Introduction

Pancreatic cancer (PC) is a devastating disease due to lack of early diagnosis and its unresponsiveness to conventional therapeutic regimens, resulting in a 5-year survival rate of less than 5% and a mortality rate of nearly 100% (1,2). Till date, tumor resection is the only potentially curative approach. But unfortunately, >80% patients are presented with unresectable tumor with distant organ metastasis at the time of diagnosis (3). Furthermore, the mortality rate for PC has remained unchanged over the past few decades (4). As there are no tests for early screening, and once detected, therapeutic options are limited, the only prospect for reducing mortality from PC is prevention. Understanding the etiology and identifying the risk factors associated with PC are essential for the prevention of this deadly disease.

Although several risk factors, such as higher body mass index, alcohol consumption, and history of diabetes, have been associated with PC, cigarette smoking is the only unequivocal risk factor that has been identified (5). Cigarette smoking is also an independent risk factor for developing chronic pancreatitis, a disease with a subsequent high risk of progression to PC (6). In addition, the cigarette smoke (CS) causes pancreatic damage by inducing alteration in pancreatic enzyme secretions and leading to acinar cell destruction by aggravating the ongoing pancreatic inflammatory events (7). CS-induced pancreatic damage is a multifactorial event caused by various tobacco-derived components, including nicotine and various other carcinogenic components, such as polycyclic aromatic hydrocarbons (PAHs), nitrosamines, aromatic amines (AA), and heterocyclic amines (HCA). Herein, we discuss the effects of these tobacco-derived components on the pancreas through the cross-talk between the CS-mediated signaling pathways and gene alterations and thus decipher the direct causal relationship between cigarette smoking and PC.

Correlation between cigarette smoking and PC incidence: epidemiological evidence

Smoking is the most common method of consuming tobacco, and tobacco is the most common substance smoked. There are no international definitions of light/heavy smoking. However, smoking <20 pack years is considered light/moderate smoking, whereas one pack-year is smoking one pack per day for 1 year. Any amount above this value is considered heavy smoking and smoking >30 pack years significantly increases the risk of developing all smoking-related diseases. The association of long-term smoking and lung cancer is well established through various studies, and it has been validated that tobacco smoking is responsible for 90% of all lung cancers (8). However, smoking also increases the risk of developing cancers of the esophagus, uterine cervix, kidney, bladder, stomach, and pancreas. Cigarette smoking is the only environmental factor that is strongly associated with risk of developing PC, and it is estimated to account for approximately 25–30% of all pancreatic tumors (9).

An association between smoking and PC was first noted through several studies during the 1960s and 1970s. These survey-based studies showed that cigarette smokers had a 70% greater risk of developing PC in comparison to non-smokers (10,11). Also, a recent meta-analysis of 82 studies published between 1950 and 2007 on smoking and PC found that current smokers have a 1.74-fold (95% confidence interval, 1.61–1.87) increased risk of developing PC (12). An ongoing multicenter prospective cohort study, European Prospective Investigation into Cancer and Nutrition (EPIC), undertaken by维利埃等, including 10 European countries, revealed that both active cigarette smoking as well as exposure to environmental tobacco smoke is associated with increased risk of PC and that risk is reduced to levels of never smokers within 5 years of quitting (13). A recent hospital-based case-control study by塔拉米尼等人. on PC in Northern Italy, between 1991 and 2008, found that current smokers have a 1.74-fold (95% confidence interval, 1.61–1.87) increased risk of developing PC (12). An ongoing multicenter prospective cohort study, European Prospective Investigation into Cancer and Nutrition (EPIC), undertaken by维利埃等, including 10 European countries, revealed that both active cigarette smoking as well as exposure to environmental tobacco smoke is associated with increased risk of PC and that risk is reduced to levels of never smokers within 5 years of quitting (13). A recent hospital-based case-control study by塔拉米尼等人. on PC in Northern Italy, between 1991 and 2008, found that current smokers have a 1.74-fold (95% confidence interval, 1.61–1.87) increased risk of developing PC (12). An ongoing multicenter prospective cohort study, European Prospective Investigation into Cancer and Nutrition (EPIC), undertaken by维利埃等, including 10 European countries, revealed that both active cigarette smoking as well as exposure to environmental tobacco smoke is associated with increased risk of PC and that risk is reduced to levels of never smokers within 5 years of quitting (13). A recent hospital-based case-control study by塔拉米尼等人. on PC in Northern Italy, between 1991 and 2008, found that current smokers have a 1.74-fold (95% confidence interval, 1.61–1.87) increased risk of developing PC (12). An ongoing multicenter prospective cohort study, European Prospective Investigation into Cancer and Nutrition (EPIC), undertaken by维利埃等, including 10 European countries, revealed that both active cigarette smoking as well as exposure to environmental tobacco smoke is associated with increased risk of PC and that risk is reduced to levels of never smokers within 5 years of quitting (13).
PC, if they were active smokers, they were noted to develop PC on an average of 20 years earlier than non-smokers (17). A plethora of studies have also developed race-/sex-dependent trends in cancer incidence. A cohort study of the US population reflected a higher incidence of tobacco-mediated PC in Black and non-Hispanic populations (18). Between 2001 and 2005, Blacks were diagnosed with PC with a 33% higher incidence compared with Whites. Moreover, the PC mortality rates were 27% greater in Black men and 38% greater in Black women as compared to their White counterparts (19).

