

REVIEW ARTICLE

Possible Involvement of Pancreatic Fatty Infiltration in Pancreatic Carcinogenesis

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ABSTRACT

Pancreatic cancer is difficult to diagnose in its early stage and is one of the most lethal human cancers. Thus, it is important to clarify its major risk factors, predictive factors and etiology. Here, we focus on fatty infiltration of the pancreas and suggest that it could be a risk factor for pancreatic cancer. Fatty infiltration of the pancreas is observed as ectopic adipocytes infiltrating the pancreatic tissue and is positively correlated with obesity and the prevalence of diabetes mellitus, which are risk factors for pancreatic cancer. However, whether fatty infiltration is a major risk factor for pancreatic cancer has not been established. Recent clinical studies show there is a positive correlation between fatty infiltration of the pancreas and pancreatic precancerous lesions or ductal adenocarcinomas. Animal experimental studies also show an association between fatty infiltration of the pancreas and pancreatic precancerous lesions or ductal adenocarcinomas development. Syrian golden hamsters, which are sensitive to chemical carcinogens in the pancreas, develop fatty infiltration of the pancreas with age. The combination of a high-fat diet and a chemical carcinogen that induces a *K-ras* mutation increases the severity of fatty infiltration of the pancreas. Thus, fatty infiltration of the pancreas is suggested to promote pancreatic carcinogenesis via a *K-ras* activating mutation. It is assumed that increased expression of adipokines and of inflammatory and proliferation-associated factors elicited by fatty infiltration of the pancreas may contribute to pancreatic precancerous lesions or ductal adenocarcinomas development. Accumulating evidence suggests that in addition to suppression of Ras activation, methods to modulate fatty infiltration in the pancreas can be considered as a strategy for preventing pancreatic cancer.

INTRODUCTION

Obesity causes accumulation of visceral and ectopic fats. Recently, ectopic fats, which are defined as excess adipose tissue in locations not classically associated with adipose tissue storage [1], have been gathering attention. Ectopic fats are commonly observed in the liver, heart, muscle and pancreas and directly exhibit lipotoxicity or indirectly secrete cytokines when accompanied by inflammation in these organs. Of note, fat deposition into hepatocytes is found in hepatic

steatosis. The pathophysiology, diagnostic criteria and clinical implications of hepatic steatosis have already been established [2]. Obesity, diabetes and alcohol consumption are known to be strongly associated with hepatic steatosis [3, 4]. Obesity and diabetes are the leading causes of non-alcoholic fatty liver diseases (NAFLD) / non-alcoholic steatohepatitis (NASH), and NASH is known to promote hepatocellular carcinoma development [5]. Ectopic fat deposition in the pancreas is synonymous with “fatty pancreas”, “nonalcoholic fatty pancreas disease (NAFPD)”, “fatty infiltration (FI)” or “fatty replacement”. In FI of the pancreas, which is distinct from hepatic steatosis, triglycerides accumulate in adipocytes in the pancreatic tissue.

In this review, we focus on pancreatic FI and its possible contribution to carcinogenesis and development of pancreatic ductal adenocarcinoma (PDAC) in humans and animal models. In addition, we review genetic background of metabolic syndrome and carcinogenesis of PDAC, and discuss the mechanisms behind the pathological consequences of FI of the pancreas.

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Keywords Diabetes Mellitus; Obesity; Pancreatic Neoplasms

Abbreviations FI fatty infiltration; NAFLD non-alcoholic fatty liver diseases; NAFPD nonalcoholic fatty pancreas disease; NASH non-alcoholic steatohepatitis; PDAC pancreatic ductal adenocarcinoma

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FATTY INFILTRATION OF THE PANCREAS

Pathology

FI of the pancreas has been observed pathologically for many years. This phenomenon is characterized by adipocyte infiltration of the area between pancreatic lobules, accumulating around great vessels (interlobular fat), and the lobules themselves, accumulating with a scattered pattern (intralobular fat). In humans, pancreatic FI appears mainly in the interlobular fat rather than in intralobular fat [6-8]. Histopathologically, only pancreatic islet cells are observed in massive adipocyte depots, and thus, pancreatic islet cells may be resistant to FI [9].

