

Neutrophil Gelatinase-Associated Lipocalin: A Novel Suppressor of Invasion and Angiogenesis in Pancreatic Cancer

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Background: Gene expression analyses of pancreatic cancer (PaCa) had identified neutrophil gelatinase-associated lipocalin (NGAL) as a potential early biomarker. Recently, proteomic analysis of pancreatic exocrine secretions showed significantly high secreted NGAL levels in PaCa patients. NGAL, a 25-kD lipocalin, is a secreted acute-phase protein, which is also upregulated in multiple cancers including breast, lung, and pancreas cancers. NGAL is a prosurvival factor in breast and lung cancer cells when challenged with xenotoxic agents. However, its role in pancreatic cancer is completely unknown.

Aim: To determine the functional role of NGAL in PaCa and correlate that with survival, proliferation, invasion, and/or metastasis of PaCa cells.

Methods and Results: Well to moderately differentiated PaCa cells, including AsPC-1, BxPC-3, and Capan-2 had very high expression of NGAL, while the poorly differentiated PaCa cells including Panc-1 and MIA PaCa-2 had no detectable levels of NGAL as detected by reverse transcription polymerase chain reaction (RT-PCR) and western blots.

Immortalized HPDE (human pancreatic ductal epithelium) cells had no NGAL expression but HPDE cells transformed with K-RasG12V (HPDE-Kras) showed significant NGAL expression. Immunohistochemistry (IHC) of untreated human PaCa tissue microarrays (150 PaCa) showed 45% positive luminal and cytosolic NGAL expression (Fisher's test; $P=.016$ vs. control). NGAL was detected in adjacent chronic pancreatitis sections (26%) and also in PanIN1-2 but not in PanIN3 lesions. Also, normal pancreatic ductal cells and invasive PaCa tissues showed no NGAL expression by IHC. We created four stable PaCa clones: two over-expressing NGAL (Panc-1-NGAL and MIA PaCa-2-NGAL) and two down-regulating NGAL (BxPC-3 shRNA and AsPC-1 shRNA). There was no effect of NGAL over-expression or down-regulation on proliferation (CellTiter 96 AQ One Solution Cell Proliferation assay) or viability (AnnexinV-FITC assay) of PaCa cells. However, NGAL down-regulation significantly increased adhesion to fibronectin (static adhesion assay). NGAL over-expression significantly suppressed (55%), and conversely, NGAL down-regulation significantly increased (45%), invasion of PaCa cells through matrigel in vitro. NGAL over-expression or down-regulation did not alter matrix metalloproteinase (MMP)-2/MMP-9 activity. But, NGAL over-expression decreased tyrosine-397 phosphorylation of focal adhesion kinase (FAK) in PaCa. Further, NGAL over-expression potently decreased angiogenesis in vitro (Matrigel™ assay) through reduction in vascular endothelial growth factor (VEGF) production.

Conclusion: We demonstrated for the first time that NGAL is selectively expressed in well to moderately differentiated PaCa including PanIN lesions. NGAL inhibits adhesion/invasion of PaCa cells by suppressing FAK activation and reduces angiogenesis by blocking VEGF production. Thus, *loss* of NGAL expression in the exocrine pancreatic secretions will be an early indicator for locally advanced or metastatic PaCa.