

Published in final edited form as:

*Int J Oncol.* 2012 July ; 41(1): 5–14. doi:10.3892/ijo.2012.1414.

## The pathobiological impact of cigarette smoke on pancreatic cancer development (Review)

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### Abstract

Despite extensive efforts, pancreatic cancer remains incurable. Most risk factors, such as genetic disposition, metabolic diseases or chronic pancreatitis cannot be influenced. By contrast, cigarette smoking, an important risk factor for pancreatic cancer, can be controlled. Despite the epidemiological evidence of the detrimental effects of cigarette smoking with regard to pancreatic cancer development and its unique property of being influenceable, our understanding of cigarette smoke-induced pancreatic carcinogenesis is limited. Current data on cigarette smoke-induced pancreatic carcinogenesis indicate multifactorial events that are triggered by nicotine, which is the major pharmacologically active constituent of tobacco smoke. In addition to nicotine, a vast number of carcinogens have the potential to reach the pancreatic gland, where they are metabolized, in some instances to even more toxic compounds. These metabolic events are not restricted to pancreatic ductal cells. Several studies show that acinar cells are also greatly affected. Furthermore, pancreatic cancer progenitor cells do not only derive from the ductal epithelial lineage, but also from acinar cells. This sheds new light on cigarette smoke-induced acinar cell damage. On this background, our objective is to outline a multifactorial model of tobacco smoke-induced pancreatic carcinogenesis.

### Keywords

pancreatic cancer; nicotine; nnk; cigarette smoke; carcinogenesis; carcinogens; chronic pancreatitis

### 1. Introduction

Pancreatic cancer is the fourth leading cause of death in the United States and the sixth leading cause in Europe affecting between 7 and 9 per 100,000 in men and between 4.5 and 6 per 100,000 in women (1). The cumulative mortality or lifetime risk of dying from pancreatic cancer is approximately 1.3% (2,3). However, the worldwide mortality/incidence ratio of pancreatic cancer is 98% indicating that almost all patients diagnosed with pancreatic cancer die of this disease (1). Pancreatic cancer is a rapidly progressive, therapy-resistant disease with a 1-year-survival rate of 25% and a 5-year-survival rate of less than

5% (4). Thus, primary prevention plays a pivotal role in disease management. However, only few risk factors are clearly established (2,5).

Late-onset diabetes correlates with a relative risk for pancreatic cancer of 2.1 for patients with diabetes persisting at least for 1 year (6,7). For this estimation patients with shortly diagnosed diabetes must be excluded because 40% of pancreatic cancer patients are diabetic, and there is an increased hazard ratio for developing pancreatic cancer in patients with newly diagnosed diabetes (8–11). The high coincidence of diabetes and pancreatic cancer may provide for new screening strategies. In fact, diagnosing an altered glucagon/insulin ratio may be the first step in identifying pancreatic cancer patients (12,13). In any case, late-onset and early-onset diabetes persisting for more than 5 years prior to cancer diagnosis are risk factors for developing pancreatic cancer (14–17).

Similar to diabetes, obesity has been reported to increase the risk of developing pancreatic cancer (18). Most case-control studies found an increased risk in men and women exceeding a body mass index of 30 (19–21). However, in some cohorts obesity showed this correlation only in men (22), while in other cohorts there was no significant association in either group (23). Therefore, a disturbance in glucose and/or lipid metabolism might be responsible for the association between obesity and pancreatic cancer. This is corroborated by the observation that patients treated with statins to lower cholesterol levels or with metformin to lower pathological glucose levels show a significantly decreased risk for developing pancreatic cancer (24). However, in the case of metformin, the reduction of pancreatic cancer risk does not seem to be related to the normalization of blood glucose levels because this reduction was absent in patients treated with insulin or insulin secretagogues (25).

A clearly identified, strong risk-factor for pancreatic cancer is chronic pancreatitis (26–30). The cumulative risk of chronic hereditary pancreatitis patients to develop pancreatic cancer by age 70 has been assessed at almost 40%. Furthermore, in a multinational study including 2,016 patients, the standardized incidence ratio was roughly 16.5 (28). These data point at a role of chronic inflammation in the initiation and progression of pancreatic cancer (31). However, chronic pancreatitis accounts for only 3–4 % of the pancreatic cancer cases observed (32).

