

Pancreatic Cancer

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Systemic Delivery of Liposome-Incorporated Adrenomedullin Receptor (ADMR) Small Interfering RNA Against Tumor and Its Microenvironment Reduced Pancreatic Tumor Growth

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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 role of specific AM receptors on tumor and cells resembling the tumor microenvironment (human pancreatic stellate - HPSC, human umbilical vein – HUVEC, and mouse lung endothelial cells - MLEC) and investigates the receptor involved in the autocrine role of AM.

Methods: Expression levels of AM and its receptors ADMR and CRLR were assessed by RT-PCR, ELISA, and western blotting. ADMR and CRLR were silenced using siRNA. ADMR-silenced and control cells were used to prepare orthotopic tumors and compared for growth and metastasis *in vivo*. Angiogenesis was assessed by CD31 staining *in vivo* and polygon formation *in vitro*. *In vivo* studies were conducted using neutral nanoliposomes to systemically deliver human/mouse siRNA to ADMR.

Results: AM receptors ADMR and CRLR were present in HPSC, HUVEC, and MLECs, while PDAC cells possessed only ADMR receptors as assessed by RT-PCR and western blotting. All cell lines expressed and secreted AM as indicated by ELISA. The basal growth of each of the cell lines was stimulated by exogenous AM [HPSCs - $29 \pm 1.8\%$, HUVECs - $25 \pm 3.7\%$, and MLECs - $33 \pm 4.5\%$ ($P < .05$)] and was inhibited by the antagonist AMA [HPSCs - $21 \pm 5.3\%$, HUVECs - $21 \pm 0.4\%$, and MLECs - $25 \pm 5.1\%$ ($P < .05$)]. AM also stimulated *in vitro* angiogenesis assessed by polygon formation of endothelial cell lines. SiRNA-mediated silencing of ADMR, but not CRLR, reduced basal growth of all

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cells examined [HPSC by $21 \pm 3.4\%$, HUVECs by $24 \pm 1.8\%$, and MLECs by $26 \pm 4.3\%$ ($P < .05$)] and reduced polygon formation of endothelial cells *in vitro*. Orthotopic tumors developed with shADMR bearing cancer cells had dramatically reduced primary tumor volume (MPanc96 by $92 \pm 0.5\%$ and BxPC3 by $83 \pm 0.6\%$) and lung (ShControl - 67% vs. ShADMR – 22%) and liver (ShControl - 100% vs. ShADMR – 43%) metastasis compared to shControl bearing cells. To validate ADMR as a potential therapeutic target, *in vivo* studies were conducted using neutral nanoliposomes to systemically deliver human siRNA to ADMR to silence human cancer cells, and mouse siRNA to ADMR to silence mouse tumor stromal cells. Systemic silencing of both human and mouse ADMR had no obvious adverse effects but strongly reduced tumor development [$88 \pm 0.4\%$ ($P < .05$)] and also resulted in significant reduction in open blood vessels when compared to their control tissues.

Conclusion: ADMR mediates the stimulatory effects of AM on cancer cells and on endothelial and stellate cells within the tumor microenvironment. These data support the further development of ADMR as a useful target treatment of pancreatic cancer.

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.