Apart from obesity or age-related pancreatic FI, extreme degrees of pancreatic adiposity have been traditionally observed in infants [10], young adults [11] and elderly adults [12], and have been described as lipomatous pseudohypertrophy of the pancreas. Lipomatous pseudohypertrophy of the pancreas is benign and characterized by an enlarged pancreas with a massive replacement of pancreatic exocrine tissue by adipose tissue; however, the shape of the pancreas and the islets of Langerhans are preserved.

Detection and Assessment

FI of the pancreas is relatively common and is identified based on magnetic resonance imaging (MRI) [13], magnetic resonance spectroscopy [14], endoscopic ultrasound (comparison the echogenicity of the pancreas with that of the normal liver) [6, 15] or computed tomography (CT; a decrease in the density of the circumscribed areas) [16]. Marks *et al.* reported that increased pancreatic echogenicity on abdominal ultrasonography, which they termed "bright pancreas", might represent a fatty change of the pancreas [6].

As quantitative methods to measure FI of the pancreas, the use of MRI (percentage decreases in pancreatic signal intensity on opposed phase images relative to those on in-phase images) or CT (mean Hounsfield unit (HU) of three pancreatic regions (head, body and tail), a threshold of -190 to -30 HU) has been reported to correlate well with histopathological findings [17, 18]. However, these methods did not show sufficient resolution for quantitative measurement of FI of the pancreas. To date, Kim *et al.* have reported an association between histologic pancreatic fat and CT attenuation indices, such as the difference between pancreatic and splenic attenuation (P-S) and the pancreas-to-spleen attenuation ratio (P/S) on unenhanced CT images [19]. The conventional P-S and P/S measurement could be used to evaluate FI of the pancreas on unenhanced CT images. Recently, pancreatic volume and fat deposition have also been quantitatively assessed using a multidetector CT (histogram analysis, CT attenuation, a threshold of -190 to -30 HU) [20].

A quantitative MRI study revealed a correlation between pancreatic fat and obesity [21]. In addition, FI of the pancreas measured by sonographic analysis was associated with higher levels of total cholesterol, LDL-

cholesterol (LDL-C), triglycerides, FFAs, insulin and HOMA-IR [22, 23]. Kim *et al.* reported that the degree of pancreatic FI was significantly associated with a clinical presence of impaired glucose metabolism measured with the CT attenuation index [19].

Etiology

The etiology of FI of the pancreas is complicated and multifactorial. FI of the pancreas is positively correlated with age, BMI, a history of diabetic mellitus (DM) and fatty liver [9, 22, 24-27] (**Table 1**). The severity of FI in the pancreas increases with age or obesity [9, 28]. It is known that obesity-related FI of the pancreas could be improved after weight reduction. Van der Zijl *et al.* described that pancreatic fat increases with impaired glucose metabolism independent of aging and obesity [29]. However, they could not establish a direct relationship between pancreatic fat and diminished β -cell function in subjects with impaired glucose metabolism [29]. Thus, whether lipid accumulation occurs in pancreatic islets and whether this contributes to β -cell dysfunction in humans is still under debate [30, 31].

Ischemia from impaired pancreatic arteries promotes FI [32]. Pancreatic ischemia is spontaneously induced by atherosclerosis or blood clots in the pancreatic artery. Risk factors for atherosclerosis include smoking, high LDL-C levels, insulin resistance, DM [33], obesity, lack of physical activity [34] and aging. These risk factors are also associated with FI of the pancreas and pancreatic cancer. Thus, FI of the pancreas and pancreatic cancer may be associated with ischemia.

FI represents replacement of acinar cells by adipocytes, which is induced by acinar cell death or by fat accumulation promoted by obesity and metabolic syndrome [35]. Loss of acinar cells resulting from various types of damage is a more significant contributor to pancreatic FI than obesity. Induction of pancreatic FI after ligation of the pancreatic duct along with parenchymal loss has been observed in experimental animal models [36, 37]. In a genetic mouse model of pancreatic duct malformation in which the Notch signaling gene *Jagged1* is knocked out, abnormal pancreatic ducts are accompanied by acinar cell death, pancreatitis, FI and/or fibrosis in the pancreatic parenchyma [38]. Thus, FI of the pancreas can be induced secondarily by tumor occupation of pancreatic ducts or by obesity and metabolic syndrome. Although the genomic changes responsible for the formation of FI of the pancreas are not clearly known, some genes are addressed in the next section.