Although various studies have established smoking as undeniable risk factor for development of PC, detailed studies on impact of smoking intensity and smoking duration on PC initiation, progression, and development are still limited. Also, data on passive smoking and the use of smokeless tobacco are inconsistent, proving an insufficient evidence for increased risk of PC, hence demanding further examination. Further studies are required to find the direct causal relationship between cigarette-smoking and PC. It is a well-established fact that CS is a cocktail of many toxic constituents (20–22) and it contributes to PC pathogenesis due to its complexity.

Components of CS: their contribution to pancreatic damage

More than 4000 chemicals are generated during cigarette combustion (20–22) that include butadiene, aldehydes, nicotine, bacterial endotoxin, a large amount of free radicals such as alkyl, alkoxy, peroxyl, and guinone/hydroquinone, PAHs such as benzo[a]pyrene (BaP), tobacco-specific N-nitrosamine such as 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and nitrosornicotine, and a large amount of nitric oxide (NO). Carcinogenic AA and tobacco-specific nitrosamines detected in CS are one of the best examined factors in the pathology of various cancers (23). Table I briefly covers the chemical nature and structure of the major CS components discussed in the review and provides information about their doses per cigarette.

Abundant evidence has been provided both through experimental and epidemiological studies for the CS-mediated pancreatic injury. Nicotine is the major psychoactive component of tobacco and CS. It is an addictive agent and has been characterized as a drug of abuse by the US Surgeon General (24). Although it is not carcinogenic by itself, it has been shown to play a key role in PC pathogenesis by leading to an uncontrolled increase of pancreatic protein synthesis in isolated acini (25). A study using the experimental mouse model of intraepithelial lesions induced by 7,12-dimethylbenzanthracene (DMBA) showed that nicotine promotes pancreatic ductal adenocarcinoma in these mice (26). Thus, smoking not only exhibits the regulatory effect on the pancreatic acinar cells but also influences the ductal cell function. A recent study has shown an upregulation of pro-collagen type 1 in pancreatic tissue with an induction of morphologic changes due to high dose of smoke exposure, which is an indicator for fibrotic tissue replacement (27). Cheeburgh et al. showed that nicotine itself induces cytoplasmic vacuolation and cellular edema in the exocrine pancreas and increases total cellular amylase content (28). It was observed that the increase in pancreatic enzymes in nicotine-treated rats may be the causative factor in nicotine-induced pancreatic cell pathology (6). Studies have shown that the exposure to nicotine causes a significant decrease in secretion of duodenal bicarbonate in rabbits and affects the composition of the pancreatic secretions (29). On the subcellular level, nicotine exposure also activates multiple signaling pathways in cells resulting in high levels of intracellular calcium release that is paralleled by increased enzyme release, including lipase (30) and amylase (31).

CS contains various toxic chemicals, including the nitrosamine and NNK, which is a derivative of nicotine formed by nitrosation during the processing of tobacco plants into cigarettes (32). Prokopczyk et al. showed higher NNK levels in the pancreatic juice of smokers as compared with the non-smokers (33). Experimental data using female hamsters revealed that 65% of offspring develop PC by 1 year of age when injected with an intra-tracheal dose of NNK 1 day before the delivery of the pups (34). In another study, when administered in the drinking water for F344 rats, NNK induced pancreatic tumors of both the exocrine and the ductal/endoctrine type (23,35). These studies suggest that smoking during pregnancy adds risk of developing PC in early stage of life. To elicit its effects, NNK undergoes a series of enzymatic and metabolic reactions specific to human cells resulting in high levels of intracellular calcium release that activates multiple signaling pathways linked to 12 core cellular signaling pathways were in PC, and each of these pathways were altered by genetic mutations or chromosomal loss in human PC and suggested that, on average, at least 63 genetic alterations that were altered by genetic mutations or chromosomal loss in human PC and suggested that, on average, at least 63 genetic alterations were in human environment causes pancreatic tumor in rodents due to DMBA–DNA adducts (41). To measure its metabolism, DMBA was injected into the pancreas of male rat and oddily a high concentration of metabolites of DMBA such as 5, 6-epoxy-7-hydroxymethyl-12-MBA was observed in pancreatic tissue.

The active metabolites of the CS constituents, once inhaled, react with all classes of biomolecules including carbohydrates, lipids, proteins, and nucleic acids in organs that can be reached directly, such as the lung. In addition, these harmful substances are taken up by the bloodstream and also reach other organs that are not directly in contact with tobacco smoke such as the pancreas, hence causing genetic and signaling abnormalities.

CS-induced genotoxicity

A very important comprehensive genetic analysis was performed by Jones et al. on 24 cancers with sequencing of more than 20 000 protein-coding genes. The study identified core signaling pathways that were altered by genetic mutations or chromosomal loss in human PC and suggested that, on average, at least 63 genetic alterations linked to 12 core cellular signaling pathways were in PC, and each of these pathways were altered in 67–100% of pancreatic tumors (42).

