

ANGIOTENSIN II TYPE 1 RECEPTOR (AT1R) EXPRESSION IN PREMALIGNANT PANCREATIC LESIONS: AN AT1R- ERK1/2- DEPENDENT, p38- JNK-INDEPENDENT REGULATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR BY ANGIOTENSIN II IN PANCREATIC CANCER CELLS

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Background: Vascular endothelial growth factor (VEGF) is a crucial pro-angiogenic component in pancreatic ductal adenocarcinoma (PDA) and its high expression levels have been correlated with poor prognosis and early postoperative recurrence. We have recently shown that high levels of angiotensin II type 1 receptor (AT1R) correlate and colocalize with VEGF in invasive PDA, and that AngII induces VEGF expression in PDA cell lines.

Objectives: 1) To correlate AT1R and VEGF expression in two premalignant pancreatic lesions; 2) to determine the role of AT1R in mediating the AngII-mediated VEGF induction; 3) to explore the signaling mechanisms involved.

Methods: The localization of AT1R and VEGF was analyzed by immunohistochemistry in histologically confirmed intraductal papillary mucinous neoplasms (IPMNs) n= 5, pancreatic intraepithelial neoplasms (PanINs) n=5, and in normal pancreatic tissue n=5. All AT1R mRNA and protein in PDA cell lines PK9, Panc10.05, AsPC-1, and HS677T were analyzed by real time PCR and Western immunoblotting. VEGF in conditioned media and cells treated with or without AngII (10^{-7} mol/L) was measured by ELISA and real time PCR. Total- and phospho-ERK1/2, total- and phospho-p38, and total- and phospho- JNK MAP kinases were analyzed by Western immunoblotting.

Results: Increased expression of AT1R was detected in most ductal cells undergoing metaplasia. PanIN lesions showed more intense AT1R staining when compared to IPMNs, which showed heterogeneous immunoreactivity. VEGF followed the same distribution pattern of AT1R in both lesions. All PDA cell lines expressed variable basal levels of AT1R mRNA and protein. AngII-mediated induction of VEGF was significantly ($p<0.05$) inhibited by an AT1R antagonist, but was insignificantly reduced by an AT2R antagonist. AngII-VEGF induction was inhibited by the tyrosine kinase inhibitor genistein, suggesting a mitogen activated protein kinase signaling mechanism. AngII activated the phosphorylation of ERK1/2, but not p38 or JNK. Inhibition of ERK1/2 activation by a specific MEK1/2 inhibitor U0126 reduced the AngII-induced VEGF expression.

Conclusions: AT1R is expressed in the premalignant pancreatic lesions and may be involved in tumor progression and angiogenesis. Our mechanistic findings provide the first insight into an AngII-initiated signaling pathway that regulates pancreatic tumor angiogenesis. An AT1R-mediated VEGF induction suggests the possibility of AT1R blockade as a novel therapeutic strategy to control angiogenesis in pancreatic tumors.