Possible Genetic Backgrounds

FI of the pancreas has been reported in several monogenic conditions: cystic fibrosis [39], Shwachman-Diamond syndrome [40] and Johanson-Blizzard syndrome [41]. In addition, pancreatic lipomatosis has been observed in nondiabetic children possessing mutations of the carboxyl-ester lipase gene [42].

The prevalence of obesity and diabetes is partially due to a westernized lifestyle. In addition, obesity-

Table 1. Associated factors for fatty infiltration of the pancreas.

| | Odds ratio | 95% CI | Method of FI detection | Country | References |
|----------------------|-------------------------------|-------------|------------------------|-------------|----------------------------------|
| Age older than 60 yr | 2.874 | 1.537-5.372 | EUS | South Korea | Choi <i>et al.</i> 2010[25] |
| Sex (male/female) | Male gender 2.636 | 1.224-5.678 | EUS | South Korea | Choi <i>et al.</i> 2010[25] |
| BMI | 1.33 (per 1 U of BMI) | 1.13-1.55 | EUS | USA | Al-Haddad <i>et al.</i> 2009[26] |
| | 1.05 | 1.00-1.10 | EUS | USA | Sepe <i>et al.</i> 2011[27] |
| | BMI≥30 kg/m ² 2.46 | 1.33-4.54 | EUS | USA | Sepe <i>et al.</i> 2011[27] |
| HOMA-IR | 2.319 | 1.431-3.757 | Abdomen sonography | South Korea | Lee <i>et al.</i> 2009[22] |
| Fatty liver | 13.7 | 2.99-62.5 | EUS | USA | Al-Haddad <i>et al.</i> 2009[26] |
| | 2.219 | 1.226-4.016 | EUS | South Korea | Choi <i>et al.</i> 2010[25] |
| | 3.61 | 1.83-7.11 | EUS | USA | Sepe <i>et al.</i> 2011[27] |
| Alcohol | 6.49 | 2.04-20.83 | EUS | USA | Al-Haddad <i>et al.</i> 2009[26] |
| Hypertension | 2.037 | 1.018-4.072 | EUS | South Korea | Choi <i>et al.</i> 2010[25] |
| Metabolic syndrome* | 3.13 | 1.45-6.72 | EUS | USA | Sepe <i>et al.</i> 2011[27] |

EUS endoscopic ultrasonography; FI fatty infiltration

*The metabolic syndrome included BMI ≥ 30 kg/m² and any 2 of the following 3 comorbidities: DM, hypertension, hyperlipidemia.

or diabetes- susceptibility loci or single nucleotide polymorphisms (SNPs) have been discovered through genome-wide association studies [43]. SNPs near the melanocortin 4 receptor (MC4R) gene are associated with increased risks of obesity and diabetes [44, 45]. One SNP, MC4R rs17782313, has been reported to correlate with an increased risk for breast [46], colorectal [47] and endometrial [48] cancers, and these associations persist after adjustment for BMI [46-48]. MC4R plays an important role in energy metabolism, and MC4R-deficient mice develop steatohepatitis in addition to obesity, insulin resistance and dyslipidemia when fed a high-fat diet (HFD) [49]. Homozygous null mutations in proopiomelanocortin (POMC) and heterozygous loss-of-function mutations in α - and β -melanocyte-stimulating hormone (α - and β -MSH) also increase obesity risk in human [50, 51]. A common variant in the fat mass and obesity associated (FTO) gene is associated with a predisposition for diabetes by affecting BMI [52]. Variants in the patatin-like phospholipase containing domain 3 gene (PNPLA3) or glucokinase regulatory protein (GCKR) have been reported to be associated with NAFLD [53, 54]. Both NAFLD and fatty pancreas were also associated with diabetes independently of age, gender, adiposity, and other cardiometabolic risk factors [55]. To date, there is no information about associations between these genetic backgrounds and susceptibility to FI of the pancreas; however, some genetic backgrounds related to obesity/metabolic syndrome may be involved in occurrence of severe FI.

ASSOCIATION OF FI OF THE PANCREAS WITH PANCREATIC CARCINOGENESIS IN HUMANS

Pancreatic cancer occurs with a high incidence and mortality rate in developed countries [56]. In Japan, the age-adjusted mortality rate of pancreatic cancer has increased by approximately 9-fold in both males and females from 1950 to 1995 [57]. This phenomenon may be explained by an increase of obesity and DM resulting from a westernization of the common life style including HFDs and low physical activity. Indeed, from 1946 to 1996, dietary fat intake has increased more than 4-fold [58]. In

addition, becoming an aging society may also contribute to increase the total number of pancreatic cancer patients.