Smoking is associated with cancer of 11 organs (as reported by International Agency for Research on Cancer, through monographs on the evaluation of the carcinogenic risks of chemicals to humans, vol. 83), and mutations in some of these smoking-associated tumors have been identified in both oncogenes and tumor-suppressor genes. Hruban et al. observed significantly high frequency of K-ras mutations in the pancreatic carcinomas harbored from smokers than from non-smokers (43,44). Berger et al. also demonstrated an aggravated PC risk with KRAS mutational activation in cigarette smokers (45). Many investigators have observed a strong “fingerprint” of tobacco carcinogens in the DNA from PC patients. The tobacco carcinogen NNK has been implicated in the pancreatic carcinogenesis by its ability to form DNA adducts, which has been associated with activating KRAS mutations (46). Studies have revealed higher proportion of G to A transition due to the involvement of nitrosamines (47,48). These mechanisms have been extensively studied in laboratory animals.
Table 1. The major components of CS involved in PC pathogenesis

<table>
<thead>
<tr>
<th>Components of CS</th>
<th>Lowest delivery dose/cigarette</th>
<th>Highest delivery dose/cigarette</th>
<th>Description</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>1.0 mg</td>
<td>1.5 mg</td>
<td>An alcaloid (the major component of tobacco smoke)</td>
<td>![Nicotine structure]</td>
</tr>
<tr>
<td>Carbon monoxide (CO)</td>
<td>14 mg</td>
<td>20 mg</td>
<td>Gas, which is highly toxic, released during burning and charring of tobacco</td>
<td>![Carbon monoxide structure]</td>
</tr>
<tr>
<td>Polycyclic aromatic hydrocarbons (PAHs)</td>
<td>0.1 µg</td>
<td>0.2 µg</td>
<td>Highly carcinogenic constituent of tobacco smoke. They are also the known ligands of the aryl hydrocarbon receptor, a cellular xenobiotic sensor responsible for activating the metabolic machinery</td>
<td>![Polycyclic aromatic hydrocarbons structure]</td>
</tr>
<tr>
<td>4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)</td>
<td>0.3 µg</td>
<td>2 µg</td>
<td>Nitrosamine, a highly carcinogenic derivative of nicotine and related compounds, formed by a nitrosation reaction that occurs during the curing and processing of tobacco</td>
<td>![4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) structure]</td>
</tr>
<tr>
<td>Nitrogen oxides</td>
<td>0.1 mg</td>
<td>0.4 mg</td>
<td>Chemical compounds of nitrogen produced as a by-product of combustion of tobacco</td>
<td>![Nitrogen oxides structure]</td>
</tr>
</tbody>
</table>

*The values for the lowest dose/cigarette and highest dose/cigarette are listed for the individual components (dose variation depends upon the brand and source of cigarette).
Cancer research has generated a rich and complex body of knowledge, revealing cancer to be a disease involving dynamic changes in the signaling cascades. At least six major pathways must be disrupted for a transition of a normal cell into a tumor cell (85). These include self-sufficiency in growth signals, insensitivity to antigrowth signals, limitless replicative potential, evasion of apoptosis, sustained angiogenesis, tissue invasion, and metastasis. Despite the strong evidences gleaned from various epidemiological studies and genotoxic analysis for the correlation between PC incidence and cigarette smoking, the exact components responsible and the signal-transduction cascades involved require further examination. A better understanding of molecular events occurring during the CS-mediated initiation/development of PC may improve the management of patients, enabling early diagnosis in high-risk individuals and permitting the development of improved therapeutic approaches targeting specific genes and key molecules of specific pathways. Initiating events alone are not sufficient for the development of cancer. Therefore, understanding whether mixtures or specific compounds found in CS can contribute in promoting the progression phase of cancer is also very important.

Inflammatory pathways

In experimental models, nicotine has been observed to incite an acute inflammatory reaction in the pancreas without the changes that are characteristic of chronic inflammation (86). However, frequent sessions of smoking-induced acute pancreatic inflammation may progress to chronic inflammation and might even cause chronic pancreatitis. Chronic inflammation is characterized by a shift in the cell types at the site of inflammation that can cause lasting and detrimental health effects (87,88). Thus, active and consistent cigarette smoking may eventually lead to chronic intra-pancreatic inflammation and the development of cancer within the inflamed tissue (89,90).

There is evidence that proinflammatory cytokines, chemokines, and their receptors are expressed in pancreatic cells eventually leading to the infiltration of immune cells within inflamed pancreatic tissues (91). The chemokine, CCL5 (C-C motif ligand 5) also known as RANTES (Regulated upon Activation, Normal T-cell Expressed, and Secreted), and one of its receptors, CCR5 (Chemokine (C-C motif) receptor 5), are believed to play a role in antitumor immunity through immune cell recruitment (92). Studies by Goekze et al. showed that CCR5 was significantly expressed by macrophages (91). Duell et al. investigated for the possible interaction between current active smoking and CCR5-32bp deletion. The studies suggested that intact CCR5 may offer pancreatic cells protection from the damaging effects of tobacco smoking (93).

