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Cigarette Smoke Induces Stem Cell Features of Pancreatic Cancer Cells via PAF1

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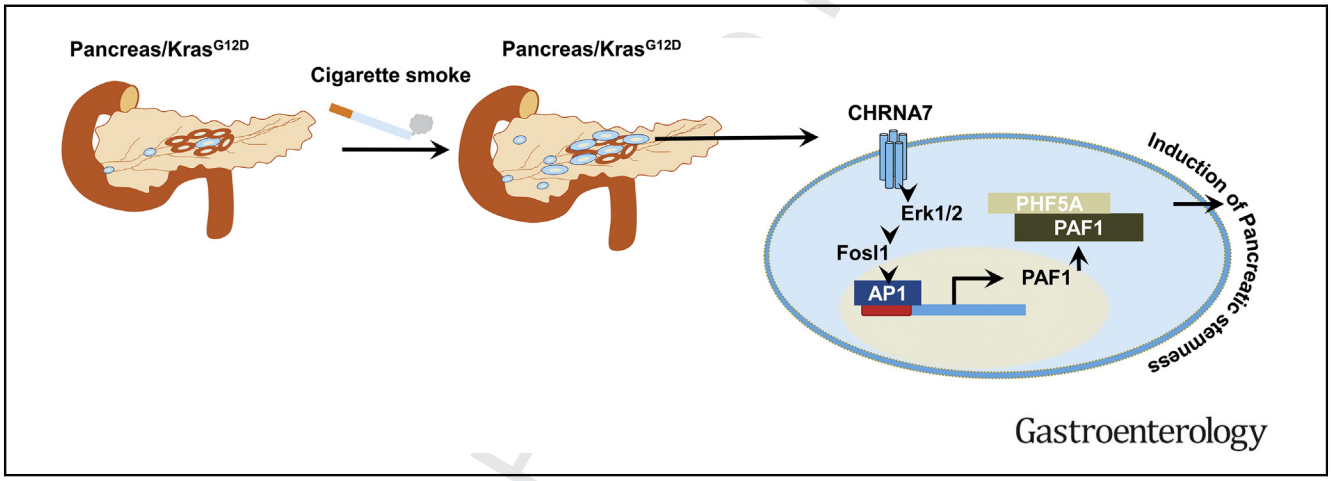

BACKGROUND & AIMS: Cigarette smoking is a major risk factor for pancreatic cancer. Aggressive pancreatic tumors contain cancer cells with stem cell features. We investigated whether cigarette smoke induces stem cell features in pancreatic cancer cells. **METHODS:** Kras^{G12D}; Pdx1-Cre mice were exposed to cigarette smoke or clean air (controls) for up to 20 weeks; pancreata were collected and analyzed by histology, quantitative reverse transcription polymerase chain reaction, and confocal immunofluorescence microscopy. HPNE and Capan1 cells were exposed to cigarette smoke extract (CSE), nicotine and nicotine-derived carcinogens (NNN or NNK), or clean air (controls) for 80 days and evaluated for stem cell markers and features using flow cytometry–based autofluorescence, sphere formation, and immunoblot assays. Proteins were knocked down in cells with small interfering RNAs. We performed RNA sequencing analyses of CSE-exposed cells. We used chromatin immunoprecipitation assays to confirm the binding of FOS-like 1, AP-1 transcription factor subunit (FOSL1) to RNA polymerase II-associated factor (*PAF1*) promoter. We obtained pancreatic ductal adenocarcinoma (PDAC) and matched nontumor tissues (n = 15) and performed immunohistochemical analyses. **RESULTS:** Chronic exposure of HPNE and Capan1 cells to CSE caused them to increase markers of stem cells, including autofluorescence and sphere formation, compared with control cells. These cells increased expression of ABCG2, SOX9, and PAF1, via cholinergic receptor nicotinic alpha 7 subunit (CHRNA7) signaling to mitogen-activated protein kinase 1 and FOSL1. Pancreatic cell lines with knockdown of PAF1 did not develop features of stem cells on exposure to CSE. Exposure of cells to NNN and NNK led to increased expression of CHRNA7, FOSL1, and PAF1 along with stem cell features. Pancreata from Kras^{G12D}; Pdx1-Cre mice exposed to cigarette smoke had increased levels of PAF1 mRNA and protein, compared with control mice, as well as increased expression of SOX9. Levels of PAF1 and FOSL1 were increased in PDAC tissues, especially those from smokers, compared with nontumor pancreatic tissue. CSE exposure increased expression of PHD-finger protein 5A, a pluripotent transcription factor and its interaction with PAF1. **CONCLUSIONS:** Exposure to cigarette smoke activates stem cell features of pancreatic cells, via CHRNA7 signaling and FOSL1 activation of PAF1 expression. Levels of PAF1 are increased in pancreatic tumors of humans and mice with chronic cigarette smoke exposure.

