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平成２５年度　総括・分担研究報告書

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REVIEW

Involvement of inflammatory factors in pancreatic carcinogenesis and preventive effects of anti-inflammatory agents

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Abstract Chronic inflammation is known to be a risk for many cancers, including pancreatic cancer. Heavy alcohol drinking and cigarette smoking are major causes of pancreatitis, and epidemiological studies have shown that smoking and chronic pancreatitis are risk factors for pancreatic cancer. Meanwhile, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) are elevated in pancreatitis and pancreatic cancer tissues in humans and in animal models. Selective inhibitors of iNOS and COX-2 suppress pancreatic cancer development in a chemical carcinogenesis model of hamsters treated with *N*-nitrosobis(2-oxopropyl)amine (BOP). In addition, hyperlipidemia, obesity, and type II diabetes are also suggested to be associated with chronic inflammation in the pancreas and involved in pancreatic cancer development. We have shown that a high-fat diet increased pancreatic cancer development in BOP-treated hamsters, along with aggravation of hyperlipidemia, severe fatty infiltration, and increased expression of adipokines and inflammatory factors in the pancreas. Of note, fatty pancreas has been observed in obese and/or diabetic cases in humans. Preventive effects of anti-hyperlipidemic/anti-diabetic agents on pancreatic cancer have also been shown in humans and animals. Taking this evidence into consideration, modulation of inflammatory factors by anti-inflammatory agents will provide useful data for prevention of pancreatic cancer.

Keywords Pancreatic cancer · Pancreatitis · Hyperlipidemia · Anti-inflammatory agents · Prevention

Introduction

Pancreatic cancer is the fifth leading cause of cancer death in Japan [1]. Pancreatic cancer incidence by age increases from the 60s and becomes higher in elders [2]. The population aging rate (percentage of the population aged 65 or elder) is increasing in Japan and passed 20 % in 2005 [3]. The quite high population aging rate contributes to an increase of death by pancreatic cancer. In addition, the age-standardized mortality rate increased markedly from 1960 to 1990 and remains at the same level or is still slightly increasing [4]. Epidemiologically, cigarette smoking, family history, chronic pancreatitis (CP), obesity, and diabetes mellitus are shown to be risk factors for pancreatic cancer [5–10]. In Japan, the cigarette smoking rate in male adults is decreasing and that in 2011 was less than half of that in 1966, although it is still higher than those in Western countries [11]. On the other hand, alcohol consumption has more than doubled from 1970 to 1996 and recently remains at the same level or is slightly decreasing [12]. Dietary fat intake has also increased more than four times from 1946 to 1996 [13]. According to these increases, patients of CP and type II diabetes are increasing [11, 14]. Such phenomena are considered to be the background responsible for the marked increase of pancreatic cancer.

Many factors associated with chronic inflammation including various cytokines, reactive oxygen species (ROS), and mediators of the inflammatory pathway produce alteration in gene expression, genomic damage, and cellular proliferation,

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which favor the malignant transformation of the ductal/ductular epithelium [15]. As a result, normal ductal/ductular epithelium is led to early neoplastic lesions, pancreatic intraepithelial neoplasias (PanINs), which eventually give rise to the major histological type of pancreatic cancer, invasive pancreatic ductal adenocarcinomas (PDACs) [16]. Multiple epidemiological studies on pancreatic inflammation have also shown an increased risk for pancreatic cancer [10]. However, invasive PDACs are usually diagnosed at an advanced incurable stage because of the absence of specific symptoms and the lack of biomarkers for early detection. Animal models of carcinoma of the pancreas induced by pancreatitis might provide new information concerning the pathways for histogenesis of the tumors. Furthermore, animal experiments of inflammation-related pancreatic carcinogenesis are needed to allow early diagnosis, to develop methods of treatment, and to aid in the development of novel and effective chemopreventive agents against pancreatic cancer.

In this paper, we focus on the roles of chronic inflammation and/or inflammatory factors in pancreatic carcinogenesis and further discuss the possible prevention of pancreatic ductal cancer by anti-inflammatory agents.

Risk factors correlated with CP

CP and pancreatic cancer risk

CP is identified as a strong risk factor for pancreatic cancer [10, 17–20]. Multinational studies including 2,015 patients revealed that the standardized incidence ratio was almost 26.3 [17]. The pooled risk estimate of seven studies for pancreatic cancer in CP was reported to be 13.3 [10]. The cumulative risk of pancreatic cancer in CP patients was reported to be 4 % after 20 years, being at least tenfold higher than those without CP [17]. Inflammation itself is considered to be not sufficient to induce cancer but able to strongly enhance carcinogenesis. Not only CP but also obesity, hyperlipidemia, and type II diabetes are shown to be risk factors for pancreatic cancer [5, 7–10, 21], and these diseases can also cause inflammation [22].

CP induces irreversible morphological changes in the parenchyma of the pancreas and the pancreatic duct due to the progressive inflammatory response of the pancreas. In the parenchyma, precedent loss of acini and in the progressive processes fibrosis and loss of islets of Langerhans are observed. In addition, stenosis and pancreatic stones are also observed in the pancreatic duct. CP is further characterized by mononuclear infiltration rather than neutrophilic inflammatory reaction, which is observed in acute pancreatitis (AP). During inflammation, the loss of acini and islets of Langerhans with fibrosis eventually leads to loss of functions and evokes diabetes. Regarding CP, its risk factors, such as environmental, disease-related, metabolic, and genetic, are described in the following sections (Fig. 1).

Environmental risk factors: social habit effects on CP

Excessive alcohol consumption

Excessive alcohol consumption increases the onset risk for both AP and CP [14, 23, 24]. Alcoholic pancreatitis is the most common type and accounts for almost two thirds of CP [14]. There are several reports elucidating the molecular mechanisms responsible for alcohol-induced pancreatic inflammation. To date, it is well established that the pancreas

