

PGCR 1:1, 2006 (Abstract 205)

Adrenomedullin Is Acting in an Autocrine Manner via the Receptor, ADMR (L1) in Pancreatic Cancer

Vijaya Ramachandran

Thiruvengadam Arumugam

Craig D. Logsdon

University of Texas M. D. Anderson Cancer Center

Houston, Texas

Diane M. Simeone

University of Michigan

Ann Arbor, Michigan

Background: Pancreatic cancer is a major oncologic challenge due to its aggressive growth and metastasis. We have shown that adrenomedullin (AM) is highly expressed in pancreatic cancer compared to normal pancreas and chronic pancreatitis.¹ In our previous study, we found that AM increases pancreatic tumor growth and invasion both in vitro and in vivo. In our current study, an attempt was made to delineate the receptor involving the impact of AM on pancreatic cancer and also peptide-based inhibitor to block the AM function.

Methods: Reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC) were done to confirm AM and expression. Growth and invasion were assessed by MTS and Matrigel invasion assay (BD Biosciences, San Jose, CA). In vivo studies were conducted by developing orthotopic tumor on SCID mice pancreas and imaged by bioluminescence method. RT-PCR was conducted to check the presence of the receptors on pancreatic cancer cells and stellate and endothelial cells. NF κ B activity was measured by reporter study. SiRNAs were used to silence the ADMR.

Results: Evaluation of a pancreatic cancer tissue array by IHC demonstrated that 90% (43/48) of pancreatic adenocarcinomas overexpressed AM compared to normal pancreas.

Expression of AM and exogenous addition of AM to Panc-1 cells led to increased cellular proliferation (MTS assay) and invasion (Matrigel invasion assay). The effects of AM on cellular proliferation and invasion were blocked by a specific peptide antagonist (AMA) and by expression of stable AM siRNA in MPanc96 cells, which express high basal levels of AM. In an in vivo orthotopic model in SCID mice, AM-overexpressing Panc-1 tumors showed increased tumor growth and invasion, while AM-silenced MPanc96 tumors showed decreased tumor growth and invasion. We further investigated whether AM has an autocrine effect on pancreatic cancer cell growth and NF- κ B activity, by inhibiting the endogenous effect of AM using its antagonist (AMA). By RT-PCR, we checked the expression of the AM receptors – CRLR and ADMR and their binding proteins – RAMP 1-3 and RCP. We found that ADMR (L1) was the only receptor expressed in pancreatic cancer, while both CRLR and ADMR and the other binding proteins were present in pancreatic stellate cells and endothelial cells. Also, silencing of ADMR by siRNA reduced the pancreatic cancer cell growth in vitro.

Conclusions: AM is highly expressed in pancreatic cancer cells and acts as an autocrine factor to stimulate pancreatic cancer cell growth, migration and invasion in vitro and in vivo. Also, pancreatic cancer has a specific receptor for AM and silencing of that reduces growth, so further ways to deliver the antagonist against AM and/or delivering the siRNAs against ADMR would be an effective tool for treatment of pancreatic cancer.

Reference

1. Logsdon CD, Simeone DM, Binkley C, et al: Molecular profiling of pancreatic adenocarcinoma and chronic pancreatitis identifies multiple genes differentially regulated in pancreatic cancer. *Cancer Res* 63(10):2649-57, 2003.