Keywords: PHF5A; ERK; Pancreatic Carcinogenesis; Nicotine Receptor Signaling.

Pancreatic cancer (PC) is recognized as one of the deadliest diseases.[1] Of various established risk factors, cigarette smoking causes 30% of all cases of PC,[1] and therefore there is an urgent need to develop novel therapeutic strategies to specifically treat patients with PC who have a history of smoking. Currently, efforts for the development of such strategies are limited because there is no mechanistic understanding of how cigarette smoking is involved in PC initiation and progression.

A previous study from our laboratory showed that cigarette smoke and its major addictive component, nicotine, induces progression and metastasis of PC through cholinergic receptor nicotinic alpha 7 subunit (CHRNA7)-mediated MUC4 up-regulation.[2] Cigarette smoke contains more than 4000 chemical components, and among them, nicotine and nicotine-derived carcinogens, 4-(methyltyramine)-1-(3-pyridyl)-1-butanone (NNK), N-Nitrosonornicotine (NNN), are associated with carcinogenesis.[3–5] Recent studies provide evidence for the association of cigarette smoke and its ingredients with the enrichment of cancer stemness population in various cancers.[6,7] In mice, nicotine promotes initiation and progression of Kras-induced PC via Gata6-dependent de-differentiation of acinar cells.[8] In our editorial commentary, we have proposed a role for nicotine in pancreatic stemness induction and acinar cell de-differentiation.[9] These studies suggest that cigarette smoke and its particular ingredients induce cancer stemness in various cancers. However, whether and how cigarette smoking induces pancreatic stemness or cancer stemness remains unexplored.

Recent studies have shown that FOS-like 1, AP-1 transcription factor subunit (FOSL1) is significantly overexpressed in PC.[10] Loss of FOSL1 reduces stemness properties in hepatocellular carcinoma.[11] FOSL1 belongs to the Fos gene family, which consists of 4 members: FOS, FOSB, FOSL1 (or FRA1), and FOSL2. FOS family members dimerize with JUN family proteins, such as c-Jun, JunB, and JunD, forming a dimeric transcription factor called Activator Protein-1 (AP-1) transcription factor. FOSL1 transcription factor levels are increased in response to a variety of stimuli, including smoking.[12] However, to our knowledge, the role of FOSL1 in smoking-induced activation of pancreatic stemness has not been investigated before.

PAF1, human RNA polymerase II-associated factor also known as pancreatic differentiation protein 2 is a subunit of the PAF1 complex, which is composed of PAF1, Ctr9, Cdc73, Rtf1, Ski8, and Leo1, and regulates multiple processes, including transcription initiation and elongation.[13–16] We and others have demonstrated that PAF1 plays an important role in the maintenance of embryonic stem cell (ESC) signature in a complex independent manner. The PAF1 maintains pluripotency and self-renewal of mouse ESCs.[17,18] PHD-finger protein 5A (PHF5A) has recently been shown to regulate the self-renewal of ESCs. The endogenous PAF1, by interacting with PHF5A activates more than 600 pluripotent genes in ESCs by regulating RNA polymerase II elongation in pluripotent loci.[19] We also showed that PAF1 maintains cancer stemness population and induces tumorigenesis and metastasis in PC.[20,21] Overall, these studies suggest that PAF1 is a major ESC maintenance factor, and its levels are increased in PC. To our knowledge, the role of PAF1 in cigarette smoke–induced PC is unknown.

In the present study, we sought to determine whether chronic exposure to cigarette smoke and its ingredients, nicotine, NNN, and NNK, could enrich pancreatic stemness. We investigated the mechanism involved in the smoking-induced promotion of pancreatic stemness and cancer stemness population, using in vitro and in vivo models. We observed that chronic exposure to cigarette smoke increases PAF1, a major ESC signature protein through CHRNA7/extracellular signal–regulated kinase (ERK)/FOSL1/cJun (AP1) signaling pathway. We concluded that chronic cigarette smoke exposure promotes pancreatic stemness and that NNN and NNK are the major contributing factors for the smoking-induced stemness induction.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

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NEW FINDINGS

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IMPACT

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Abbreviations used in this paper: **AF, autofluorescence; ALDH, aldehyde dehydrogenase; AP-1, Activator Protein-1; C-CSE, commercial CSE; ChIP, chromatin immunoprecipitation; CHRNA7, cholinergic receptor nicotinic alpha 7 subunit; CSE, cigarette smoke extract; ERK, extracellular signal–regulated kinase; ESC, embryonic stem cell; FOSL-1, FOS-like 1, AP-1 transcription factor subunit; mRNA, messenger RNA; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N-Nitrosonornicotine; PAF1, human RNA polymerase II-associated factor; PC, pancreatic cancer; PDAC, pancreatic ductal adenocarcinoma; PHF5A, PHD-finger protein 5A; Ser-2-P-Pol II, serine 2 phosphorylation of RNA polymerase II.**

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