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Immune Cell Production of Interleukin 17 Induces Stem Cell Features of Pancreatic Intraepithelial Neoplasia Cells

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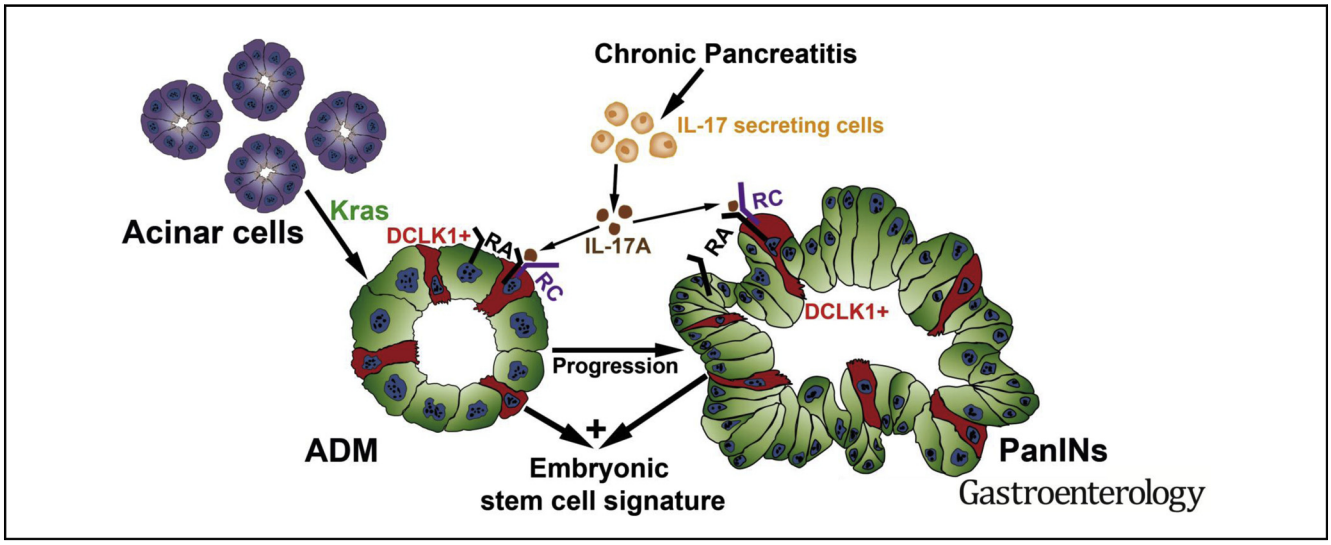

BACKGROUND & AIMS: Little is known about how the immune system affects stem cell features of pancreatic cancer cells. Immune cells that produce interleukin 17A (IL17A) in the chronically inflamed pancreas (chronic pancreatitis) contribute to pancreatic interepithelial neoplasia (PanIN) initiation and progression. We investigated the effects that IL17A signaling exerts on pancreatic cancer progenitor cells and the clinical relevance of this phenomena. **METHODS:** We performed studies with Mist1Cre;LSLKras;Rosa26mTmG (KC^{iMist};G) and Kras(G12D);Trp53(R172H);Pdx1-Cre (KPC) mice (which upon tamoxifen induction spontaneously develop PanINs) and control littermates. Some mice were injected with neutralizing antibodies against IL17A or control antibody. Pancreata were collected, PanIN epithelial cells were isolated by flow cytometry based on lineage tracing, and gene expression profiles were compared. We collected cells from pancreatic tumors of KPC mice, incubated them with IL17 or control media, measured expression of genes regulated by IL17 signaling, injected the cancer cells into immune competent mice, and measured tumor growth. IL17A was overexpressed in pancreata of KC^{iMist} mice from an adenoviral vector. Pancreata were collected from all mice and analyzed by histology and immunohistochemistry. Levels of DCLK1 and other proteins were knocked down in KPC pancreatic cancer cells using small interfering or short hairpin RNAs; cells were analyzed by immunoblotting. We obtained 65 pancreatic tumor specimens from patients, analyzed protein levels by immunohistochemistry, and compared results with patient survival times. We also analyzed gene expression levels and patient outcome using The Cancer Genome Atlas database. **RESULTS:** PanIN cells from KC^{iMist};G mice had a gene expression pattern associated with embryonic stem cells. Mice given injections of IL17-neutralizing antibodies, or with immune cells