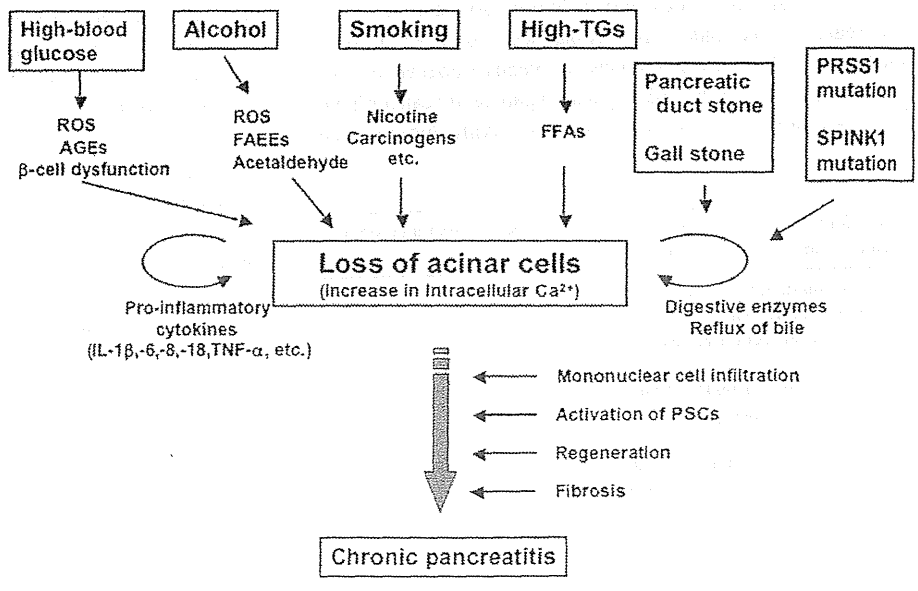

Fig. 1 Causes of chronic pancreatitis. Environmental, disease-related, metabolic, and genetic risk factors are involved in the induction of CP. *TG* triglyceride, *AGE* advanced glycation end products, *ROS* reactive oxidative species, *FAEE* fatty acid ethyl ester, *FFA* free fatty acid, *IL* interleukin, *TNF* tumor necrosis factor, *PSC* pancreatic stellate cell

can metabolize alcohol to its toxic metabolites, acetaldehyde and fatty acid ethyl esters. Moreover, ROS are produced as by-products of ethanol metabolism. These toxic metabolites and oxidative stress injure the pancreatic acinar tissue [25–27]. There are also several lines of evidence indicating the deleterious effects of alcohol and its toxic metabolites on acinar cells. Alcohol and its toxic metabolites have been shown to (1) destabilize lysosomes containing lysosomal enzymes and zymogen granules which contain digestive enzymes [25, 28], (2) increase the synthesis of digestive and lysosomal enzyme content [29], (3) activate inflammation-related transcriptional factors, such as nuclear factor (NF)-κB [30], and (4) induce a sustained increase in cytoplasmic ionic calcium, which causes mitochondrial calcium overload and resultant mitochondrial depolarization to cell apoptosis/necrosis [31].

Taking a light to pancreatic cancer, there are conflicting data for alcohol as a risk factor. Two Netherlands cohort studies, namely, Cancer Prevention Study II and the NIH-AARP Diet and Health Study, demonstrated that excess alcohol consumption was associated with an increased risk for pancreatic cancer [32–34]. It has also been reported that chronic consumption of alcoholic beverages enhances the risk of pancreatic cancer in smokers [35–37]. However, a European Prospective Investigation into Cancer and Nutrition study and a recent pooled analysis from the pancreatic cancer cohort consortium (PanScan) did not identify any overall association between total alcohol intake and pancreatic cancer [38, 39]. Therefore, it is plausible that alcohol consumption is not a direct risk factor for pancreatic cancer, but an indirect risk factor via the development of CP in heavy drinkers.

Smoking

The observation that only ~10 % of heavy drinkers develop CP suggests that other environmental factors affect CP development. Cigarette smoke, a crude bioactivator, has long been thought to be additionally associated with CP. It has been reported that cigarette smoking dose-dependently promotes the development of CP [24, 40]. Furthermore, smoking increases inflammation severity and induces pancreatic calcification [41, 42]. As for pancreatic cancer, epidemiological studies have shown that cigarette smoking doubles the risk of pancreatic cancer (relative risk (RR)=2.5), and as many as 25 % of pancreatic cancer cases are attributed to smoking [6, 8, 43, 44]. Indeed quitting smoking has been demonstrated to prevent both the development of pancreatic cancer and CP [45]. Of note, cigarette smoke is graded as a class 1 carcinogen by the World Health Organization [46]. Pancreatic cancer risk increases with duration and amount of cigarette smoke [44], similar to CP [40]. Thus, cigarette smoking has properties to increase pancreatic cancer both directly and indirectly via CP [14, 41, 47].

Carcinogen-induced pancreatic cancer animal model studies have indicated that acinar cells could transform to atypical pseudo-ductular structures [48–50]. These reports led us to speculate that acinar cells but not pancreatic ductal cells could be progenitor cells for PDACs at least partly. Recently, it has been reported that acinar cells targeting oncogenic K-*ras* in adult mice induce a spontaneous induction of mouse PanINs (mPanINs) of all histological grades [51], showing the possibility of trans-differentiation of acinar to ductal metaplasia via K-ras activation. Therefore, acinar cell damage may be the first step in pancreatic ductal carcinogenesis, and this hypothesis may link cigarette smoke-induced acinar cell damage to the development of PDACs.

Disease related to CP and metabolic risk factors

AP

During the natural course of CP, AP commonly occurs. It is assumed that the recurrence of AP could be a predictor for subsequent CP. However, its frequency and the factors affecting AP progress to CP remain unclear. It has been shown in a population-based study that the ratio of admission for the recurrence of AP by the time from the first attack of AP to a median of 40 months was almost 30 %, while CP was about 10 % [52]. In this study, recurrence of AP and progression to CP were significantly affected by alcohol etiology and tobacco abuse.

Pancreatic duct stone- and gallstone-induced pancreatitis

Pancreatic duct stones are most commonly observed in CP patients with long-term alcohol abuse [53]. Pancreatic duct stones are thought not only to be the cause of CP but also to be a risk factor for pancreatic cancer.

On the other hand, gallstones are prevailing etiologies of AP as well as alcohol. Alcohol abuse is the most common cause of AP in men, while gallstone migration into the common bile duct constitutes the leading etiology in women [54]. Fat intake induces secretion of pancreatic juice and bile, which are needed for lipid assimilation. In addition, serum triglyceride (TG) levels are associated with gallstone formation [55] and also with pancreatic cancer risk [21]. Gallstones obstruct the ampulla of Vater along with retention and stasis of pancreatic secretory fluids and result in the reflux of bile into the pancreatic duct [56, 57]. It is suggested that refluxed bile acids could bind to a cell surface bile acid receptor, Gpbar1, expressed on the luminal membrane of acinar cells [58]. In vivo study of Gpbar1-deficient mice showed reduced severity of pancreatitis, indicating that refluxed bile acids are associated with pancreatitis development. Furthermore, inflammatory cells which are recruited

locally and activated in the pancreas may produce pro-inflammatory cytokines, such as interleukin (IL)-6, -8, and -18 and tumor necrosis factor-α (TNF-α) [59–63]. In addition, such pro-inflammatory cytokines may subsequently activate pancreatic stellate cells (PSCs) and trigger both fibrin deposition and scarring [64].