Between 5 and 10% of pancreatic cancer cases can be accounted to hereditary factors. The risk depends on the number of affected first degree relatives with a hazard ratio of 32.0 when 3 family members are affected and 6.4 with 2 affected family members (33). Between 6 and 19% of familial pancreatic cancer families have mutations in the BRCA2 gene (34–36), but other germline mutations such as CDKN2A, PRSS1, STK11, or MMR genes have also been found (37). No single gene defect has been identified to date except for one family, where a mutation in the palladin gene that is only found in affected family members causes a proline to serine amino acid change (38). These results may be the first steps in identifying gene mutations responsible for familial pancreatic cancer (39).

Remarkably, the most frequent risk factor associated with pancreatic cancer development is cigarette smoking (40–43). Cigarette smoke is graded as a class 1 carcinogen by the World Health Organization (44). It is related to 25% of pancreatic cancer cases, and the risk increases with duration and amount smoked (32). Quitting smoking reduces the excessive risk for pancreatic cancer within 5–10 years (21) with an initial reduction of pancreatic cancer risk of 50% within the first 2 years (42). Smoking also elevates the risk of patients with familial predisposition to pancreatic cancer, and in patients with hereditary pancreatitis, the onset of pancreatic cancer occurs 10–20 years earlier in smokers than in non-smokers (45,46). Furthermore, cigarette smoking promotes the development of chronic pancreatitis in a dose-dependent manner (47,48), and during chronic pancreatitis smoking further increases

disease severity and induces pancreatic calcifications (49,50). Therefore, cigarette smoking is the only amenable risk factor in pancreatic carcinogenesis, and quitting smoking both prevents the development of pancreatic cancer as well as chronic pancreatitis (51).

For several reasons, the experimental work aiming at understanding the mechanisms behind cigarette smoke-mediated pancreatic damage lags behind. Cigarette smoke consists of a mixture of side stream smoke and mainstream smoke containing a blend of chemicals out of which 4,000 have been identified to date. Of the so far identified substances, more than 50 compounds act, or are likely to act as carcinogens (52). Due to this complexity, most studies investigate either the action of nicotine or tobacco-derived carcinogens. Thus causal relationships between individual compounds at high doses and pancreatic carcinogenesis, but not cigarette smoke inhalation have been established. However, most chemicals contained in cigarette smoke are likely to interact with each other or synergize in their detrimental action. Unfortunately, only very few studies reflect this pathophysiological situation. In this review, we discuss recent experimental advances in the evolving field of cigarette smoke-induced pancreatic carcinogenesis. Furthermore, we link the evidence obtained in *in vivo* and *in vitro* experiments to observations in humans and discuss possible pathomechanisms involved in cigarette smoke-mediated pancreatic carcinogenesis.

## 2. Morphological alterations

Smoking leads to distinct histomorphological alterations of the pancreas. In autopsy specimens, several investigators have reported alterations described as focal acinar cell hyperplasia or dysplasia (53). These hyperplastic acinar nodules as depicted in Fig. 1A are well demarcated from the surrounding normal acinar cells, measure 300–1,000  $\mu\text{m}$ , and are randomly distributed throughout the pancreas. Most of the lesions show only mild nuclear atypia and are therefore referred to as hyperplasia. However, severe dysplastic changes can be observed as well, but only in specimens where ductal hyperplasia or PanINs were also detected (54). It has been suggested that these lesions might be precursors of acinar cell carcinomas. However, there is a clear discrepancy between the number of observed lesions and the extremely rare incidence of acinar cell carcinoma.

In addition to cellular hyperplasia, two types of focal atypical acinar cell lesions associated with cigarette smoking have been described and differentiated by their staining properties in hematoxylin and eosin (H&E) staining, one of which shows predominantly acidophilic cells, the other basophilic cells (55). Electron microscopy reveals dilated rough endoplasmic reticulum and few zymogen granules. Interestingly, the proliferative index was lower in these lesions than in adjacent normal pancreatic acini (56,57). These acinar cell lesions were morphologically close to acinar cell nodules observed in rats treated with carcinogenic chemicals. However, their role remains speculative (58).