The link between cigarette smoking and pancreatitis is supported by the results of various animal studies (28). Smoke enhances ethanol-induced pancreatic injury and accelerates the development and progression of chronic pancreatitis, which might further lead to the development of PC (94). The article by Wittel et al. sheds further light on this by providing experimental evidence that tobacco smoke leads to focal chronic inflammatory changes in the pancreas, increased turnover of the pancreatic digestive enzymes, and reduction of the antiprotease activity (6). A recent study by Song et al. examined the carcinogenic effects of an aqueous extract of CS (tobacco smoke, TS) and Snus (the Swedish variant of oral smokeless tobacco) in an elastase-IL-1β transgenic mouse model of chronic pancreatitis. The studies showed that both TS- and Snus-treated elastase-IL-1β mice developed significant pancreatic ductal epithelial flattening and severe glandular atrophy. Also, TS-elastase-IL-1β mice had an earlier onset and a greater extent of phenotypic changes, which were associated with upregulation of tumor necrosis factor-α, IL-6, and TGF-β (95).

Inflammation can be oncogenic through multiple molecular mechanisms (Figure 1). Inflamed tissues characteristically generate NO via inducible NO synthase (iNOS) and vascular inflammatory radicals that carry the oncogenic potential of causing direct DNA and protein damage, promoting angiogenesis, inhibiting apoptosis and cellular repair.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Observed polymorphism(s) and associated genotypes/alleles</th>
<th>Polymorphism affecting the susceptibility to PC (Refs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P450 (CYP2A6)</td>
<td>Metabolization of nicotine to cotinine</td>
<td>CYP2A6<em>2, CYP2A6</em>4, CYP2A6<em>9, CYP2A6</em>12</td>
<td>CYP2A6<em>2 and CYP2A6</em>4 genotypes: slow metabolism of nicotine in Caucasian population. No association in Japanese population (54,55,69,70).</td>
</tr>
<tr>
<td>Cytochrome P450 (CYP2A13)</td>
<td>Metabolization of nicotine to cotinine, metabolic activation of nitrosamines</td>
<td>CYP2A13*1/*7 (Arg101Stop)</td>
<td>CYP2A13*1/<em>7 (Arg101Stop): Inactive enzyme, no significant association with PC risk was observed due to lack of carrier of CYP2A13</em>7 among the PC cases (158).</td>
</tr>
<tr>
<td>N-acetyl-transferase 1 (NAT1)</td>
<td>O-acetylation of aromatic and HCA</td>
<td>C1095A (NAT1<em>10, 3'UTR, C97T, Arg33Stop, NAT1</em>19), C190T (Arg45Tnp, NAT1<em>17), G445A (Val49Ile, NAT1</em>11)</td>
<td>NAT1<em>10: Rapid acetylator (4-fold higher risk of PC in female smokers in comparison to never smokers who did not carry the NAT1</em>10 allele (59,82).</td>
</tr>
<tr>
<td>N-acetyl-transferase 2 (NAT2)</td>
<td>O-acetylation of aromatic and HCA</td>
<td>G191A (Arg64Gln), C282T, T341C (Ile114Thr), C481T, G590A (Arg197Gln), A803G (Lys268Arg), G857A (Gly286Thr)</td>
<td>Slow acetylator increases the susceptibility to PC in smokers (82,159).</td>
</tr>
<tr>
<td>Glutathione S-transferase T1 (GSTT1)</td>
<td>Adding reduced glutathione</td>
<td>Wild type/heterozygous del, Null/homozygous del (deletion)</td>
<td>GSTT1-null genotype: Increases the susceptibility to PC in smokers (160).</td>
</tr>
<tr>
<td>Tumor suppressors genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p21</td>
<td>Critical mediator of G1-phase cell cycle arrest</td>