In addition to age [59], obesity [60, 61] and DM [62], epidemiological studies have shown that smoking[63, 64] and chronic pancreatitis[65, 66], which are reported as etiologies of FI of the pancreas, are also major risk factors for pancreatic cancer. Thus, an association between FI of the pancreas and pancreatic cancer is possible, but to date it is not evident whether FI in the pancreas itself is a risk factor.

Implication of K-ras Mutation

The top two common smoking-associated cancers are adenocarcinomas of the lungs and pancreas [67]. Cigarette smoke contains toxic chemicals such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). NNK is known as the most potent carcinogen and is formed under reactions with metabolites of nicotine. In mice and rats, O⁶-methyguanine formation is associated with a point mutation in the K-ras gene in NNK-induced pulmonary adenocarcinomas [68, 69]. Westra *et al.* reported more K-ras gene mutations were found in lung adenocarcinomas obtained from current and former smokers (approximately 30%, respectively) than in nonsmokers (7%; P = 0.015) [70]. Activating mutations of the K-ras gene are the most common genetic abnormality in PDAC and are present in approximately 90% of cases [71] even in low-grade PanIN [72]. Thus, they are the earliest genetic abnormalities observed in the progression of PDAC. In addition, K-ras mutations in mucous and in ductal / desquamated cells involved in pancreatic juice production are detected in patients with chronic and/or autoimmune pancreatitis [73-75]. However, an association between smoking and K-ras gene mutations in pancreatic cancer is controversial [76, 77]. Statistically, it may be impossible to detect an effect of smoking on the K-ras gene because most pancreatic cancer patients harbor the K-ras mutation. Thus, smoking remains one of the likely causes of K-ras mutation.

Data from Clinical Studies

We found and reported on a case in which PDAC developed in the head of the pancreas markedly infiltrated

by adipose tissues [78]. This patient underwent distal gastrectomy against early gastric cancer 9 years ago, and 5 years later, CT scans showed that most of the pancreas had been replaced by fat tissue. Namely, fatty pancreas was observed 4 years before PDAC development. Subsequently, we performed a case-control study of FI of the pancreas prevalence between controls (patients with distal bile duct cancer, cancer of the ampulla of Vater, gallbladder cancer, or duodenal cancer) and cases (patients with pancreatic head ductal carcinoma)[79]. The degrees of FI of the pancreas were measured histopathologically from the area occupied by adipocytes in pancreatic sections in the study. The results showed that the degree of FI of the pancreas was significantly higher in PDAC cases than in non-PDAC controls (median 26 vs. 15%, $P < 0.001$). It is also suggested that FI of the pancreas may increase the risk of pancreatic cancer beyond the effect of obesity itself (OR=6.1; adjusted with BMI, DM and other confounding factors, $P < 0.001$, **Table 2**). Rebours *et al.* have also reported that PanINs were associated with the degree of intralobular fibrosis and the intralobular fat from surgical specimens of normal pancreatic tissue [80]. The odds ratio (OR) in intralobular fat (OR=17.86) was higher than that in intralobular fibrosis (OR=5.61). The number of PanIN lesions also increased with the severity of hepatic steatosis, but not with the percentage of subcutaneous fat area or BMI [80].

ASSOCIATION OF FI OF THE PANCREAS WITH PANCREATIC CARCINOGENESIS IN ANIMAL MODELS

The initiation of pancreatic carcinogenesis results from *K-ras* activation, which induces precancerous lesions, PanINs. In addition to activation of the *K-ras* gene, either nongenetic events involving tissue damage and/or an inflammatory response or genetic events causing loss of *p16/Ink4a* or *p53* are necessary for the formation of PDAC [81]. Pancreatitis has been shown to contribute to PDAC progression by abrogating the senescence barrier characteristic of low-grade mPanIN expansion [82]. Pathologically, development of PDACs would be promoted by adipocytes during FI after the *K-ras* activation and by loss of acinar cells by carcinogens.