Cigarette smoking also induces the occurrence of atypical nuclei in pancreatic acinar and ductal cells, which were defined by variation in shape, size, and staining character. In pancreatic ducts, heavy smoking leads to an increase of atypical nuclei from 5.4 in non-smokers to 74.9% (59). Similarly, smoking 40 cigarettes per day induces an increase in atypical nuclei from 1.8 to 69.1%. However, in contrast to acinar cell alterations, the increase in smoke related ductal lesions appears to correlate with age rather than with smoking history (60).

Similar to humans, rodents treated with environmental tobacco smoke also develop acinar cell damage (61). In this experiment, animals were exposed to environmental tobacco smoke for 70 min twice a day over a period of 3 months in two different doses, 100  $\text{mg}/\text{m}^3$  total suspended particles (TSP) and 160  $\text{mg}/\text{m}^3$  TSP. In 58% of the animals treated with the higher dose of cigarette smoke, histomorphologic pancreatic damage was detected, while

animals subjected to the lower dose treatment lacked these lesions. The lesions were typically confined by sharp borders, which usually correlated with the anatomical lobular structures. They were also characterized by focal increases in extracellular matrices with a subsequent decrease in acinar cell structures (Fig. 1B). However, the occurrence of these lesions was low, and less than 5% of the pancreatic tissue was affected. Further examinations indicated that an inflammatory reaction was present and that the smoke-induced acinar cell damage may have induced chemotraction, immune responses, and tissue reorganization (62–64).

### 3. Enzyme regulation

There is a clear association of cigarette smoke exposure and alterations of the pancreatic acinar cell compartment. Its pathomechanism and how acinar cell damage may contribute to the development of pancreatic ductal adenocarcinoma remain unclear. One probable causal factor is the ratio disturbance of pancreatic digestive enzyme expression and the expression of their protective, anti-proteolytic counterparts (65). Moreover, the impact of cigarette smoke is not limited to gene expression. Altered synthesis and secretion patterns are induced as well. Thus, trypsin and chymotrypsin activity of pancreatic cell lysates of animals exposed to cigarette smoke are markedly decreased (61).

Similar to the aforementioned experimental observations, smoking induces alterations of pancreatic enzyme synthesis and secretion in humans (66,67). Current data must be interpreted carefully due to the small number of test subjects and distinct differences in the tests used. Still, it appears likely that cigarette smoking interferes with the synthesis and secretion of pancreatic digestive enzymes in humans. Upon pancreatic stimulation, increased serum concentrations of total amylase, pancreatic isoamylase, cationic trypsinogen, and pancreatic secretory trypsin inhibitor proteins were measured in cigarette smokers (68). Smokers more frequently showed a serum increase of immunoreactive cationic trypsinogen to secretin stimulation of more than 100% (69). However, a high dose of secretin was required to achieve a low response in blood enzyme concentration (70).

Additionally, secretin-independent increased basal levels of digestive enzymes in smokers have been reported. Basal serum amylase and pancreatic elastase concentrations were found to be higher in smokers than in non-smokers, and a single injection of secretin to cigarette smokers significantly increased serum amylase, trypsinogen and elastase without an observed increment in non-smokers (71). Similar results were found regarding serum lipase activity (72). These studies, together with the observation of altered gastrointestinal motility (67) and endocrine alterations (73,74) indicate that cigarette smoking induces functional alterations of pancreatic enzyme secretion that are currently not fully understood.

### 4. Nicotine and pancreatic regulation

One pathophysiological explanation for such altered secretion patterns could be the pharmacologic action of nicotine on the cholinergic system (75). In humans, pancreatic enzyme secretion is both under neurohormonal and CCK-receptor mediated control (76,77). In fact, there is a 10-fold higher level of m3-muscarinic-receptor-mRNA than CCK-receptor-mRNA. This indicates that the neurohormonal control of enzyme secretion plays a bigger role than acinar cell stimulation with CCK (78).

