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Adrenomedullin is an Autocrine Regulator via the ADMR Receptor of Cancer, Stromal, and Endothelial Cells in Pancreatic Tumors

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Background: Pancreatic cancer is a major oncologic challenge due to its aggressive growth and metastasis. Our previous study showed that adrenomedullin (AM) is highly expressed in pancreatic cancer and stimulates pancreatic cancer cells leading to increased tumor growth and metastasis.¹ The current study examines the autocrine effects of AM on cancer cells and cells within the tumor microenvironment (human primary stellate cells [HPSC], human umbilical vein endothelial cells [HUVEC]) and investigates the receptor involved in the autocrine role of AM.

Methods: In vivo studies were conducted in orthotopic tumors in the pancreas as well as lung and liver metastasis developed by tail vein and splenic injection, respectively, of cancer cells. Comparisons were made between control and adrenomedullin receptor (ADMR) shRNA-silenced cell lines. Tumor volume, incidence, and number of colonies were measured by bioluminescence imaging. In vivo angiogenesis was measured by CD31 staining. Reverse transcriptase polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) confirmed the expression of AM and its receptors in tumor microenvironment cells. Growth was assessed by MTS assay. Angiogenesis was measured by Chemicon's In Vitro Angiogenesis Assay Kit (Millipore Corporation, Billerica, MA, USA). Receptor silencing in vitro was by siRNA technique.

Results: ADMR silencing reduced primary tumor volume by 92%. ADMR-silenced tissues also showed a significant reduction in CD31 staining. ADMR silencing reduced

the numbers of lung metastases by 80% and the total volume of lung metastases by 96%. Liver metastases also showed significant reductions in tumor volume, colony formation, and detection time when ADMR was silenced. AM was present and secreted, and its receptors ADMR and calcitonin receptor-like receptor (CRLR) were present in both HPSC and HUVEC cells. Exogenous AM significantly stimulated the growth of HPSC ($129 \pm 1.8\%$, $P < .05$) and HUVEC ($125 \pm 3.7\%$, $P < .05$) cells, while an AM antagonist significantly reduced the basal growth of HPSC ($79 \pm 5.3\%$, $P < .05$) and HUVEC ($79 \pm 0.4\%$, $P < .05$) cells. AM significantly increased the HUVEC polygon formation in an angiogenesis assay while the antagonist reduced polygon formation. Silencing ADMR reduced basal growth of HPSC ($79 \pm 3.4\%$, $P < .05$) and HUVEC ($76 \pm 1.8\%$, $P < .05$) cells. ADMR silencing also reduced angiogenesis formation. In contrast, CRLR silencing showed no reduction on growth or angiogenesis assays.

Conclusion: AM has an autocrine effect on pancreatic cancer cells as well as on important cells of the tumor microenvironment. The autocrine effects of AM are mediated by the ADMR receptor. AM and/or the ADMR receptor may be useful targets for the development of novel therapies for pancreatic cancer that inhibit both the cancer cells and the cells of the tumor microenvironment.

Reference

1. Ramachandran V, Arumugam T, Rosa FH, et al: Adrenomedullin is expressed in pancreatic cancer and stimulates cell proliferation and invasion in an autocrine manner via the adrenomedullin receptor, ADMR. *Cancer Res* 67:2666-2675, 2007