The evidence that a delay in cholecystectomy after an attack of AP increases the risk of recurrence [65] indicates that early cholecystectomy should be considered in all patients with biliary AP to prevent progression of AP to CP and pancreatic cancer.

Dyslipidemia

Hypertriglyceridemia is a well-recognized and the most common cause for AP related to gall bladder disease and alcohol consumption [66, 67]. Hypertriglyceridemia is reported to account for ~10 % of all AP episodes [67, 68]. In general, it is believed that a serum TG level of more than 1,000 mg/dL triggers pancreatitis [66]. In addition, disorders of lipoprotein metabolism, such as diabetes, obesity, and hypothyroidism, may be correlated with hypertriglyceridemic pancreatitis. One of the mechanisms accounting for the development of hypertriglyceridemic pancreatitis may be the formation of free fatty acids (FFAs) that induce inflammatory changes following hydrolysis of TGs by lipases. The very high concentrations of FFAs bind to plasma albumin to form micellar structures with detergent properties. Attack from these FFA micelles to the vascular endothelium leads to induction of ischemia in acinar cells and cell damage. The resultant ischemia creates an acidic microenvironment, which further enhances FFA toxicity [69].

Type II diabetes

Diabetes mellitus is a very common metabolic disorder with hyperglycemia, eventually affecting all systems in the body, and it is highly prevalent in the world. Although there are two types of diabetes mellitus, type I and type II, type II diabetes mellitus accounts for 90–95 % of all diagnosed cases of diabetes mellitus in adults.

In both type I and type II diabetes patients, blood glucose concentrations are high compared with healthy subjects. It has been reported that glucose converted into dicarbonyl compounds in a non-enzymatical manner under physiological conditions. Well-known products are methylglyoxal and glyceraldehyde. The dicarbonyl compounds react irreversibly with protein, DNA, and lipids by a Maillard reaction and result in forming glycation adducts, the so-called advanced glycation end products (AGEs) [70, 71]. The signals from cellular AGE receptors are implicated to activate pathways involved in the pathogenesis of vascular complications in diabetes [72]. In AP patients, the plasma-soluble form of the receptor for AGEs is significantly higher in patients who develop multiple organ dysfunction than in patients without multiple organ dysfunction [73]. Among AGEs, glycation products of DNA induce mutations in mammalian cells [74], which may lead to the development of neoplastic lesions. For instance, N^2-(1-carboxyethyl)-2′-deoxyguanosine produced by the reaction of 2′-deoxyguanosine with methylglyoxal induce G:C to C:G and G:C to T:A transversions in *supF* gene in simian kidney cells [74].

ROS production from mitochondria is susceptible to the damaging effects of high glucose conditions. It has been reported that ROS affects apoptotic cell signaling via modification of gene expression caused by the activation of NF-κB [75]. Free and ester forms of unsaturated fatty acids and cholesterol are easily attacked by ROS and oxidized. These lipid peroxidation products could also activate transcriptional factors [76]. As a result, activation of NF-κB turns to induce inflammatory cytokines and growth factors, which promotes inflammation status.

Emerging evidence indicates that type II diabetes mellitus is positively associated with an increased risk of pancreatic cancer with an odds ratio (OR) of 1.5–2.0 in long-term diseases (≥ 5 years) [5, 9, 77], but much higher relative risks were observed in patients with a short-term history of diabetes mellitus (OR 2.9 for ≤ 2 years) [77].

Obesity

Obese patients were found to be associated with a higher risk of developing severe pancreatitis [78, 79]. The mechanism by which obesity increases the severity of AP is unclear, but one hypothesis might be that obese patients have an increased inflammatory response within the pancreas [80, 81]. Recent experiments also show increased mortality and morbidity in obese rats and mice with AP and the implication of adipokines such as leptin and adiponectin. Such models are important in investigating whether the inflammatory response of the disease is enhanced by obesity [82, 83]. On the other hand, it is now clear that obesity is a pro-inflammatory condition. In obese patients, the abdominal fat tissues produce several chemokines, such as monocyte chemoattractant protein-1 (MCP-1), which attract macrophages and form crown-like structures in fat tissue. These activated macrophages are known to produce ROS and inflammatory cytokines and may produce basal conditions, which easily develop a severe inflammatory status.

The World Cancer Research Fund and American Institute for Cancer Research have evaluated causal relationships between body fat and cancer and provided strong evidence for roles in, for example, pancreatic cancers [84]. The fat content of the daily diet also plays an important role in the risk for cancer of the pancreas [85]. Furthermore, elevated serum TGs and a high intake of cholesterol may exert potential promotion on pancreatic carcinogenesis [21, 86].

Genetic risk factors: gene mutations affect CP

As mentioned earlier, environmental factor-inducing CP is one of the major risk factors for pancreatic cancer. However, cancers arising from CP were not only induced from after-birth daily customs but also from genetic alterations. Hereditary pancreatitis (HP) is a dominant autosomal disease. Mutations causing HP have been identified mainly in the *PRSS1* gene: cationic trypsinogen [87–90]. HP is usually characterized by exocrine and/or endocrine insufficiency and recurrent episodes of acute pancreatic attacks, which can progress to CP. The problem of the disease is that its symptoms begin at an early age, at less than 20 years old. These clinical spectra of HP were first reported in 1952 by Comfort and Steinberg [91].

HP patients have been examined for the risk of developing pancreatic cancer in a cohort from ten countries, as reported by the International Hereditary Pancreatitis Study Group [19]. Two hundred forty-six patients were surveyed with over 14 years of follow-up, and PDACs were found in eight patients, indicating a standardized incidence ratio of 53.3. The pooled risk estimate of three studies for pancreatic cancer in HP was reported to be 69.0 [10]. The total risk of pancreatic cancer incidence in the patients with chronic HP has been assessed at around 40 % [19]. These data demonstrated that HP gives a markedly higher risk of developing cancer than the sporadic disease, indicating that the genetic changes in HP could be closely related to pancreatic cancer induction.

Mutations in cationic trypsinogen gene (PRSS1)

The specific mutations responsible for HP were mapped to chromosome 7q35 in five families [92, 93] and identified as cationic trypsinogen gene (*PRSS1*) in 1996 [94].