Several studies examined the action of nicotine on the pancreatic gland. Biologically active nicotine and its inactive metabolites cotinine and norcotinine can be detected in the pancreas. *In vitro* pancreatic stimulation even increases acinar cell uptake of nicotine (61,79,80). The pathophysiological action of nicotine on the pancreas has been reviewed recently (79,81,82). In rodents, nicotine induces a widespread action on acinar cells. After

adding nicotine to the drinking water of rats in concentrations ranging from 50–200 mg/l over a period of 16 weeks, acinar cell amylase release upon CCK-8 stimulation is decreased (83). This effect appears to occur *in vivo* as well where pancreatic secretion is reduced while transcription of digestive enzymes remains unaltered, leading to increased pancreatic content of digestive enzymes upon nicotine treatment (84). Furthermore, nicotine induces morphological and functional cell lesions regardless of the application method (83,85,86).

Interestingly, nicotine also increases acinar cell proliferation in AR42J cells, an immortal cell line-derived from acinar cells with retained secretory capacity (81). This proliferative effect was dependent on p-ERK 1/2 activation, but neither ERK activation nor cell proliferation was truncated by the nAChR antagonist mecamylamine, indicating this effect being independent of nicotinic acetylcholine receptors (nAChR) (87). In freshly isolated pancreatic acini, a similar proliferative response upon nicotine treatment was observed. However, in contrast to AR42J cells, this response was mediated by interaction of nicotine with nAChRs and b-ARs (88).

While the data on nicotinic action in pancreatic cells are limited, the effect of nicotine has been investigated in greater detail in other types of tissue. In these studies, nicotine exerted an effect on a variety of human cell types such as endothelial cells and even tumor cells (89). There is a plethora of experimental evidence demonstrating various effects of nicotine, such as an increased survival of tumor cells (90), an increased rate of tumor metastasis (91,92), a decrease in patient survival (93–95), and a reduced response to chemotherapy (96–100).

One of the nicotine-related effects that was studied in detail, is the angiogenic property of nicotine which is mediated at least in part by nAChRs (101–103). The endothelial cell tube formation assay using human umbilical vein cells (HUVEC) showed that nicotine stimulated the release of basic fibroblast growth factor (b-FGF) in HUVECS (104). In accordance, nicotine treatment increased human endothelial cell tube formation in a dose-dependent manner. Similarly, nicotine stimulated angiogenesis through b-FGF in the chick chorioallantoic membrane (CAM) tumor implant model. These effects were completely blocked by  $\alpha v \beta 3$  integrin antibodies and by the blockade of the non-neuronal nicotine receptor, indicating that FGF and nACh-receptors are involved in nicotine mediated angiogenesis (103).

In cervical cancer cell lines, nicotine induces increased cell proliferation and EGFR over-expression (105). Furthermore, after serum starvation, long-term exposure of mouse epithelial lung cells to nicotine disrupted the cell cycle restriction machinery in a nAChR-dependent manner through *ras* activation and subsequent increase in Raf/MAP kinase activity, which further induced a significant increase in cyclin D1 promoter activity (106). Intriguingly, significant differences were observed between short- and long-term exposure of immortal cell lines to nicotine. While brief nicotine application induces protein kinase C and phosphoinositide 3-OH-kinase activation, long-term exposure influenced *ras* activation and ERK 1/2 expression (107). In parallel to increasing proliferation, nicotine also reduces the rate of apoptosis by NF- $\kappa$ B up-regulation (108). These observations were reproduced *in vivo* where nicotine treatment stimulated the growth of pancreatic xenograft tumors (109). Taking the above-mentioned experimental evidence into consideration, it appears without a doubt that nicotine alters neurotransmitter levels in pancreatic cancer, exerts direct proliferative effects on tumor cells and increases neo-angiogenesis (110).

## 5. Cigarette smoke-related carcinogens and the pancreatic gland

In cigarette smoke, many substances have been detected that directly induce malignant tumors through DNA interference. Several of these compounds have been proven to reach



the pancreas and are likely to influence carcinogenesis. In a study examining the carcinogenic burden of the pancreatic juice of 18 smokers and 9 non-smokers, the mean level of the nicotine derivative 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a nitrosamine (111), smoking significantly elevated the NNK concentration in pancreatic juice. Another nicotine derivative, N'-nitrosornicotine (NNN), was found in two out of 18 samples of the pancreatic juice of smokers, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) was detected more frequently as well (112).