BOP-Treated Hamster Model for Pancreatic Carcinogenesis

The hamster is a unique model animal that can develop PDAC by the subcutaneous injections of *N*-nitrosobis(2-oxopropyl)amine (BOP)[83]. Moreover, similar to human PDACs [84, 85], point mutations in codon 12 of the *K-ras* gene are frequently observed and expression of the fragile histidine triad gene is aberrant in BOP-treated hamsters [86]. The *p16* gene is one of the most frequently inactivated tumor-suppressor genes in human PDACs [87], and loss of *p16* expression has also been found in hamster PDAC lesions [88]. Syrian golden hamsters have hyperlipidemia. The effect of a HFD on hyperlipidemia and pancreatic carcinogenesis was examined in BOP-treated hamsters, and a HFD was shown to enhance PDAC formation with

a corresponding increase of FI of the pancreas, BW, and serum lipid and leptin levels [89].

Mouse and Rat Models for Pancreatic Carcinogenesis

Unlike hamsters, mice and rats are not susceptible to chemically induced pancreatic carcinogenesis. They do not develop PDAC upon treatment with BOP [90]. The Syrian golden hamster shows hyperlipidemia because hepatic lipoprotein lipase activity is low compared with that of mice and rats. Our previous study has shown that the OLETF rat, a model of T2DM accompanied by hypertriglyceridemia, failed to develop BOP-induced PDAC [91]. It was notable that FI of the pancreas of OLETF rats did not develop to the same degree as that observed in Syrian golden hamsters, and the data indicated that hypertriglyceridemia and/or hyperinsulinemia in T2DM are not sufficient to increase susceptibility to pancreatic carcinogenesis. It is assumed that the enzymatic activation and detoxification of BOP as well as the ability of BOP metabolites to induce formation of DNA adducts is different between hamsters and mice/rats. Another difference is the basal potential to develop FI. Hamsters have more scattered FI in the pancreas than mice/rats. Thus, it is possible that sensitivity to pancreatic carcinogens depends on the degree of pancreatic FI.

Despite the fact that mice are less susceptible to PDAC, many genetically engineered mouse models of PDAC have been developed [92]. A mouse strain carrying a conditional knocked-in of a *KRas*^{G12D} allele silenced by a floxed STOP transcriptional cassette (*LSL-KRas*^{G12D}) was crossed to transgenic strains that expressed the bacterial Cre recombinase under the control of gene promoters of pancreas-specific factors, such as *Pdx1*, *Ptf1a/P48* [93] or elastase. These transgenic mice develop acinar-to-ductal metaplasia and PanINs via *K-ras* activation [93, 94]. A small proportion of these mice develop PDAC after a prolonged latency period (>12 months) because additional alterations, including *p16* [95], *p53* [96] and *dpc4* [97, 98], are necessary for development of PDAC in *LSL-KRas*^{G12D} mice.

Pigment epithelium-derived factor (PEDF) is a multifunctional secreted protein, for example, it has antiangiogenic, antiproliferative and neurotrophic activities [99, 100]. It has been reported that *Elastase-Kras*^{G12D}/*PEDF* deficient mice developed invasive PDAC and increased FI of the pancreas accompanied by stromal expression of two lipid droplet associated proteins, tail-interacting protein 47 (TIP47, perilipin 3) and adipose differentiation-related protein (ADRP, perilipin 2) [101]. Ras oncogenes themselves are sufficient to induce differentiation of 3T3-L1 fibroblasts into adipocytes [102], and additional PEDF deficiency may promote adipogenesis by cancelling its suppressive role on master adipogenic transcription factors such as CCAAT-enhancer-binding protein α (C/EBP- α), and peroxisome proliferator-activated receptor γ (PPAR γ) that are upregulated through the Ras/MAPK pathway[103].