<td>p21 exon 2 (C/A) Ser31Arg</td>
<td>p21 C-to-A SNP: Increases the susceptibility to PC among homozygous wild-type carrier of p21 especially among non-smokers (71).</td>
</tr>
<tr>
<td>Methylene-tetrahydrofolate reductase (MTHFR)</td>
<td>Catalyzes conversion of THF to 5-methyl THF during folate metabolism</td>
<td>MTHFR C677T (exon 4) (Ala/Val) MTHFR A1298C (exon 7) Gln429Ala</td>
<td>MTHFR 677T: Increases susceptibility to PC in ever-smoker MTHFR 1298C: Significantly reduced risk for PC (161,162).</td>
</tr>
<tr>
<td>Somatostatin receptor 5 (SSTR5)</td>
<td>Inhibits proliferation of normal and neoplastic cells</td>
<td>Pro109Ser (CC, CT) Leu48Met (CC, AC) Pro351Leu (TT, TC)</td>
<td>Leu48Met: Increases the risk for PC in combination with smoking (73).</td>
</tr>
<tr>
<td>Proliferator-activated receptor gamma (PPARG)</td>
<td>Regulate the adipose cell differentiation and inhibits the invasive behavior of PC cells in vitro</td>
<td>PPARG exon 2(C/G) Pro12Ala</td>
<td>Pro12Ala: Increases the risk for PC among high-risk smokers in vitamin-administered subjects in comparison to placebo-administered subjects (163).</td>
</tr>
<tr>
<td>Pro-inflammatory genes</td>
<td></td>
<td></td>
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<tr>
<td>Cyclooxygenase-2 (COX-2)</td>
<td>Converts free arachidonic acid into PGH2</td>
<td>Promoter region—765G/C—1195G/A—1290AG</td>
<td>765C: Increased COX2 promoter activity upon cigarette smoking in comparison to −765G allele (164).</td>
</tr>
<tr>
<td>DNA repair genes</td>
<td></td>
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<td></td>
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<tr>
<td>XRCC3</td>
<td>Double-strand break repair gene</td>
<td>XRCC2 exon 3 (G/A) Arg101His, Arg/Arg, Arg/His, His/His</td>
<td>XRCC2 Arg101His: Increased risk of PC among ever-smokers in comparison to never-smokers (79,165).</td>
</tr>
<tr>
<td>XERCC3</td>
<td>Double-strand break repair gene</td>
<td>XRCC3 exon 7 (CT) Thr241Thr, Thr241Met, Met (241) Met, XERCC3.241C/T</td>
<td>Combination of smoking and XERCC3 variant (XERCC3.241* and XERCC3 Thr241Met) associated with increased risk of PC (80)</td>
</tr>
<tr>
<td>XPD</td>
<td>Member of the human transcriptional initiation factor TFIIH with ATP-dependent helicase activity</td>
<td>XPD exon 10 (G/A) Asp312Asn and exon 23 (A/C) Lys203 Gly</td>
<td>Asp312Asn: Reduced risk of PC in ever smokers in comparison to carriers of Asp312/Asp* allele (81,166).</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase 2 (ALDH2)</td>
<td>Catalyzes the chemical transformation of acetaldehyde to carboxyl acid in mitochondria</td>
<td>Glu504 homoyzogotes (ALDH2<em>1/1), Glu504/Lys504 (active enzyme) heterozyogotes (ALDH2</em>1/2 (inactive enzyme)</td>
<td>The OR of smoking patients with ALDH2<em>1/2</em>2 polymorphism was more than 7-fold higher than that of non-smoking patients with the active form of ALDH2 (84).</td>
</tr>
<tr>
<td>ABO</td>
<td>A, B, and O glycosyl-transferases transfer GalNac, Gal, and no sugar residue, respectively, to H histo-blood group antigen expressed by red blood cells, endothelial cells, and epithelial cells</td>
<td>Genotype derived from ABO, O, A, B, AB</td>
<td>In a joint model with smoking, current smokers with non-O blood type had an adjusted OR of 2.68 (95% CI, 2.03–3.54) compared with non-smokers of blood type O (167, 168).</td>
</tr>
</tbody>
</table>