In rodents several tobacco-derived carcinogens have been found to induce pancreatic tumors of ductal phenotype (Table I). NNK and NNAL, a carbonyl reduction metabolite of NNK, induces pancreatic tumors in mice, rats and in hamsters. In 10% of F344 rats, life-time treatment with 1 ppm NNK in the drinking water induced pancreatic tumors of ductal phenotype. Strikingly, lung tumors were observed in 25% (113). The tumorigenicity was diet-dependent and increased in animals with high fat intake. However, F344 rats tend to develop spontaneous pancreatic tumors with increasing age independent of their diet which indicates that the diet accelerated tumor formation (114).

The formation of DNA adducts and their subsequent interference with gene function plays a key role in carcinogenesis. Cigarette smoking increases these DNA adducts in the human pancreas. In a <sup>32</sup>P post-labeling analysis of pancreatic DNA of smokers 102±21 DNA adducts/10<sup>8</sup> nucleotides were detected. This contrasts sharply with the <sup>32</sup>P-post-labeling analysis of DNA-derived from non-smoking control subjects which reveals only 13±1 DNA adducts/10<sup>8</sup> nucleotides (115). The chemical nature of these adducts found in human smokers is heterogeneous, and there is no evidence that one specific DNA adduct is induced by cigarette smoking. One possible explanation may be that nitrosamines are metabolized to reactive electrophils in order to react with DNA, and this metabolic process may be influenced by individual and organ specific factors (116,117). In laboratory animals, carcinogen metabolism has been extensively studied, and it has been found that the hydroxylation of NNK by cytochrome P450 isoenzymes induces DNA methylation (118). Additional experiments showed that unspecific inhibition of NNK hydroxylation through isothiocyanate inhibits the generation of NNAL and decreases its carcinogenic potential (119,120). Whether these mechanisms are solely responsible for the tumor formation is a matter of debate, and several studies propose additional mechanisms of NNK action. The observation that DNA methylation patterns in rats, mice, and hamsters did not correlate with the organotropy of NNK mediated carcinogenesis corroborates this view (121). A second observation supporting additional factors to the P450 catalysed  $\alpha$  hydroxylation in NNK-induced tumorigenesis is the influence of ethanol treatment on NNK mediated pancreatic tumor development. *In vitro*, human and hamster ductal epithelial cells can be transformed to cells with malignant properties by NNK alone. Ethanol treatment enhances the metabolism and carcinogenicity of NNK (122,123). Strangely, these observations cannot be reproduced *in vivo*. In a hamster model where NNK is administered s.c. to pregnant female hamsters together with oral ethanol treatment, pancreatic tumors are induced only when ethanol is administered, while NNK treatment alone mostly induces lung tumors (124–126). This shift in organotropy cannot be explained by the expression pattern of cytochrome P450 isoenzymes or the rate of NNK metabolism. Nor was the concentration of metabolites altered by ethanol treatment. This indicates that in contrast to the *in vitro* experiments with pancreatic ductal cells, alterations in enzyme activity induced by ethanol have less impact on the development of NNK-induced pancreatic tumors *in vivo* (127).

Several factors that influence the carcinogenic action of NNK in a P450-independent fashion have been described to date. The metabolism of nitrosamines may be affected by anatomic factors (128), NAD<sup>+</sup> glycohydrolase-catalysed transglycosylation reactions in pancreatic microsomes (129), extensive tissue retention of the (S)-NNAL enantiomer

followed by sequestration and re-oxidation to NNK in the target tissue (130) or COX2 mediated inflammatory events (131,132). Another likely explanation for the discrepancy between NNK-dependent adduct formation and the induction of malignancies could be the interference of the nitrosamines with regulatory pathways, for neither NNK itself nor its metabolites are able to bind and activate nicotinic acetyl choline receptors (nAChR) and beta adrenergic receptors (b-AR).

