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Insights Into the Role of Nicotine in Pancreatic Stem Cell Activation and Acinar Dedifferentiation

See "Nicotine promotes initiation and progression of KRAS-induced pancreatic cancer via *Gata6*-dependent dedifferentiation of acinar cells in mice," by Hermann PC, Sancho P, Cañamero M, et al, on page 1119.

Pancreatic ductal adenocarcinoma (PDAC) is highly lethal. Owing to its asymptomatic nature during early stages, this disease has generally already progressed to its advanced stage at the time of diagnosis. Emerging evidence demonstrates that stage-wise progression of PDAC from preinvasive lesions involves significant alterations in

the ductal epithelium. In particular, there are 3 major types of preinvasive lesions categorized in PDAC progression: (i) Pancreatic intraepithelial neoplasms (PanINs), (ii) intraductal papillary mucinous neoplasms, and (iii) mucinous cystic neoplasms.[1] Furthermore, stage-specific genetic alterations and signature gene mutations have also been observed during the development and progression of PDAC. For example, *Kras* is the primary genetic mutation identified during the early stages of PDAC, but many other later mutations, such as *p16*, *p53*, *DPC4*, and *BRCA2*, have also been identified in conjunction with the initiation and progression of PDAC. In addition to specific gene mutations, several risk factors have been identified in PDAC progression, such as pancreatitis, obesity, diabetes, alcohol use, and smoking. According to estimates from the American Cancer Society for 2014, 20%–30% of exocrine pancreatic cancer cases in the United States are linked to lifestyles that included smoking; similarly, 37% of pancreatic cancer cases in the United Kingdom in 2014 correlate with smoking as a risk factor (www.cancer.org/).

In addition to cancer, tobacco use/smoking is a substantial risk factor for pancreatitis, which is an inflammatory lesion caused by carcinogenic exposure. Pancreatitis involves alterations in pancreatic enzyme secretion and acinar cell destruction, subsequently leading to PDAC development.[2,3] For example, during tobacco use/smoking, different carcinogenic multifactorial compounds that cause gene alterations may lead to development of PDAC.[4] It is well established that nicotine is the major addictive agent in tobacco. Nicotine results in different functional alterations to cancer cells, such as increased proliferation and angiogenesis.[5,6] Previous studies show that nicotine and cotinine, the metabolic derivative of nicotine, are significantly present in pancrease of animals exposed to tobacco smoke.[2,3] Furthermore, a recent study demonstrates that smoking and nicotine treatment up-regulates the MUC4 mucin in pancreatic cancer cells through the activation of the α-7 nicotinic receptor (α7nAChR)/JAK2-STAT3 pathway; this activation leads to increased metastatic properties.[7] However, the role of nicotine in the initiation and progression of genetically mutated PDAC is poorly understood. Thus, it is important to investigate the mechanistic role of nicotine with respect to PDAC. In this issue of Gastroenterology, the research article from Hermann et al[8] demonstrates that nicotine alters the acinar cell compartment in the *Kras*-activated initiation and progression of PDAC. Using a *Kras*-mutated PDAC mouse model, the authors demonstrate a clear mechanism for how nicotine mediates acinar dedifferentiation through to changes to the AKT/ERK/MYC-mediated signaling pathways, resulting from down-regulation of the *Gata6* gene.

The goal of the study in this issue of *Gastroenterology* by Hermann et al primarily focuses on analyzing the role of nicotine in the development of *Kras*-mediated PDAC. The authors found that *Kras* animals exposed to cigarette smoke for 86 weeks maintained the same weight but underwent significant morphologic changes in their pancreases. PanIN lesions and acinar-to-ductal metaplasia were also observed in nicotine-treated *Kras* animals. Acinar-to-ductal metaplasia is an initiating event in PDAC; mutated *Kras*, when present in acinar cells, promotes PanIN and acinar-to-ductal metaplasia formation.[9,10] Additionally, polarized macrophages were also observed in the nicotine-treated *Kras* animals. Nicotine itself is not a carcinogenic agent, which could be the possible reason for the failure of neoplastic transformation in nicotine-treated normal mice. However, Hermann et al observed significant alterations of acinar-specific genes in these non–oncogene-bearing mice. Acinar cell differentiation is an important cellular process and is initiated by Gata6 and Mist1 molecules.[11,12] In nicotine-treated normal mice, the authors show significantly decreased expression of *Gata6* and *Mist1*, increased acinar cell proliferation, and increased numbers of Sox9-positive stem/progenitor cells (Figure 1*A*). These findings suggest that nicotine plays a critical role in pancreatic tissue homeostasis. For example, Sox9 is an established marker for stem-like centroacinar cells. Sox9 has also been shown to play a role in initiation of premalignant lesions.[13] Therefore, these results offer an approach for identifying risk factors that initiate and/or activate stem/progenitor cells, which can further transform into cancer stem cell populations. Hermann et al also confirmed a previous study[7] that nicotine acts through α7-nAChR in pancreatic cancer cells. From a mechanistic perspective, Hermann et al show that nicotine inhibits *Gata6* by the ERK/C-MYC pathway and C-MYC–binding sites; this was also confirmed in the *Gata6* promoter. Results show that nicotine treatment increases the level of C-MYC and its corresponding upstream signaling pathways, such as ERK and AKT. These results suggest that C-MYC serves as *Gata6* repressor, in turn leading to PDAC development in *Kras*-mutated animals (Figure 1*B*). A previous study by Smith et al establishes the interaction of *Gata6* and MYC[14]; the results of the Hermann et al study further strengthen the concept of MYC-mediated Gata6 repression by nicotine treatment.

Overexpression of the Gata6 molecule further confirmed nicotine-mediated acinar dedifferentiation. In particular, Gata6 overexpression caused a morphologic alteration that resulted in an epithelial-like phenotype, with decreased expression of stemness genes and reduced tumorigenic potential. These results suggest that *Gata6* is a key player in the nicotine-mediated self-renewal process and tumorigenic activity. Furthermore, Hermann et al observed that ERK and AKT inhibitors reversed alterations in nicotine-mediated epithelial–mesenchymal transition. Taken together, these results suggest that nicotine induces the epithelial–mesenchymal transition process and increases circulating pancreatic cells with a metastatic phenotype. Moreover, these results confirm a previous study by Momi et al, which found that nicotine mediates pancreatic cancer cell metastasis.[7]

Another significant goal for the study of Hermann et al was to investigate the role of metformin in dedifferentiation and preneoplastic development of PDAC. Metformin is an antidiabetic drug currently undergoing phase II clinical trials for pancreatic cancer.[15] Hermann et al observed that metformin treatment does not ameliorate the development of nicotine-induced low-grade PanINs. However, high-grade PanINs significantly decrease with metformin treatment