It is known that HF and high-calorie diets promote pancreatic neoplasia in *Pdx-1-KRas*^{G12D} mice [104]. The pancreas of these mice have an increased number of

Table 2. Possible risk factors for pancreatic cancer.

| | | Relative Risk | 95% CI | Method of FI Detection | Country | References |
|----------------------|----------------------------------|---------------|-----------|------------------------|---------|-------------------------------|
| BMI | BMI≥30 kg/m ² | 2.08 | 1.48-2.93 | - | USA | Patel <i>et al.</i> 2005[60] |
| DM | | 2.1(HR) | 1.3-3.5 | - | Japan | Luo <i>et al.</i> 2007[61] |
| Smoking | ever | 2.04 | 1.09-3.83 | - | USA | Schenk <i>et al.</i> 2001[63] |
| | ≥40 cigarettes/day, male smokers | 3.3 | 1.38-8.1 | - | Japan | Lin <i>et al.</i> 2002[64] |
| Family history | | 2.49 | 1.32-4.69 | - | USA | Schenk <i>et al.</i> 2001[63] |
| Chronic pancreatitis | | 19.5 | 3.08-124 | - | USA | Risch <i>et al.</i> 2015[66] |
| FI of the pancreas | | 6.1 (OR) | 2.4-15.2 | histopathology | Japan | Hori <i>et al.</i> 2014[79] |

DM diabetes mellitus; FI fatty infiltration

infiltrating inflammatory cells, increased stromal fibrosis and more advanced PanIN lesions; however, FI of the pancreas has not been described in these mice.

ADIPOCYTE-ASSOCIATED FACTORS CONTRIBUTING TO TUMOR PROMOTION

Possible mechanisms of tumor promotion by FI of the pancreas include an increase of inflammatory- and proliferation-associated factors derived from infiltrated adipocytes.

Inflammatory Factors

Adipose tissue has been characterized as an organ that secretes cytokines such as TNF- α , IL-6, and monocyte chemoattractant protein-1 (MCP-1). In turn, macrophages accumulate around adipose tissue and produce cytokines including IL-1 β thereby aggravating a state of inflammation. NAFLD presents with inflammation accompanied with FI of the pancreas, and inflammatory cells such as macrophages can accumulate around adipose cells. In BOP-treated hamsters, a HFD increased or tended to increase the pancreatic expression of MCP-1, IL-1 β and COX-2 as well as aggravate FI and PDAC [89]. These cytokines could promote inflammation and contribute to tumor progression. Overproduction of PGE₂ by COX-2 has been suggested to activate cell proliferation, angiogenesis and anti-apoptotic mechanisms [105, 106]. PGE₂ has the ability to differentiate 3T3-L1 cells to adipocytes [107]. Additionally, COX-2 expression is regulated by Wnt and Ras signaling [108]. Oncogenic K-ras increases COX-2 levels in intestinal epithelial cells [109] and NF- κ B-mediated positive feedback involving COX-2 expression could increase in pancreatic duct cells with oncogenic K-ras [110].

Adipokines

Adipose tissue has been described as an endocrine organ that produces adipokines such as leptin and adiponectin. The serum levels of adipokines such as leptin and adiponectin in BOP-treated hamsters fed HFDs were higher than those in the BOP-treated hamsters fed a standard diet (STD) [89]. Leptin is a hormone whose level increases in the serum with the onset of obesity. There are reports about allografted tumor growth in obese mice. To determine whether diet-induced obesity (DIO) could result in FI of the pancreas in mice, C57BL/6 mice were fed HFDs. In addition to insulin resistance and/or increased

serum leptin levels, FI of the pancreas was observed in the mice with DIO [111-113]. DIO increased the proliferation of orthotopically implanted pancreatic tumor cells *in vivo* via an activation of the PI3K/Akt pathway by leptin signaling [111]. *In vitro*, leptin directly stimulates the phosphorylation of Akt, with increased pancreatic cancer cell proliferation and migration. When lean (C57BL/6J) and obese (Lep^{ob} and Lep^{db}) mice were inoculated with murine pancreatic cancer cells, obese mice developed larger tumors, metastases, intratumoral adipocyte masses, and greater mortality than lean mice [114]. These findings suggest that both insulin resistance and an altered adipokine milieu could lead directly to changes in the microenvironment.

Free Fatty Acids

Fatty tissue that accumulates during FI of the pancreas contains high levels of triglycerides and FFAs. Serum FFA levels are correlated with the degree of FI of the pancreas in BOP-treated hamsters. Moreover, the expression of fatty acid synthase, representing *de novo* FFA synthesis, is enhanced in the pancreas of BOP+HFD-treated hamsters compared with BOP+STD-treated hamsters [89]. A high level of FFAs is toxic to cells due to its peroxidation [115, 116], suggesting that FFAs could damage pancreatic acinar cells and induce FI.