When NNK acts as a  $\beta$ 1- and  $\beta$ 2-adrenergic receptor agonist, it induces the release of arachidonic acid in lung adenocarcinoma cell lines (133). In colon carcinoma cell lines, binding of NNK to b-ARs induces intracellular c-AMP elevation, NF- $\kappa$ B mediated cyclooxygenase-2 up-regulation, and prostaglandin E2 release (134,135). In addition to NNK-mediated stimulation of prostaglandin syntheses, NNK increased cell proliferation in immortalized human pancreatic duct epithelium as well as airway epithelial cells by b-AR-mediated transactivation of EGFR (136,137). Recent studies have shown that in addition to b-AR binding, NNK binds predominantly to nAChRs  $\alpha$ 7 with a binding affinity that is several magnitudes greater than the receptor affinity of nicotine to nAChRs (138–140). The nAChR  $\alpha$ 7 is functionally expressed in a variety of malignant and non-malignant tissues. Upon activation of nAChR  $\alpha$ 7 by NNK, cell proliferation is stimulated and apoptosis is inhibited involving several intracellular pathways (141–145). For example, in human lung cancer cells, NNK induced functional cooperation of Bcl2 and c-Myc in promoting cell survival and proliferation, while at the same time the cell migration and invasion was increased in an Erk1/2-dependent manner (146,147).

Other tobacco-derived carcinogens, such as N-nitrosobis(2-oxopropyl)amine (BOP) applied subcutaneously, can induce pancreatic adenocarcinomas in an experimental setting. Co-treatment with NNAL induced a synergistic, carcinogenic action (148–151).

Furthermore, dimethylbenzanthracene (DMBA) induces pancreatic tumors in rodents and resembles a model in which transdifferential events in pancreatic carcinogenesis may play a role. When DMBA crystals are implanted in the area of the pancreatic head of mice, these mice quickly develop pancreatic duct alterations in a Notch signalling-dependent manner (152). These changes are characterized by transdifferentiation of acinar cells to tubular lesions starting as early as 2 weeks after DMBA implantation. One month post-implantation, PanIN lesions and some adenocarcinomas were observed. Sixty percent of the animals developed pancreatic tumors within three months after implantation (152,153). Intriguingly, DMBA induces pancreatic carcinogenesis in rats as well (58,154,155). DMBA-induced carcinogenesis was increased through pancreatic hyper-stimulation as well as systemic immunosuppression through immunosuppressive acidic protein (156,157). Metabolization of DMBA has been investigated in rats. High concentrations of DMBA metabolites, such as 5,6-epoxy-7-hydroxymethyl-12-methylbenzanthracene were formed by pancreatic tissue *in vitro*, and measured DMBA-DNA adducts correlated with tumorigenesis in F344 rats (158).

## 6. Acinar cell damage and pancreatic ductal adenocarcinoma

It seems that cigarette smoke as well as the DMBA model primarily affect the acinar cell compartment. This appears to be in contrast to the ductal phenotype of human pancreatic cancer. Experimental evidence suggests that cigarette smoke exposure induces a differential expression of genes related to pancreatic ducts only when histomorphological acinar cell damage is present (65). Recent data on carcinogen-induced transformation of acinar cells to atypical pseudo-ductular structures in the DMBA model indicate that neither centroacinar cells nor pancreatic ductal cells are the only progenitors from which the pancreatic ductal adenocarcinoma develops (58,159,160). In genetic mouse models of *K-ras*, nestin positive progenitor cells were the source of ductal adenocarcinomas (161). Nestin is expressed in

both endocrine and exocrine lineages. In additional experiments the selective expression of endogenous *K-ras* oncogene in embryonic acinar cells and in the centroacinar lineage resulted in pancreatic intraepithelial neoplasias and invasive pancreatic ductal adenocarcinoma (162,163). However, since nestin is not expressed in cells of the centroacinar lineage and since beta cell transdifferentiation does not contribute to metaplastic ductal lesions, the possibility of acinar to ductal transdifferentiation in early pancreatic carcinogenesis became likely (164). In accord with this hypothesis, Cre-loxP-based genetic lineage tracing provides direct evidence of acinar to ductal metaplasia in a minority of mucinous metaplastic lesions induced by pancreatic hyperstimulation (165). Independently, acinar cell targeting of oncogenic *K-ras* in adult mice induces a spontaneous induction of mPanINs of all histological grades (166). Therefore, if transdifferentiation of acinar cells to duct-like structures is thus induced, acinar cell damage may be the first step in cigarette smoke-induced pancreatic adenocarcinoma.