A single-point mutation at the third exon of the *PRSS1* gene was demonstrated to exist in HP patients. This mutation changes amino acid at the 122nd position of cationic trypsinogen from arginine (CGC) to histidine (CAC): R122H. A second mutation in this gene was identified at exon 2, resulting in an amino acid change at the 29th position, from asparagine (ACC) to isoleucine (ATC): N29I [87]. These two mutations (R122H and N29I) have now been identified in families with HP from many countries [87, 89, 90, 94]. Some minor mutations, such as A16V, D22G, K23R, and E79A, K92A, R122C, etc., were also found at exons 2 and 3, respectively.

The majority of patients with cationic trypsinogen gene mutations have obvious family histories of pancreatitis. Vice versa, an investigation of patients with alcoholic CP showed no evidence of the R122H or N29I mutation [95].

Trypsinogen is secreted by pancreatic acinar cells [96] and is activated to trypsin by enterokinase. Trypsin activates many other digestive enzymes in a cascade fashion and finally is inactivated by trypsin inhibitor or digested by trypsin itself. R122H is thought to alter this trypsin digestive site which is recognized by trypsin, and the N29I mutation is also speculated to affect pancreatic secretory trypsin inhibitor recognition [88]. Both of these mutations may delay inactivation and prolong protease activity and are proposed to act as over-self digestive attacks to the pancreas.

Probably, CP, brought by *PRSS1* gene mutation and HP, is a highly influential cause for pancreatic cancer; however, it is not completely clear whether the risk of cancer is due to sustained inflammatory conditions or whether it is related to the presence of a cationic trypsinogen mutation [97]. For example, no specific relationship was observed between the *PRSS1*–R122H mutation and pancreatic cancer in 34 sporadic PDAC samples [98]. This evidence indicated that mutations in *PRSS1* are not causative for pancreatic cancer, but only for CP. On the other hand, PRSS1 and PRSS2 have been found to be overexpressed in pancreatic cancer cell lines and tissues [99, 100], and transfection of PRSS1 in pancreatic cancer cells, which lack PRSS1 expression, has been shown to increase the invasiveness of the carcinoma cells through activation of matrix metalloproteinases (MMPs) [101]. In addition, PRSS3 has shown to be overexpressed in metastatic pancreatic cancer cell lines and tissues, but not in non-metastatic cancer cells [102]. These findings indicate that an increase in the activity of trypsin by mutations that inhibit its inactivation or by overexpression can promote pancreatic cancer progression.

Mutations in pancreatic secretory trypsin inhibitor: serine protease inhibitor, Kazal type 1 gene (SPINK1)

Mutations at the cationic trypsinogen gene are the most frequent responsible gene alterations for HP; however, fewer cases were found in idiopathic CP without family history. As for gene mutations other than the cationic trypsinogen gene, the mutation N34S in the pancreatic secretory trypsin inhibitor (SPINK1 or PST1) was reported to be associated with idiopathic CP [103].

SPINK1 product specifically inactivates pancreatic trypsin. In this gene, some amino acid mutations alter the conformational molecular structure of this protein product and diminish the secretion of mature protease inhibitor [104]. This inhibitor activity deficiency results in the continuous activation of trypsin protease activity and run-on self-digestion, the same as the cited mutation mechanism of *PRSS1* [103]. However, the functional defect in the N34S mutant was not observed [105]. *Spink3*, a mouse homolog gene of human *SPINK1*, has been shown to act not only as trypsin inhibitor but also as a negative regulator of autophagy. A deficiency of *Spink3* showed excess autophagy, followed by enhanced trypsin activity in the exocrine pancreas, suggesting that CP caused by mutations of the *SPINK1* gene is due to autophagy induction, but not to loss of binding to trypsin [106, 107].

The mutation of *SPINK1* was found to be the most frequent mutation in idiopathic CP (~25 %) [103, 108]. However, the presence of N34S mutation was not associated with early disease onset or disease severity, indicating that this mutation was not sufficient to be responsible for pancreatitis but could induce pancreatitis with other additional environmental factors [108, 109].

Other gene mutations

In CP, mutations of the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) and α1-antitrypsin gene (*SERPINA1*) were often detected [110, 111]. As plugging of the small pancreatic ducts occurs in cystic fibrosis with loss of CFTR function, and the α1-antitrypsin gene acts as physiological trypsin inhibitor in blood, mutations of these genes might be suspected to correlate with CP. In a gene mutation survey for pancreatitis risk, some were detected, but the ratio was not significant, and so genetic screening for these genes is generally not recommended to date [112].

Roles of inflammation in pancreatic carcinogenesis

DNA damage

ROS derived from either inflammatory/immune phagocyte cells or the mitochondria of epithelial cells act as central endogenous carcinogens and induce DNA damage in epithelial cells. Inflammatory cells, especially tumor-associated macrophages, also release cytokines that can promote chronic oxidative stress in the affected tissue. 8-OHdG elevated by oxidative stress induces mutations in replicating cells by preferentially mispairing with adenine during DNA synthesis, resulting in G:C to T:A transversions [113], while tobacco-derived carcinogenic nitrosamines such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) produce O^6-methyl-guanine and induce G:C to A:T transition [114]. Mutations of K-*ras* and other genes, such as *Dpc4*, could have accumulated already in non-malignant, inflammatory pancreatic tissue [115]. K-*ras* mutations in CP were only found after a disease duration of 3 years [116]. These data suggest that CP could induce gene mutations. Of note, some CP patients with K-*ras* mutations were observed prospectively and reported to develop pancreatic cancer [117, 118].

Cell proliferation/anti-apoptosis

Epidermal growth factor receptor

Epidermal growth factor receptor (EGFR) is a transmembrane receptor that binds epidermal growth factor (EGF) and transforming growth factor (TGF)-α then stimulates phospholipase Cγ1 activity, leading to cell proliferation. TNF-α-induced protein 8 (TNFAIP8) expression is positively correlated with EGFR levels in pancreatic cancer [119]. In CP and pancreatic cancer, expressions of EGFR and TGF-α are up-regulated in ductal and acinar cells, and TGF-α is considered to act through autocrine and paracrine mechanisms to excessively activate the overexpressed EGFR [120, 121]. However, EGF is expressed at the late phase of pancreatitis and is involved in pancreatic repair and regeneration [122]. Expression of epiregunin, a member of the EGF family, is also up-regulated in the ductal and acinar cells in CP and pancreatic cancer cells [123].

