receptors, and leads to the exchange of GDP for GTP on RhoA, activating the small GTPase. RhoA•GTP binds and translocates PLCε to the plasma membrane, where it hydrolyzes membrane phosphatidylinositol-4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol triphosphate (IP_3). The increased DAG activates PKC, which ultimately inhibits mitochondrial apoptosis and prevents cardiomyocyte death. Despite the critical role of RhoA and PLCε in driving the cardioprotective response, little is known about how these proteins interact to increase lipase activity. Functional studies implicated an insertion within the catalytic TIM barrel domain, known as the Y-box, as a requirement for RhoA-dependent activation of PLCε. However, the Y-box does not bind the GTPase. Our goal is to identify the molecular mechanism by which RhoA binds to PLCε and increases its activity using structural and functional studies. The successful completion of these studies will map the interaction between these two critical signaling proteins, as well as identify elements in PLCε required for activation at the membrane. This knowledge can be ultimately exploited to develop lead therapeutic compounds that modulate this interaction to improve cardiovascular health.