## 7. Conclusions and perspectives

Cigarette smoke inhalation leads to pancreatic acinar cell damage and increases the risk of developing pancreatic cancer and chronic pancreatitis in humans. So far, the precise underlying mechanisms have not been defined. The influence of cigarette smoke constituents on pancreatic carcinogenesis can be divided into two major modes of action, first through the interference with physiological pathways and second through the interaction with DNA. Nicotine and its derivatives greatly interfere with physiological regulatory pathways in terms of altering secretion, increasing proliferation and reducing apoptosis. These alterations result in inflammatory lesions to pancreatic acinar cells. The second class of substances that induces pancreatic carcinogenesis interferes with pancreatic DNA. The data reviewed strongly suggest that cigarette smoke induced pancreatic carcinogenesis is a multifactorial event consisting of DNA damage as well as DNA independent alterations. Of these events, the transdifferentiation of acinar cells to duct-like structures may be the link between cigarette smoke induced acinar cell damage and the development of ductal adenocarcinoma. Additionally, tobacco-derived carcinogens may cause genomic mutations that lead to a malignant phenotype (Fig. 2). Further studies that are not limited to acinar or ductal cells are therefore needed to investigate the pathogenesis of cigarette smoke induced pancreatic damage.

## Acknowledgments

The authors (S.K.B. and N.M.) on this work are supported by grants from the National Institutes of Health (CA78590, CA133774 and CA13194).

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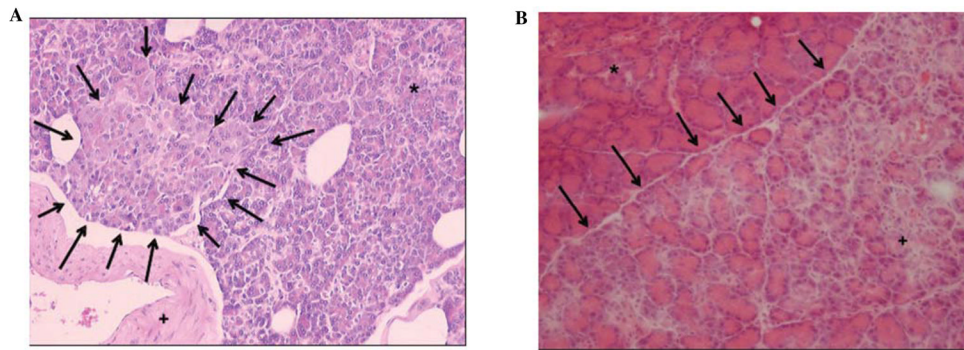