The regenerating protein family

Regenerating (Reg) protein family members, such as Reg1α, 3β, 3δ, and 4, have anti-inflammatory, anti-apoptic, and mitogenic roles and are associated with various pathologies, including pancreatitis, diabetes, and forms of gastrointestinal cancer.

Reg1, also known as pancreatic stone protein, is expressed in acinar cells despite normal conditions and AP or CP, and cancer. Reg1 may play a role in the transdifferentiation of acinar cells to islets in CP because Reg1 is not observed in healthy islets, but only when the islets are damaged, and it increases as observed in regenerating or hyperplastic islets after damage [124, 125]. Moreover, Reg1α protein is preferentially expressed in carcinoma cells of pancreatic cancer patients with diabetes over that in subjects without diabetes [126].

Reg3β, also known as pancreatitis-associated protein, is secreted as a stress protein through NF-κB activation by TNF-α and inhibits acinar cell apoptosis [124]. Reg3β protein is strongly expressed in acini adjacent to invasive adenocarcinomas, and Reg3β levels in both serum and pancreatic juices are significantly higher in pancreatic cancer patients compared with those of a control group [127].

Islet neogenesis-associated protein (INGAP), a hamster homolog of mouse Reg3δ, is expressed in acinar cells during islet neogenesis, and INGAP protein could initiate duct cell proliferation, a prerequisite for islet neogenesis. INGAP may also act on islet genes involved in β-cell metabolism and insulin secretion [128].

Reg4 is expressed in rat acinar cells and human insulin-producing β cells. Reg4 is up-regulated via the overexpression of glioma-associated oncogene homologue GLI1, a key transcription factor in the Hedgehog signaling pathway, in pancreatic cancer cells [124, 128].

Other growth factors

Fibroblast growth factor (FGF)-1 and 2, insulin-like growth factor (IGF)-1, and hepatocyte growth factor are also markedly increased in pancreatitis tissue [129]. Expression of FGF-2 is strongly associated with the proliferation of tumor cells and

intratumor endothelial cells, suggesting that its increased expression may give tumors a growth advantage [130].

Angiogenesis

The number of blood vessels is significantly higher in PDACs, CP with or without a mutant K-*ras* genome, compared to surrounding normal areas. The fact that microvessel density was significantly elevated in CP patients with mutant K-*ras* in their genome, compared to patients with a normal K-*ras* genome, suggests that oncogenic K-*ras* may modulate tumor angiogenesis [131].

Vascular endothelial growth factor (VEGF) is a key component in pancreatic tumorigenesis, and high expression levels are correlated with poor prognosis. Even if in CP, VEGF is strongly expressed in ductal cells as observed in carcinoma cells [132]. Serum levels of VEGF and another factor, angiopoietin-2, have also been shown to be significantly higher in CP and pancreatic cancer patients than in a control group [133]. In addition, angiotensin II increases the production of VEGF, and VEGF production could be prevented by an angiotensin II type 1 receptor antagonist, losartane, or an angiotensin 1-covering enzyme inhibitor, captopril [134].

Fibrosis

The development of pancreatic fibrosis is a major component of pancreatic diseases including CP, type II diabetes, and pancreatic cancer. In the progression of pancreatic fibrosis, PSCs have been identified as a key mediator. PSCs are normally quiescent cells and characterized by star-like morphology. When activated by tissue injury, oxidative stress, growth factors, or cytokines, they are transformed to a myofibroblast-like phenotype and produce excess extracellular matrices (ECMs), such as collagen and fibronectin. Regarding growth factors and cytokines, TGF-β and platelet-derived growth factor are the strongest mediators of PSC activation. The heparin-binding epidermal growth factor-like growth factor overexpressed in pancreatic islet cells also facilitates fibrosis through secretion of ECMs [135]. Hypoxia also induces migration, type I collagen expression, and VEGF production in PSCs to stimulate fibrosis and angiogenesis [136].

Epithelial to mesenchymal transition

Epithelial to mesenchymal transition (EMT) is closely related with tumor growth and metastasis. During EMT, cells lose polarized epithelial phenotypes and acquire highly motile fibroblastoid or mesenchymal phenotypes, invasive properties, and stem cell-like features. TGF-β induces EMT along with overexpression of EMT-relating transcription factors Snail and Slug which repress E-cadherin expression. On the other hand, overexpression of Twist increases N-cadherin expression. It is interesting that these EMT markers are expressed in PDACs [137]. In a mouse model expressing oncogenic K-*ras*, inflammation was shown to enhance EMT in premalignant lesions. In such situations, inflammatory stroma were considered to be necessary for EMT and dissemination [138].

Major molecules and molecular pathways involved in inflammation-related pancreatic cancer

ROS

Oxidative stress is caused by an imbalance between the production of ROS, such as peroxides and free radicals, and the detoxification of reactive intermediates in a biological system. Smoking generates ROS [139]. Obesity also induces systemic oxidative stress. Several observations from human and animal models have suggested that obesity accelerates lipid peroxidation, which is an oxidative stress marker [140]. Oxidative stress could induce DNA damage, inflammation, apoptosis, and insulin resistance, resulting in the development of cancer [141, 142]. ROS can also prolong chronic inflammation by activation of NF-κB and could alter the regulation of adiponectin in adipose tissue [140, 143]. Thus, obesity-induced oxidative stress may contribute to an increased risk of pancreatic cancer.

Oxygen radicals react most readily with polyunsaturated fatty acids, resulting in peroxidation of lipids. Several experimental models of AP demonstrated the development of lipid peroxidation in pancreatic tissue [144–146]. In CP patients, tissue levels of lipid peroxidation products, such as conjugated dienes and malondialdehyde concentrations, were significantly elevated [147, 148]. The activation of oxygen-derived free radicals also occurs in CP tissues [149]. Thus, most studies have shown increased oxidative stress in patients with CP.

Cytokines

In the presence of chronic inflammation, large amounts of pro-inflammatory cytokines, growth factors, and proteases, such as TNF-α, IL-1β, IL-6, TGF-β, and MMPs, are secreted and activate oncogenic pathways in pancreatic cancer cells [150]. Single-nucleotide polymorphisms in TNF-α, IL-1β, and IL-6 have been reported to affect inflammatory response [151].

TNF-α

TNF-α is a pro-inflammatory cytokine and mainly secreted by activated macrophages. Significantly higher levels of TNF-α have been found in serum of CP and pancreatic cancer patients [152]. TNF-α production is also increased in obese adipose tissues and is involved in the development of insulin resistance [153]. TNF-α binds its receptors and activates NF-κB

through IκB kinase activation. TNF-α also activates c-Jun N-terminal kinases (JNKs), which regulate cell growth, differentiation, survival, and apoptosis.