CS, Cigarette smoke; THF, 5,6,7,8-tetrahydrofolate; PGH2, prostaglandin H2; ERCC2, excision repair cross-complementing repair deficiency; GalNac, N-acetyl-D-galactosamine; Gal, D-galactose; OR, odd ratio; PC, pancreatic cancer.

*The variant alleles of the gene.
*aNo amino acid change due to the alteration in the wobble nucleotide.
Fig. 1. Interconnected signaling cascades for CS-mediated pancreatic injury. CS produces various components, including nicotine, nitrosamines (NNK), and PAH that cause the pancreatic injury. (A) Both nicotine and NNK are selective agonists for \( \alpha_7 \)nAChR, which upon binding causes the cell depolarization and lead to an enhanced influx of cations including calcium ions through voltage-dependent channels. Calcium ions mediate the nicotine entry into the pancreatic cells inducing altered exocrine pancreatic secretions associated with pancreatic injury. Enhanced calcium influx triggers the release and synthesis of excitatory neurotransmitters (adrenalin and noradrenalin) that activates the adenyl cyclase downstream of G\( \alpha_s \)-coupled receptors. GABA normally balances these effects by inhibiting adenyl cyclase downstream of the G\( \alpha_i \)-coupled GABA receptor through a \( \alpha_4 \beta_2 \)nAChR-dependent mechanism. Both nicotine and NNK desensitizes the \( \alpha_4 \beta_2 \)nAChR, hence inhibiting the release and synthesis of GABA and virtually shutting down all inhibitory GABA signaling. (B) NNK and nicotine leads to the stimulation of the cell proliferation, migration, and angiogenesis either by the direct activation of the \( \beta \)-adrenoreceptor-mediated signaling via cAMP/PKA/p-CREB or indirectly by regulating the release and synthesis of EGF, VEGF, or arachidonic acid and PKA-mediated transactivation of EGFR. This leads to the nicotine/NNK-mediated indirect induction of the Ras-Raf-MEK ERK pathway. (C) MAPK pathway and nicotine/\( \alpha_7 \)nAChR-mediated JAK2/STAT3 pathway further stimulates the upregulation of the genes such as MYC, CYP, KRAS, FOS, and JUN in the nucleus, inhibiting the apoptosis of the cells and causing cells to proliferate and grow. (D) Nicotine elicits a prometastatic response in pancreatic cells by stimulation of osteopontin production through \( \alpha_7 \) nAChR-dependent mechanism. Nicotine can also induce through EGFR/AKT/NFkB-mediated pathways, changes in gene expression consistent with epithelial to mesenchymal transition (EMT). PAH also contributes to the cell metastasis by inducing the release of arachidonic acid and inhibiting the gap junctional intercellular communication. (E) The carcinogens NNK and PAH can be metabolically activated to intermediates that react with DNA, forming DNA adducts resulting in the mutation of crucial genes such as KRAS gene. If the DNA adducts are repaired by cellular repair enzymes, DNA is returned to its normal undamaged state. Nicotine is able to enhance the production of ROS, which plays an important role in inhibiting the DNA repair mechanism of the cell. Also, increase in the ROS has been directly linked to the lipid peroxidation leading to the pancreatic injury. Cells with damaged or mutated DNA can be removed by apoptosis, which is inhibited by nicotine as it regulates EGFR leading to activation of the serine threonine kinase, AKT, and other factors such as NFkB and Bcl2, causing decreased apoptosis. The red arrows merge the various pathways defining their specific role in modulating the functional properties of PC cells such as proliferation, angiogenesis, migration, EMT, and reduced apoptosis. AA, aromatic amines; AKT, cAMP; Cyclic adenosine monophosphate; COX-2, cyclooxygenase-2; CREB, “http://en.wikipedia.org/wiki/Cyclic_adenosine_monophosphate” \( \alpha \) Cyclic adenosine monophosphate” cAMP response element-binding; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; GABA, \( \gamma \)-aminobutyric acid; GPCRs, G-protein coupled receptors; JAK2, Janus kinase 2; MAPK, mitogen activated protein kinase; nAChR, nicotinic acetylcholine receptor; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol:OPN, osteopontin; PAH’s, polycyclic aromatic hydrocarbons; STAT3, signal transducer and activator of transcription 3; TGF-\( \beta \), transforming growth factor- \( \beta \); VEGF, Vascular endothelial growth factor.
functions (96). Various studies have shown an increased expression of iNOS and increased protein tyrosine nitration during the development and progression of PC (97,98). Also, an increased expression of iNOS and CS-induced pathogenesis in various cancers are significantly correlated (99,100). A recent study has shown that nicotine is able to enhance oxidative stress and CCK-stimulated anlyase release in AR42J pancreatic acinar cells via the XOD pathway and that these events trigger pathophysiological changes in the pancreas (56). Studies by Tai et al. in a pluripotent rat liver epithelial stem cell line indicate that a distinct structural configuration of the methylenethreanones could be a potential etiological agent contributing to the epigenetic events of PC, such as an induced release of arachidonic acid (49). Cyclooxygenase enzymes catalyze a critical step in the conversion of arachidonic acid to prostaglandins, which are important mediators of acute and chronic inflammation (101). A recent study by Lazar et al. showed that cigarette smoking contribute to PC inflammation by inducing monocyte chemoattractant protein-1 and provided evidence for osteopontin (OPN) being a downstream effector of nicotine, capable of mediating these pro-inflammatory effects in PC cells (102).

Nicotinic acetylcholine receptor-mediated pathways

The nicotinic acetylcholine receptor (nAChR), first characterized in 1970 as a membrane spanning protein and a ligand-gated ion channel, is known to be localized both in the neuro-muscular junctions as well as in a large variety of non-neuronal cells where they serve diverse functions (103). A very recent study by Sullivan et al. demonstrated the expression and regulation of nAChRs in PC (104). These receptors are usually activated by the neurotransmitter acetylcholine, but significant evidence exists in the literature suggesting that they are the primary site of nicotine action in the central nervous system (105,106). Several laboratories have demonstrated that prolonged exposure of mice and rats to nicotine and other nicotinic agonists produce a significant increase in the number of agonist binding sites in many brain regions (107,108). The binding of nicotine to nAChR causes a conformational change that either opens or closes the receptor ion channels, thereby changing the receptors functional state. If the nicotine remains bound for a longer time, a second conformational change occurs termed as desensitization in which the channel is closed. Each nAChR is made up of five subunits, arranged symmetrically around a central pore. These subunits belong to a multigene family and the different combinations of subunits results in a great functional diversity of the receptors.

The nAChRs have been demonstrated to play a key role in smoking-induced pathogenesis in various types of human cancers. Recent studies have shown that treatment of several PC and colon cancer cell lines with nicotine induces proliferation and metastasis in a receptor-dependent manner and that α7 is the main nAChR subunit that mediates this effect (109–111). Studies by Chen et al. have shown that nicotine causes α7nAChRs-mediated rapid activation of STAT3 and Erk1/2 leading to cell proliferation in human bladder cancer cells (112). Our recent studies have also demonstrated that α7nAChR modulate JAK2/STAT3 pathway in PC both in vitro and in vivo (unpublished data). The α7nAChR, being a pivotal subunit involved for the nicotine-mediated effects, has received profound interest as a valuable molecular target for cancer therapeutics (113,114). Recently, multiple single-nucleotide polymorphisms were identified in the gene clusters encoding for α3, α5, and β4nAChR subunits, which are associated with an increased risk for nicotine dependence and lung cancer (115). Also, studies by Dasgupta et al. have shown antiapoptotic effects of nicotine in non-small cell lung cancer (NSCLC) mediated by α3-containing nAChRs that imparts resistance against various chemotherapeutic agents (116).