Growth Factors

The mRNA levels of insulin, IGF-I and cyclin D1 were elevated in the pancreas of BOP-treated hamsters with HFD [89]. Insulin and IGF-I promote the differentiation of 3T3-L1 cells to adipocytes [117], and thus, FI may increase in the pancreas. IGF-I is known to stimulate cell proliferation [118]. In *KrasInk4a*^{+/-} mice, IGF-I levels clearly represent the diet-dependent changes in body fat and PDAC development compared with other markers, such as adiponectin, leptin and insulin [119]. Leptin, insulin and IGF-I could promote cancer development thorough induction of inflammation- and growth-related gene expression.

Angiogenesis Factors

Serum levels of VEGF, VEGF-C and VEGF-D are elevated in overweight/obese subjects [120]. Leptin secreted by adipocytes increases VEGF levels by activating STAT3. VEGF induces angiogenesis and promotes tumor growth [121]. Thus, FI has the potential to promote tumor angiogenesis in the pancreas. Angiotensin II, which is

secreted by adipocytes, also increases the production of VEGF. VEGF-C and VEGF-D are reported to enhance lymphatic metastasis of PDAC [122].

CHALLENGES FOR THE FUTURE

Prospective Study

We cannot distinguish whether FI is a risk factor or a consequence of pancreatic cancer by case-control studies or retrospective clinical studies. The only way to demonstrate that FI is a risk factor for PDAC is to perform a prospective cohort study that examines whether individuals with severe FI in the pancreas could develop PDAC. The implementation of a large cohort is needed because the incidence of pancreatic cancer is low. As described above, the conventional CT attenuation index on unenhanced CT images has been recently established for the detection of fatty pancreas [19]. Mass screening of individuals with severe FI of the pancreas (so-called “fatty pancreas”) using such conventional methods may be useful for a future prospective study to assess fatty pancreas, as a high-risk marker for pancreatic cancer. In addition to the image diagnosis, the establishment of useful biomarkers for fatty pancreas is also desired to support the screening.

Prevention Study

If fatty pancreas is determined to be a risk for pancreatic cancer, therapeutic intervention of FI of the pancreas could increase prevention of pancreatic cancer development. Indeed, weight reduction, high physical activity, and reducing intake of HF foods are possible ways to lower the risk of both fatty pancreas and pancreatic cancer.

Obora *et al.* detected a “bright pancreas” in 776 subjects of 1,993 participants (38.6%) who were followed up for a mean of 8.7 years. This study found that the “bright pancreas” disappeared in approximately 30% of both sexes from baseline “bright pancreas” detection (detected at the follow-up) [123], indicating that “bright pancreas” is reversible and could be improved by changes of lifestyle. It is known that obesity-related FI of the pancreas could be improved after weight reduction [124].

Obesity and DM are associated with both FI and increased risk of pancreatic cancer. The use of metformin, an insulin-lowering agent for T2DM, has been shown to be associated with a decreased risk of pancreatic cancer in epidemiological studies [125, 126]. Metformin has also been shown to decrease the growth of PDAC in a xenograft model [127] and to lower the number of PDACs in BOP-treated hamsters with HFD [128]; however, there are currently no data on the effect of metformin on FI of the pancreas.

One anti-diabetic drug, pioglitazone, which is a ligand for PPAR γ , improved hyperlipidemia and suppressed the incidence of pancreatic tumors in BOP-treated hamsters [129]. Another PPAR γ ligand, rosiglitazone, also reduced fasting glucose and insulin levels in mice fed a HF plus high-sucrose diet, however, these PPAR γ ligands induced weight gain and remarkable FI of the intralobular space [113]. This finding seems to be inconsistent with the expected roles of FI of the pancreas in pancreatic carcinogenesis. Recently, it has been reported that pioglitazone use in people with diabetes increased their risk for developing pancreatic and prostate cancers [130], though the increased risk for pancreatic cancer was observed in short time users