IL-1β

Together with TNF-α, IL-1β also plays central roles in the regulation of immune responses and the inflammatory process. Caspase-1, which has been designated as IL-1β-converting enzyme, is also up-regulated in CP and pancreatic cancer [154]. IL-1β secreted by malignant cells or infiltrating leukocytes contributes to increased tumor adhesiveness and invasion, angiogenesis, and immune suppression [155] through activation of NF-κB [156] and JNK [157].

IL-6

Significantly higher levels of IL-6 have also been found in serum of obese people, type II diabetes, CP, and pancreatic cancer patients [151, 152]. In obese mice, IL-6 seems not to contribute to the increased severity of pancreatitis but delays its recovery from acute inflammation, which is explained by the prolonged activation of Stat3, induction of MMP-7, and sustained production of chemokines [158].

TGF-β

TGF-β is a multifunctional cytokine involved in embryonic development, cell proliferation, differentiation, angiogenesis, and wound healing. TGF-β also plays a role as a tumor suppressor via growth inhibition of premalignant cells, induction of autophagy, senescence and apoptosis, and suppression of inflammation. On the other hand, TGF-β, especially TGF-β1, is overproduced in a variety of human tumors, including pancreatic tumors, and is involved in tumor progression and metastasis via immunosuppression, induction of EMT, and angiogenesis [159]. TGF-β signaling is mediated by Smad-dependent and Smad-independent pathways. In pancreatic tumors, type I and II TGF-β receptors (Tgfbr1, Tgfbr2) or Smad4 are often mutated or deleted. Therefore, Smad-independent pathways could mediate the tumor-promoting effects of TGF-β signaling. In addition, TGF-β enhances motility and stimulates the recruitment of monocytes, macrophages, natural killer cells, neutrophiles, and T cells while directly inhibiting their anti-effector functions [160].

Inducible enzymes

Inducible nitric oxide synthase

Nitric oxide (NO) is an important bioregulatory mediator involved in a variety of processes in the cardiovascular, nervous, and immune systems [161]. Sustained release of NO causes immune cell cytotoxicity. Chronic infection and inflammation are associated with release of many cytokines along with activation of NF-κB, resulting in the expression of inducible nitric oxide synthase (iNOS). iNOS is a calcium-independent enzyme and induced by bacterial endotoxins and cytokines. NO is not only an important mediator in benign diseases but it is also implicated in cancer, considered as an endogenous mutagen, an angiogenesis factor, an enhancer of protooncogene expression, and an inhibitor of apoptosis [162, 163]. These observations suggest that iNOS overexpression with high levels of NO generation provides a plausible link between inflammation and cancer initiation, progression, and promotion.

Increased expression of iNOS has been detected in more than half of human PDACs and in severe AP tissues [164, 165]. In cerulein-induced rat AP, expression of iNOS produces a large amount of NO [166]. We also confirmed that iNOS expression was observed in hamster PDACs and atypical hyperplasia [167]. Our previous study revealed that iNOS expression can be markedly elevated by transfection of K-*ras* mutant cDNA into IEC-6 rat intestinal epithelial cells in the presence of IL-1β or lipopolysaccharides through the activation of promoters at NF-κB, C/EBP, and CRE-like sites. The growth of tumors formed in nude mice by a subcutaneous injection of the K-*ras* mutant-transfected cells can be suppressed by feeding diets containing NOS inhibitors [168]. It is feasible that iNOS expression in PDACs could also be associated with K-*ras* activation since human PDACs frequently harbor K-*ras* mutations [169] and show increased iNOS expression [164]. Chemically induced hamster carcinomas also quite frequently harbor G to A transitions at the second base of codon 12 of the K-*ras* gene [170]. Thus, NO produced by iNOS may be generally involved in tumor development by activated K-*ras*.

Cyclooxygenase 2

Cyclooxygenase 2 (COX-2) is an inducible immediate early gene involved not only in inflammation and cell proliferation but also in differentiation, anti-apoptosis, metastasis, immunologic surveillance, and angiogenesis [171]. COX-2 expression is regulated via the Wnt and Ras signaling pathways. Mutant β-catenin expression up-regulates COX-2 promoter activity, and induction of mutated K-*ras* increases COX-2 levels through stabilization of COX-2 mRNA [172, 173].

As for pancreatitis, elevated COX-2 activity appears to correlate with chronic inflammation. COX-2 plays an important pro-inflammatory role in experimental pancreatitis models [174, 175]. COX-2 is also strongly increased in pancreatic specimens from patients with CP [176, 177]. These studies revealed that COX-2 was localized to atrophic pancreatic acinar cells, islets, and duct cells, providing a potential target for treatment of patients with CP. The beneficial effects of inhibition of COX-2 or the knock-out of its gene in experimental AP

have been demonstrated [174, 175, 178]. Song et al., using a mouse model of experimental cerulein-induced AP, showed that the severity of pancreatitis was reduced in COX-2-deficient mice compared with the noninhibited strains of COX-2-sufficient mice [174]. Up-regulation of COX-2 has been also observed in PanIN lesions and PDACs in both humans and BOP-treated hamsters [179].

Transcriptional factors

NF-κB

NF-κB is known to be a master inflammatory transcriptional regulator and is highly activated in macrophages. Targets of NF-κB include genes regulating immune response, inflammation, cell proliferation, cell migration, and apoptosis. The nuclear translocation of NF-κB can activate target genes involved in carcinogenesis [180]. NF-κB has the potential to lead the amplification of the inflammatory response in the tumor environment. The expression of both IL-1β and TNF-α is stimulated by NF-κB, suggesting an autoregulatory loop that can amplify the inflammatory response. As mentioned previously, NF-κB also stimulates the expression of iNOS and COX-2 [15].

STAT3

The inflammatory mediator, signal transducer, and activator of transcription 3 (Stat3), a critical component of pancreatitis, has been shown to accelerate PDAC precursor formation. Stat3 is activated by phosphorylation of key tyrosine (Tyr705) and serine (Ser727) residues. The activation is triggered by binding of IL-6 to IL-6 receptors and subsequent activation of receptor-associated tyrosine kinase, Janus kinase. In the nucleus of tumor cells and infiltrating immune cells within the tumor microenvironment, Stat3 and NF-κB co-regulate numerous genes controlling cell proliferation and survival, such as c-Myc, cyclin D1, and Bcl-2, and contribute to metaplasia during inflammation-associated neoplastic development [150].