Various in vitro and in vivo animal studies show that homo-pentameric α7nAChR-specific inhibitors, such as methyllycaconitine and α-bungarotoxin, can attenuate nicotine-induced proliferative, angiogenic, and metastatic effects on lung, colon, and bladder cancer cells. Studies have demonstrated that α7nAChR is critical in breast cancer and that several α9nAChR-specific antagonists (such as α-ImI, α-ImI, Vc1.1, RglA, and It14a) produce an analgesic effect in many in vivo studies. Also, for cancer therapy, natural compounds such as garcinol have been found to inhibit the α9-nACh signaling pathway, thereby blocking the nicotine and estrogen-induced breast cancer cell proliferation (117). Interestingly, it has also been shown in PC that nicotine-mediated upregulation of α7nAChRs and desensitization of α4β2nAChR in smokers shifts the balance in favor of α7nAChR signaling with strong stimulatory effects on cancer cells. On the other hand, γ-aminobutyric acid (GABA) balances these effects by inhibiting adenylyl cyclase downstream of the Gs-coupled GABA receptor (GABAR) through a α4β2nAChR-dependent mechanism (118,119).

Recently, studies have reported that, similar to nicotine, NNK may also modulate the nAChRs, α7nAChR sensitization, and desensitization of the α4nAChR (120), causing increased MAPK/CREB signaling and a concomitant increase in stress neurotransmitters and decrease in inhibitory neurotransmitters (GABA) (118). Both nicotine and NNK are selective agonists for α7nAChR, which upon binding causes the cell depolarization and leads to an enhanced influx of cations including calcium ions through voltage-dependent channels. Enhanced calcium influx triggers the release and synthesis of excitatory neurotransmitters, which activates the adenylyl cyclase downstream of Gs-coupled receptors. These findings suggest that nicotine and NNK-induced alterations in regulatory nAChRs may contribute to the development of smoking-associated PC by disturbing the balance between cancer stimulating and inhibiting neurotransmitters (118,121) (Figure 1).

Other intracellular pathways

Nicotine induces cell proliferation, invasion, and epithelial–mesenchymal transition (EMT) in a variety of human cancer cell lines (110). Studies by Chipitsyna et al. showed that nicotine significantly upregulates the OPN messenger RNA (mRNA) and protein secretion in PC and further activates MAPK signaling pathways that induce cell survival, proliferation, invasion, and metastasis (122). A recent study has shown that a pro-metastatic splice variant of OPN, OPnc, confers a migratory phenotype in PC (104). Later, the same group showed that matrix metalloproteinase-9 (MMP-9) mRNA levels were significantly higher in smokers compared with non-smokers in invasive PC lesions. Also, the vascular endothelial growth factor (VEGF) protein co-localized with MMP-9 and OPN in the malignant ducts correlated with their higher levels in invasive PC lesions in smokers. Hence, this study proved that cigarette-smoking and nicotine-induced OPN plays a central role in mediating PC metastasis through the induction of MMP-9 and VEGF (123). Nicotine, through EGFR/AKT/ NFκB-mediated pathways, can also induce changes in gene expression consistent with EMT, characterized by a reduction of epithelial markers like E-cadherin expression and concomitant increase in levels of mesenchymal proteins like vimentin and fibronectin (110). Nicotine increases the cellular proliferation of the AR42J PC cells by activating pERK-1/2 signaling. This process is also known to occur through an independent pathway, that is, the stimulation of the G-protein coupled receptor (GPCR)-mediated secretory response of nicotine (124). Also, studies by Dasgupta et al. showed that nicotine induces cell proliferation by activation of the Src and Rb-Raf-1 pathways upon stimulation of GPCRs. They illustrated that Src forms a complex with the scaffolding protein β-arrestin-1 and gets recruited to the nAChRs. The mitogenic effects of nicotine were mediated via the α7nAChR subunit and resulted in enhanced recruitment of E2F1 and Raf-1 on proliferative promoters (cdc25A and cdc6) and dissociation of Rb from these promoters, in NSCLC cell lines and human lung tumors, correlating with transcriptional activation and S-phase entry (125).