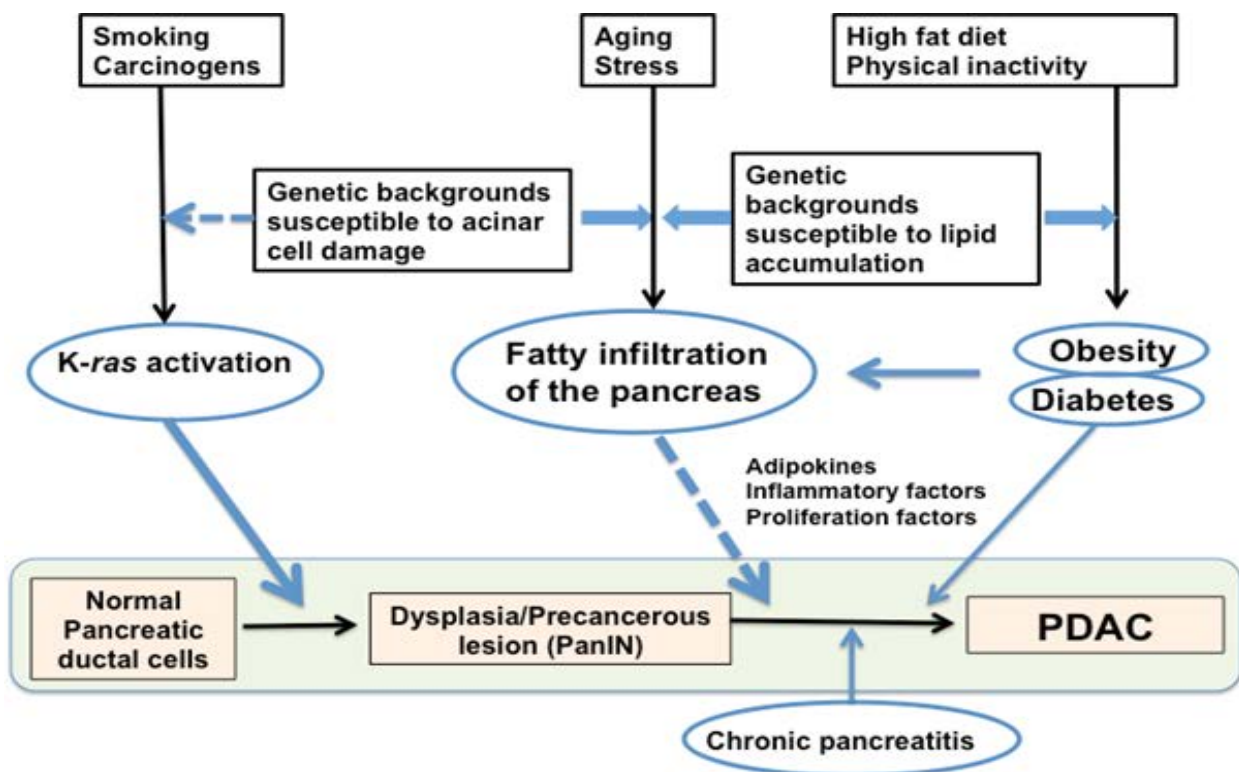


Figure 1. Diagram of pancreatic fatty infiltration involved in pancreatic carcinogenesis. PDAC pancreatic ductal adenocarcinoma; PanIN pancreatic intraductal neoplasm

(≤ 12 months since initiation). Therefore, further studies are required to clarify the effects of pioglitazone on fatty pancreas and pancreatic cancer.

FI, especially NAFFPD, is accompanied by inflammation in the pancreas. Clinical and animal experimental data show that COX-2 inhibitors have the potential to prevent pancreatic cancer prolongation [131, 132].

CONCLUSION

FI of the pancreas, which is associated with age, obesity and DM, is fundamentally caused by acinar cell damage. A *K-ras* mutation is essential for pancreatic cancer development in humans and in animal models. FI of the pancreas combined with *K-ras* mutation is strongly correlated with pancreatic cancer development in humans and animal models (**Figure 1**). Hamsters are likely to have a high sensitivity to pancreatic carcinogens, and their acinar cells can be easily damaged compared with those of mice and rats. The difference of such sensitivity may be explained in part by occurrence of severe FI of the pancreas. In human cases, severe FI of the pancreas is associated with PDAC. Severe FI of the pancreas presumes acinar cell damage, while proliferation- and inflammation-related factors secreted from adipocytes are considered to promote pancreatic carcinogenesis. Therefore, FI of the pancreas is a possible pancreatic risk factor and its modulation could be a useful method for preventing pancreatic cancer. Further prospective studies on these points are warranted.

Author contributions

Hori M, Mutoh M, Imai T, Nakagama H and Takahashi M solely contributed to this paper.

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Conflict of Interest

The authors have no conflict of interest.

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