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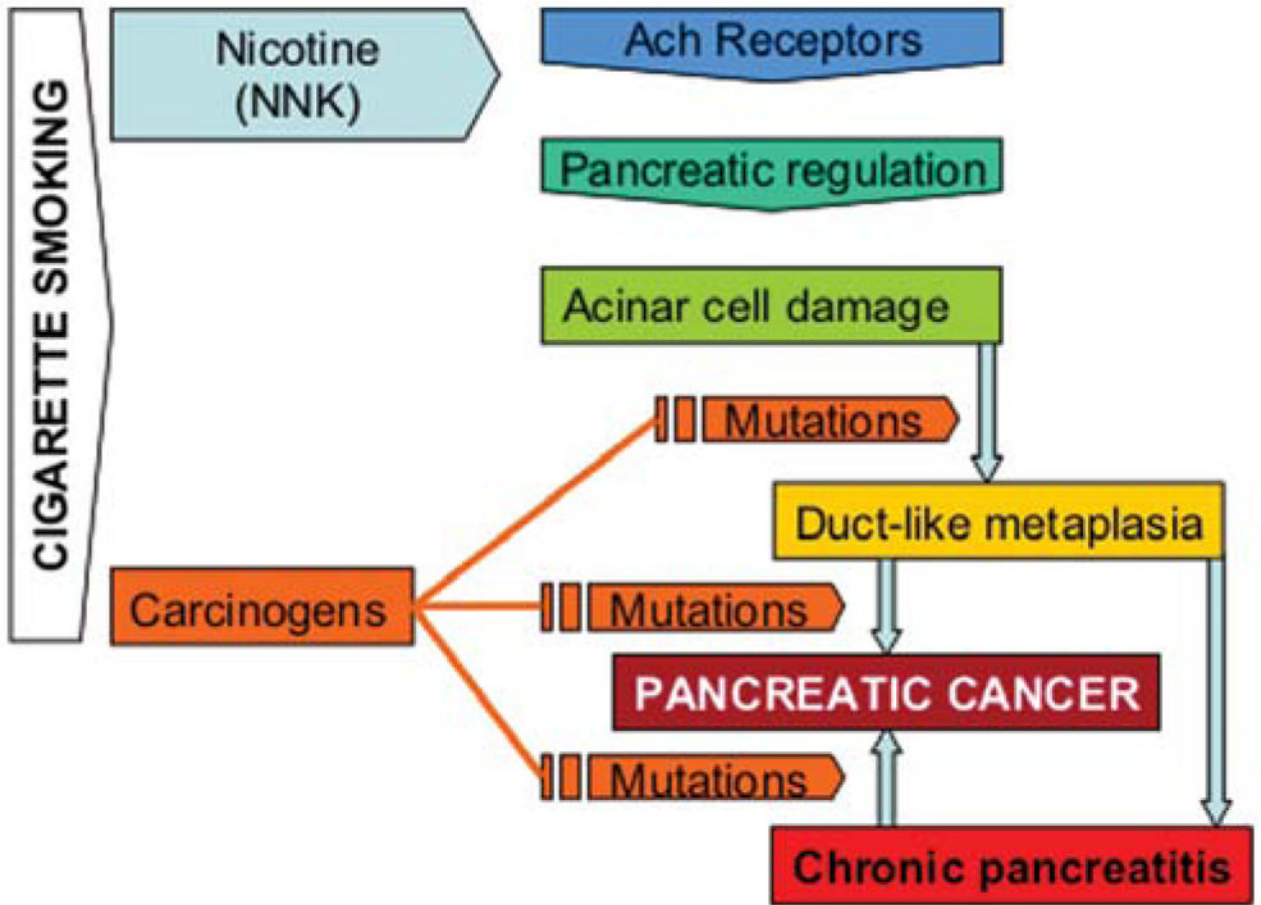
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**Figure 1.**

(A) Human acinar cell alterations in a tissue section-derived from a patient with pancreatic cancer. The arrows indicate the focal hyperplasia of acinar cells (asterisk) being well demarcated from normal acinar cells. A blood vessel (+) is shown in the lower left of the picture. H&E, original magnification x100. (B) Section of the pancreas of a rat treated with cigarette smoke for 12 weeks. The arrows indicate focal acinar cell damage compared to normal acinar cells marked by an asterisk. Area of acinar cell damage is indicated by the cross. H&E, original magnification x200.





**Figure 2.** Schematic view of proposed mechanism of pancreatic cancer induction by cigarette smoke.

**Table I**

*In vitro* and *in vivo* action of tobacco-derived carcinogens (A acinar phenotype; D, ductal phenotype; P, precancerous lesion).

Carcinogen	Tumorigenic in pancreas	Detected in human pancreas	Increased DNA adducts in smokers
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) NNAL	Rats D (113)	Pancreatic juice	No
N-nitroso-bis(2-oxopropyl)amine (BOP)	Hamsters D (148,167)	No	No
N-nitroso-bis(2-hydroxypropyl)amine (BHP)	(168)		
N-nitroso-(2-hydroxypropyl)(2-oxopropyl)amine (HPOP)			
N-nitroso-bis(2-acetoxypopyl)amine (BAP)			
7,12-dimethyl-1,2-benzanthracene (DMBA)	Mouse D (153) Rat D (169)	No	No
3-methylcholanthrene (3-MC)	Hamsters D (170)		
N-nitroso-2,6-dimethylmorpholine (cis-NNDM)	Rat D (171) Hamster D (168,172)		
N-nitrosomethyl(2-oxopropyl)amine (MOP)	Hamster D (173)		
n-methyl-N-nitrosourea (MNU)	Mouse P (174) Guinea Pig D/P (175)		
2-amino-1-methyl-6-phenylimidazo [4,5-b]-pyridine (PhIP)	Rat A (176)		Yes (177)
Dimethylhydrazine	Rabbit P (178)		