that did not secrete IL17, lost this expression pattern and had significantly decreased expression of DCLK1 and POU2F3, which regulate tuft cell development. KC^{iMist} mice that overexpressed IL17 formed more PanINs, with more DCLK1-positive cells, than control mice. Pancreatic tumor cells from KPC mice and human Capan-2 cells exposed to IL17A had increased activation of NF-κB and mitogen-activated protein kinase signaling and increased expression of DCLK1 and ALDH1A1 (a marker of embryonic stem cells) compared with cells in control media. These cells also formed tumors faster that cells not exposed to IL17 when they were injected into immunocompetent mice. KPC cells with knockdown of DCLK1 expressed lower levels of ALDH1A1 after incubation with IL17 than cells without knockdown. Expression of the IL17 receptor C was higher in DCLK1-positive PanIN cells from mice compared with DCLK1-negative PanIN cells. In human pancreatic tumor tissues, high levels of DCLK1 associated with a shorter median survival time of patients (17.7 months, compared with 26.6 months of patients whose tumors had low levels of DCLK1). Tumor levels of POU2F3 and LAMC2 were also associated with patient survival time. **CONCLUSIONS:** In studies of mouse and human pancreatic tumors and precursors, we found that immune cell-derived IL17 regulated development of tuft cells and stem cell features of pancreatic cancer cells via increased expression of DCLK1, POU2F3, ALDH1A1, and IL17RC. Strategies to disrupt this pathway might be developed to prevent pancreatic tumor growth and progression.

Keywords: Immune Response; Kras; PDAC; Tumorigenesis.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Chronic pancreatitis is a well-established risk factor for pancreatic cancer but the mechanistic link between them is not fully established.

NEW FINDINGS

In studies of mouse and human pancreatic tumors and precursors, immune cell-derived IL17 regulates development of tuft cells and stem cell features of pancreatic cancer cells via increased expression of DCLK1, POU2F3, ALDH1A1, and IL17RC.

LIMITATIONS

Further work should be done blocking the inducible IL-17RC in Kras-induced pancreatic cancer mouse models as the restricted expression of this receptor may result in lower toxicity.

IMPACT

The study finds a novel link between immune components of chronic pancreatitis and pancreatic tumorigenesis. Novel IL-17-target genes with prognostic and potential therapeutic relevance were discovered.

The aggressive nature and poor prognosis of pancreatic cancer are well known.[1] Recently, genomic analysis of hundreds of pancreatic cancers showed molecular subtypes that include an immunogenic type characterized by up-regulation of immune networks.[2] Further understanding of the early immunologic events surrounding pancreatic tumor initiation and progression could result in effective novel immunopreventive strategies, potentially resulting in a stronger impact on this disease.

The interaction between immune cells and tumor cells can result in either tumor development and progression or effective immunosurveillance. In particular, interleukin (IL) 17-secreting immune cells have been described as having mostly tumor-promoting activity[3,4] although there are reports of anti-tumoral activity in specific contexts and model systems.[5] In pancreatic cancer, using a genetically engineered mouse model that mimics the human disease,[6] we previously reported that IL17-producing $CD4^+$ (T helper type 17 cells) and γδ T cells (γδ/IL17 cells) induced pancreatic premalignant lesion development and progression.[7] These phenomena are further augmented in the presence of concomitant chronic inflammation, a well-recognized risk factor for pancreatic cancer. However, the mechanisms by which IL17-secreting immune cells ultimately accelerate pancreatic intraepithelial neoplasia (PanIN) initiation and progression are not well defined.

IL17-secreting immune cells play an essential role in host defense at mucosal surfaces by inducing acute phase proteins (IL6, LCN2), neutrophil-recruiting chemokines, and antimicrobial peptides (defensins, mucins).[8,9] Recent evidence suggests that the immune component of the microenvironment can affect the stem cell niche.[10–12] As examples of this, T helper type 22 cells, $CD4^+$ T cells capable of secreting IL22, have been recently found to promote colorectal cancer stemness through activation of STAT3 and expression of the methyltransferase DOT1L, which induces core stem cell genes NANOG, SOX2, and POU5F1.[10] In a similar manner, IL17B secreted by nontumor cells has been reported to promote gastric cancer stemness by signaling with IL17 receptor (IL17R) B through activation of the protein kinase B (AKT)/β-catenin pathway.[12]

The presence and role of stem cells in pancreatic cancer is well established,[13,14] contributing to tumor relapse[15] and resistance to chemotherapy.[16] Tuft cells have been described originally as unique solitary chemosensory cells that are analogous to taste cells,[17] with tumor-initiating capacity in the colon,[18] and their presence in both normal and preneoplastic pancreatic lesions has been recently rigorously described.[17,19,20] Delgiorno et al have shown that KrasG12D activation is associated with aberrant genesis of pancreatic tuft cells, which are identified by a specific array of markers.[17] DCLK1 has been proposed as a marker of tuft cells in the stomach, intestine, and pancreas[18,21] Furthermore, the role of DCLK1-expressing cells as long-lived

Abbreviations used in this paper: **ADM, Acinar-ductal metaplasia; AKT, protein kinase B; ERK, extracellular signal–regulated kinase; ESC, embryonic stem cell; GFP, green fluorescent protein; IL, interleukin; IL17R, interleukin 17 receptor; JNK, c-Jun-*N*-terminal kinase; KO, knockout; mPanIN, murine PanIN; NF-κB, nuclear factor-κB; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; shRNA, short hairpin RNA; siRNA, small interfering RNA.**

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