Stat3 regulates the expression of MMP7, a member of a family of zinc-dependent endopeptidases, during mPanIN development following pancreatitis. Furthermore, MMP7 in carcinoma cells contributes to tumor size and metastasis in mice [181]. In humans, MMP7 is overexpressed in PanINs and PDACs [182, 183] and correlates with decreased survival and possibly tumor size, lymph node metastasis, and distant metastasis [182, 184, 185]. In vitro studies also support the idea that MMP7 is involved in cancer progression by dictating the invasive and metastatic capacity of PDAC cells [182]. Thus, Stat3 and MMP7 are thought to be key mediators of both initiation and progression of pancreatic carcinogenesis. In addition, both Stat3 and MMP7 could be potential novel targets for future therapies since serum MMP7 levels in PDAC patients were correlated with metastatic disease and survival [181].

Evidence of involvement of inflammation in pancreatic carcinogenesis in experimental animals

Animal models for pancreatitis

Ethanol

Excessive alcohol consumption is a major risk factor for developing CP [186]. However, the pathobiology of this disease remains unclear because of a lack of animal models representing alcohol-induced CP. Indeed ethanol feeding by itself does not affect pancreatic injury in animal models. Its metabolites on acinar cells may promote CP changes by its toxic effects [187]. Moreover, oxidant stress [188] and activation of PSCs promote inflammation [186]. Thus, administration of ethanol in combination with caerulein, cyclosporin A, or high-fat diet has been shown to enhance pancreatitis [189, 190].

Caerulein

A caerulein-induced rodent pancreatitis model is widely used and is one of the best-characterized experimental varieties. Caerulein is an analog peptide of cholecystokinin (CCK), and its frequent intraperitoneal (i.p.) injection causes pancreatic hypertrophy, characterized by increased pancreatic weight, increased amylase content, and acinar cell hyperplasia. This model is useful because either AP or CP could be induced by modulating the treatment schedule and its doses [191, 192].

Choline-deficient ethionine-supplemented diet

Choline-deficient ethionine-supplemented (CDE) diet is known to induce AP [193–195]. Ethionine is the analog of the essential amino acid methionine and induces exocrine pancreatic insufficiency by inhibiting the activation of phospholipase C in pancreatic acinar cells [196–198]. Moreover, ethionine diet alone can increase the amount of digestive enzymes by decreasing the digestive enzyme discharge [199]. As a result, AP will develop. Pancreatitis induced by CDE diet is an ideal model because of its natural history, histological features, and biochemical changes that are similar to those of humans [193]. In addition, the method of this model is relatively simple and not expensive. This model could also develop CP by repeating a CDE diet and standard diet by turn [200].

High-fat diet

A long-term high-fat diet can induce rat CP [201]. In mice, a high-fat diet with treatment of IL-12 plus IL-18 can induce

AP [202]. A high-fat diet with caerulein can also increase plasma amylase activity in mice [203]. Moreover, a high-fat diet leads to obesity [204], which enhances the severity of AP [205]. Thus, a high-fat intake may play a pivotal role in the development of CP.

Up-regulation of K-ras activity

Ras signaling pathways are activated by several stimuli, including CCK, known to cause CP [206], or TGF-α [207, 208]. On the other hand, elevation of Ras activity in acinar cells in a genetically engineered mouse (GEM) model has been reported to induce inflammation and fibrosis resembling the histological features of human CP, characterized by loss of acinar cells, acinar-to-ductal metaplasia, leukocyte infiltration, replacement by stroma with collagen, and activated PSCs [209, 210].

Animal models for pancreatic carcinogenesis

Chemically induced PDAC model in hamsters and other rodents

The Syrian golden hamster is in a hyperlipidemic state even under normal diet conditions because the lipoprotein lipase activity in the liver is low compared with mice and rats [211]. The hamster is a unique model animal for the development of PDACs induced by subcutaneous (s.c.) injections of BOP [212]. Histopathologically, the induced lesions possess close similarities to pancreatic cancer in humans. Moreover, point mutations in codon 12 of the K-*ras* gene are frequently observed, and expression of the *fragile histidine triad* (*Fhit*) gene is aberrant in BOP-treated hamsters [213, 214], as was also observed in human PDACs [215, 216]. The *p16* gene is one of the most frequently inactivated tumor suppressor genes in human PDACs [217], and loss of *p16* expression has also been found in hamster PDAC lesions [218].

The intrapancreatic implantation of 7,12-dimethyl-1,2-benzanthracene (DMBA) model in rats and mice is known to cause tubular complexes and produce mPanIN lesions and PDACs derived from acinar cells [219, 220]. The tumors developed in this model have also been shown to harbor K-*ras* mutations [221], and activated PSCs surround precancerous duct cells as they do in human pancreatic cancers [222]. Thus, pancreatitis induced by implantation of the chemical carcinogen could develop fibrosis and enhance K-*ras* activation.

GEM pancreatic cancer models

In 2003, Hingorani et al. developed a GEM model that specifically expressed an oncogenenic K-*ras*^{G12D} mutation from its endogenous gene locus in pancreatic progenitor cells during embryologic development through *Cre*-mediated recombination driven by *Pdx1* regulatory elements [223]. Since then, several GEM models of pancreatic exocrine neoplasia have been developed. An activating mutation of the K-*ras* is the most frequent genetic alteration associated with pancreatic carcinogenesis, having been identified in up to 90 % of all PDACs [224, 225]. K-*ras* mutations are also observed in early lesions, such as atypical ductal hyperplasia and PanIN, in humans [226]. Therefore, the GEM models are commonly based on K-*ras* mutations such as with Pdx-*Cre*/Lox-Stop-Lox (LSL)-*Kras*G12D or p48^{Cre}/LSL-*K-ras*^{G12V} mice. Unlike in the case of humans, acinar to ductal metaplasia is the predominant precursor lesion for pre-neoplastic and neoplastic lesions in a GEM model. However, pancreatic cancer arising from mPanINs is very similar to human pancreatic carcinogenesis [227]. Furthermore, these mice were modified by conditional deletions or mutations of *p16* [228], *p53* [229], *dpc4* [230], and *TGF−β receptor II* [231]. These combined genotypes cause multiple preinvasive lesions of all grades, invasive adenocarcinomas, and metastasis to other organs, ultimately leading to a significantly reduced median survival. Pancreatic carcinogenesis induced by pancreatitis in K-*ras*-based models is hoped to provide preclinical model systems to analyze the molecular biology of this disease and to evaluate the benefit of new therapies.

