

**NFKB-DEPENDENT GENE TRANSCRIPTION IN CCK- AND TNF-STIMULATED ISOLATED ACINAR CELLS IS MEDIATED BY P38 MAP KINASE.**

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Mitogen activated protein (MAP) kinases are implicated in early stages of acute pancreatitis pathogenesis. Nuclear factor kappa-B (NFkB) is a transcription factor necessary for induction of many proinflammatory cytokines. We have shown that the MAP kinase p38 plays a role in NFkB-dependent gene transcription in an exocrine pancreatic cancer cell line (AR42J). Here we evaluate the role of p38 in regulating NFkB-dependent gene transcription in CCK- and TNF-stimulated acinar cells isolated from pancreata of healthy rodents (mice/rats). Following infection with an adenoviral luciferase expression vector containing a promoter driven only by NFkB, we infected isolated acinar cells with a replication-deficient adenovirus containing either an empty vector or a dominant negative (DN) p38 expression vector. Stimulation of native CCK-A or TNF- $\alpha$  receptors promoted a significant increase in NFkB-dependent gene expression, as measured by luciferase activity, in cells expressing the empty vector. In CCK- or TNF-stimulated cells, over expression of DN p38 significantly abrogated NFkB-dependent luciferase activity. These findings indicate that CCK- or TNF- $\alpha$ -stimulated NFkB-dependent transcription in isolated acinar cells is attenuated by p38 MAP kinase inhibition. In our studies, viability of isolated acinar cells was confirmed with an ATP assay. Preliminary studies confirmed that adenoviral infection of isolated acinar cells was successful: a) cells infected with adeno-GFP showed excellent infection efficiency with fluorescent microscopy; b) cells infected with adeno-DN-p38 showed attenuated phospho-p38 signal on immunoblots compared to empty vector controls, confirming functional effectiveness of the DN p38 expression vector. These findings support our hypothesis that p38 MAP kinase is involved in activation of proinflammatory nuclear transcription factors, such as NFkB, in pancreatic exocrine cells. In addition, isolated acinar cells provide a model to study NFkB-dependent gene expression mechanistically prior to experimental in vivo studies of pancreatitis.