Studies have shown that NNK is also a selective agonist for β-adrenergic receptor and that it stimulates the MAP kinase pathway by activation of Src tyrosine kinase (126). Binding of NNK to the β-adrenergic receptor may also regulate the release and synthesis of endothelial growth factor (EGF), VEGF, or arachidonic
acid that acts as a second messenger, leading to the formation of mitogenic metabolites that stimulate DNA synthesis and cell proliferation (126). Moreover, NNK and nicotine indirectly lead to the stimulation of the cell proliferation, migration, and angiogenesis through protein kinase A (PKA)-mediated transactivation of EGFR. The NNK-β-adrenergic receptor-mediated transactivation of the EGFR and phosphorylation of Erk1/2 in immortalized human pancreatic ductal epithelial cells contribute to the development of tobacco-mediated pancreatic carcinogenesis (127). This leads to the nicotine/NNK-mediated indirect induction of the Ras-Raf-MEK ERK pathway. MAPK-mediated JAK2/STAT3 pathway further stimulates the upregulation of the genes such as MYC, CYP, KRAS, FOS, JUN, etc., in the nucleus, inhibiting the apoptosis of the cells and causing cells to proliferate and grow (Figure 1).

The carcinogens NNK and PAH are metabolically activated to intermediates that react with DNA-forming DNA adducts. If the DNA adducts are repaired by cellular repair enzymes, DNA is returned to its normal undamaged state. Cells with damaged or mutated DNA can be removed by apoptosis, which is blocked by nicotine-mediated effects. Nicotine induces oxidative stress and activates NFκB leading to decrease in apoptosis in colon cancer cells (128). Several studies have shown that the NFκB pathway confers an antiapoptotic trait to the PC cells (129–131). Nicotine regulates EGFR leading to activation of the serine threonine kinase, AKT, and other factors such as NFκB, Bcl2, and inactivates XIAP and survivin, thereby causing decreased apoptosis. PAH also contributes to the cell metastasis by inducing the release of arachidonic acid and inhibiting the gap junctional intercellular communication. Experimental evidence suggest that upon implantation of a PAH component namely DMBA, into the area of the pancreatic head of mice, the mice quickly developed pancreatic duct alterations in a notch signaling-dependent manner (132). Thus, the individual components of CS have adverse effects on the pancreas by altering various processes such as increasing the proliferation rate, angiogenesis,
migration potential, and EMT phenotype as well as decreasing the apoptosis of the pancreatic cells (Figure 1).

Future implications and clinical perspectives

PC is a silent killer because it remains undiagnosed at an early stage due to lack of symptoms. Cigarette smoking is the most well-established environmental factor that has been associated with high risk of PC. To quit or avoid smoking remains the best prevention from this malignancy, but apart from that, there is an urgent need for the design of therapies that are more effective than the current regimens. For this, a deeper insight into the genetic alterations and the molecular mechanisms that contribute to the aggressive nature of PC is highly essential. This aforementioned review hints that the interplay between the susceptible polymorphisms, genotoxicity, and altered signaling pathways caused by the various constituents contained in CS contributes to the CS-induced pathogenesis. This knowledge will be helpful to target the specific key molecules and key genes that are altered in the CS-mediated regulatory pathways, hence inhibiting the cancer promoting events (Figure 2). Overall, our review has attempted for better understanding of the smoke-induced PC, which must overcome the limitations in PC therapeutics and provide a better survival benefit.

One of the evolving therapeutic strategies is the usage of $\alpha_7$nAChR as a molecular target for combating cancers (113,114). Previous studies involving lung adenocarcinoma cell lines have shown that the antagonists of $\alpha_7$nAChR, $\alpha$-bungarotoxin or methyllycaconitine, can attenuate the proliferative effects of nicotine (133–135). The antagonists of $\alpha_7$nAChR might be used with caution because these receptors also regulate many vital cellular and organelle functions, such as inflammatory reactions and respiratory and cardiovascular functions, thereby leading to mental and psychological problems. Future studies involving the design of nAChR antagonists with improved selectivity might identify novel strategies for the treatment of tobacco-related PC.

In smokers with PC, the balance shifts in favor of the $\alpha_7$nAChR-mediated upregulation of stimulatory neurotransmitter, imparting strong stimulatory effects on cancer cells, with concurrent decrease in the inhibitory neurotransmitter, GABA (118). GABA or baclofen-mediated stimulation of the GABA-B-R inhibiting the isoproterenol-induced cyclic adenosine 3',5'-monophosphate (cAMP) signaling suggests GABA-B-R as a potential target for the treatment and prevention of PC (136). Blockers of voltage-gated Ca$^{2+}$ channels might be yet another alternative for desensitization of the hyperactive $\alpha_7$nAChR.

Experimental evidence has shown that the treatment of the adenocarcinoma cells with the $\beta$-adrenergic receptor blockers, such as propranolol, atenolol, or ICI-118551, reduces the mitogenic response to NNK. Studies by Hussein et al. have shown that propranolol prevents development of PC in hamsters by reversing the NNK-mediated regulatory pathways, hence inhibiting the cancer promoting events (Figure 2). Overall, our review has attempted for better understanding of the smoke-induced PC, which must overcome the limitations in PC therapeutics and provide a better survival benefit.

We foresee PC research and a therapeutic outlook toward it developing into much understandable form. This can be achieved thorough intensive investigation and the future challenge of chemotherapy against PC relies on the identification of molecular and genetic markers that are predictive of response. It is to be expected that combinatorial or sequential treatment with different drugs might prove to be more promising with a better survival rate. However, the current knowledge of the side effects for various inhibitors is limited and requires further investigation.

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Smoking-mediated pathogenesis of pancreatic cancer


