

Pancreatic Cancer

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Anterior Gradient 2 (AGR2) Is Expressed and Secreted During the Development of Pancreatic Cancer and Promotes Cancer Cell Survival

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Background: Pancreatic cancer is a major oncologic challenge due to its aggressive growth and metastasis. Our microarray data demonstrated overexpression of the human homologue of anterior gradient-2 (AGR2) in pancreatic cancer as compared with its expression in normal pancreas and chronic pancreatitis. AGR2, a gene of differentiation, is associated with estrogen receptor (ER)-positive breast tumors; however, nothing is known about the biologic significance of its expression in pancreatic cancer. The current study investigated the role of AGR2 in pancreatic cancer.

Methods: Immunohistochemistry, quantitative RT-PCR (Q-RT-PCR) and western blotting (WB) were used to assess AGR2 expression. The influence of AGR2 was evaluated by silencing with siRNA and shRNA in pancreatic cancer cells. Proliferation was assessed using MTS; invasion using a Boyden chamber assay; and apoptosis by FACS. MPanc96 cells bearing stably silenced AGR2 cells and luciferase gene were used to develop orthotopic tumors in athymic nude mice. Tumor growth and metastasis with AGR2-silenced cells with and without the chemotherapeutic drug, gemcitabine, was studied by non-invasive bioluminescence imaging. Analysis of apoptotic cells in tumor tissue was done using commercially available TUNEL kit.

Results: AGR2 mRNA was 14-fold higher in pancreatic cancer tissues compared with normal pancreas and pancreatitis tissues. In tumor tissues, AGR2 levels were very high in

pancreatic cancer cells but not in normal duct, acini, islets, stroma, or in foci of chronic pancreatitis. AGR2 was also expressed in the dysplastic duct cells in all grades of PanIN lesions. To evaluate the frequency of AGR2 expression in pancreatic tumors, we analyzed a tissue microarray and observed that 98% (56/57) of pancreatic cancer tissues were positive, of which staining was high in 60%, moderate in 22%, and low in 18%. RT-PCR and WB demonstrated elevated AGR2 expression in 6/8 pancreatic cancer cell lines. Analysis of AGR2 levels in conditioned media from cancer cells indicated that it was secreted. Transient and stable silencing of AGR2 in pancreatic cancer cell lines significantly reduced cell proliferation and invasion and improved gemcitabine sensitivity, in vitro. In vivo studies with AGR2-silenced cells led to a four-fold reduction in tumor volume, while combination with gemcitabine treatment led to a further significant reduction in tumor volume. TUNEL analysis indicated significant increase of apoptotic cells on tissues silenced with AGR2 and treated with gemcitabine when compared with all other tissues. AGR2 silencing alone did not reduce the incidence of metastases, but in combination with gemcitabine there was a complete reduction of lung metastasis (100%) and 60% reduction of liver metastasis incidence. The number of metastatic foci was reduced by silencing AGR2 itself (lung-47%; liver-64%), and gemcitabine treatment further significantly reduced the number of metastatic foci in lung (100%) and liver (80%).

Conclusion: The current study describes the expression, secretion, and function of AGR2 in pancreatic cancer. This molecule was found to stimulate growth, invasiveness, and survival in vitro, and tumor growth and sensitivity to chemotherapy in vivo, thus suggesting that AGR2 could be a better therapeutic target.