DIVERGENT ROLES OF A NOVEL PHOSPHOLIPASE A_2 IN CELL DEATH

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Phospholipase A_2 (s) are esterases that hydrolyze the *sn-2* ester bond in phospholipids, releasing a fatty acid and a lysophospholipid. We previously showed that most PLA_2 activity in rabbit renal proximal tubule cells (RPTC) was Ca^{2+}-independent, localized to the endoplasmic reticulum (ER-$iPLA_2$), and inhibited by the specific Ca^{2+}-independent PLA_2 inhibitor bromoenol lactone (BEL). Our recent molecular biology experiments suggest that ER-$iPLA_2$ is similar to a novel human $iPLA_2$ called $iPLA_2\gamma$. Numerous investigators have implicated phospholipase A_2 in cell injury and death. The goal of these studies was to determine the role ER-$iPLA_2$ in cell necrosis and apoptosis. Using primary cultures of RPTC, we examined the role of ER-$iPLA_2$ in oxidant-induced necrosis by inhibiting ER-$iPLA_2$ with BEL. The model oxidants *t*-butyl-hydroperoxide, cumene-hydroperoxide, menadione, duraquinone and cisplatin (a common chemotherapy drug) produced RPTC lipid peroxidation and necrosis. Inhibition of ER-$iPLA_2$ prior to oxidant exposure, potentiated oxidant-induced lipid peroxidation and necrosis. In contrast, necrosis produced by the non-oxidant antimycin A was not potentiated by ER-$iPLA_2$ inhibition. These results suggest that ER-$iPLA_2$ plays a protective in cell injury produced by diverse oxidants.

In a second series of studies we determined the role of ER-$iPLA_2$ in apoptosis. Low concentrations of cisplatin produce time- and concentration-dependent RPTC apoptosis, causing p53 nuclear translocation, caspase 3 activation, annexin V labeling, chromatin condensation and DNA hypoploidy. Using primary cultures of RPTC, we examined the role of ER-$iPLA_2$ in cisplatin-induced apoptosis by inhibiting ER-$iPLA_2$ with BEL. Inhibition of ER-iPLA2 decreased cisplatin-induced caspase 3 activation, annexin V labeling, chromatin condensation and DNA hypoploidy but not p53 nuclear translocation. Caspase 8 and 9 activities did not increase following cisplatin exposure. The contribution arachidonic acid metabolism downstream of ER-$iPLA_2$ in cisplatin-induced apoptosis was investigated using cyclooxygenase (indomethacin), 5-lipoxygenase (MK886), and 12-lipoxygenase (baicalien) inhibitors. MK886, but not indomethacin or baicalein, decreased cisplatin-induced apoptosis. These results suggest that ER-$iPLA_2$ is a mediator of apoptosis and acts subsequent to p53 nuclear translocation and prior to downstream markers of apoptosis. In addition, an arachidonic acid metabolite of 5-lipoxygenase may be the active molecule.

In summary, ER-$iPLA_2$ appears to play a complex, critical and divergent role in cell injury and death. Under oxidative stress leading to necrosis, ER-$iPLA_2$ is cytoprotective and may act as a phospholipid repair enzyme that removes oxidized arachidonic acid from damaged ER phospholipids. In contrast, ER-$iPLA_2$ signals apoptosis downstream of p53 and prior to caspase 3 activation.