Animal models with combination of inflammation and carcinogenesis

Exposure to ethanol

Transplacental treatment with NNK plus ethanol in Syrian golden hamster to induce PDACs is an interesting model to confirm the synergistic effect of cigarette smoking and alcohol drinking on fetuses [232]. Nicotine-derived NNK is one of the most potent tobacco-specific carcinogens and thus is an excellent model compound for studies of the potential carcinogenic effects of cigarette smoke [233]. NNK is also known to be an active transplacental carcinogen in Syrian golden hamsters. Although the main target site for NNK-induced tumor development is the respiratory tract (nasal cavity, lungs, trachea, and larynx) in adult hamsters and offspring, focal ductular hyperplasias and/or small ductal adenocarcinomas of the pancreas were also occasionally found in offspring [234]. The simultaneous treatment of pregnant hamsters with ethanol and NNK significantly elevates the carcinogenic response in their offspring compared to animals exposed in utero to NNK alone. Especially, the simultaneous in utero exposure to ethanol and NNK resulted in the induction of PDACs, while in utero exposure to ethanol alone induces pancreatitis in the offspring [232]. These findings indicate that alcoholic pancreatitis itself does not induce PDACs but markedly enhances smoking-induced PDAC development.

Wendt et al. reported the influence of ethanol on pancreatic carcinogenesis using a DMBA-induced mouse pancreatic carcinogenesis model. The mice received water or 6 % ethanol in their drinking bottle, and 1 mg of DMBA was implanted into the head of the pancreas. The ethanol-drinking group had a significantly greater incidence of invasive adenocarcinomas than the water-drinking group [235]. Thus, association between ethanol and pancreatic carcinoma development was evident.

In a BOP-induced hamster pancreatic carcinogenesis model, administration of 20 % ethanol in their drinking water slightly increased the multiplicity of PDACs, although there were statistically no significant differences regarding lesion incidence [236]. On the other hand, an inhibitory effect of ethanol on BOP-induced pancreatic carcinogenesis in hamsters when given in the initiation phase was reported [237]. The administration period might be important for investigating the modulating effects of ethanol.

Caerulein-induced pancreatitis

Administration of caerulein in hamster pancreatic carcinogenesis model is a beneficial model for inflammation-related pancreatic carcinogenesis. Two groups of hamsters received BOP at a dose of 5 mg/kg once weekly for life by s.c. injection. One group also received exogenous CCK 30 IDU/kgs.c. for 3 days per week for 6 weeks on the day before, the day of, and the day after BOP injection. In the CCK-treated group, a significant excess of panlobular ductular proliferation was found at 10 weeks after the first BOP treatment. At 15 weeks, the incidence of adenocarcinoma in a BOP + CCK group and BOP-alone group was 71 and 20 %, respectively [238]. Thus, CCK treatment enhanced pancreatic carcinogenesis, acting as co-carcinogen or promoter of pancreatic carcinogenesis.

Some pancreatic carcinogenesis experiments in GEM have demonstrated that caerulein-induced pancreatitis can enhance carcinoma development. In LSL-K*ras*^{G12D}/Pdx1-Cre mice, chronic treatment with caerulein (a single daily i.p. injection of 5 μg/animal at 5 days per week during the duration of the study) induces CP and significantly enhances the development of PDACs [206]. Acute chemical pancreatitis induced by caerulein (seven-hourly i.p. injections at a dose of 50 μg/kg of body weight repeated 48 h later) has also been demonstrated to cause rapid acinar-to-ductal metaplasia and mPanIN progression and accelerate PDAC development in a GEM model in which oncogenic K-*ras* is activated in all pancreatic cell types [239]. Thus, even a brief inflammatory insult to the pancreas, when occurring in the context of oncogenic K-*ras*^{G12D}, can initiate a cascade of events that dramatically enhances the risk for malignant transformation.

In a K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mouse model, oncogenic K-*ras* expression in acinar cells of adult mice did not cause any pancreatic lesions, even when loss of $p16^{Ink4A}$/$p19^{Arf}$ or *Trp53* tumor suppressors were introduced. The findings indicate that adult acinar cells are extremely resistant to malignant transformation. However, chronic caerulein treatment induced mPanINs and PDACs in mice expressing K-*ras*^{G12V} in adult acinar cells, suggesting that pancreatitis contributes to tumor progression by abrogating the senescence barrier characteristic of low-grade mPanINs. Even though pancreatitis occurs before the activation of K-*ras* oncogenes, PDACs can also develop in injured acinar cells expressing oncogenic K-*ras* [240]. These findings show that CP could give irreversible or prolonged changes to promote pancreatic carcinogenesis stronger than loss of tumor suppressors.

In response to caerulein-induced AP, acini dedifferentiate to develop duct-like structures with transient expression of embryonic factors, a characteristic of pancreatic embryonic progenitors. In the absence of mutant K-*ras*, re-differentiation into functional acinar cells occurs rapidly within a week [241–245]. In contrast, mutant K-*ras* locks damaged acinar cells in a persistently dedifferentiated ductal state that can rapidly give rise to PanINs [244, 245]. Thus, pancreatitis provides a permissive environment for K-*ras*-driven neoplasia. Moreover, Stat3 activation in pancreatic epithelial cells may play a significant role in the process [181]. Pancreatitis induction in K-*ras*-driven mouse pancreatic carcinogenesis models has revealed that inflammatory damage can promote and/or accelerate mPanIN and carcinoma formation.

Augmentation of cancer development by CDE diet

Long-term administration of a CDE diet can induce CP. The set of 7 days was defined as one cycle and the cycles were repeated to develop CP in mice. In this set of 7 days, mice were starved for 24 h (day 0) and then fed a CDE diet for 72 h (days 1 to 4) and after that fed regular laboratory chow for 72 h (days 5 to 7). After 24 weeks, CP-like features characterized by acinar atrophy, fibrosis, and well-developed tubular complexes were established. Key events in the process of pancreatic carcinogenesis, such as EGFR overexpression, FGF receptor phosphorylation, and K-*ras* gene mutation, were also detected in the mice fed the long-term CDE diet [200]. However, CP in this model is not sufficient for the development of PDACs. A further combination of the CDE diet with the administration of other promoting insults or genetic alterations may result in PDAC development.

A rapid production model, called "augmentation pressure," was established for PDAC development in Syrian hamsters [246, 247]. In this method, Syrian hamsters received s.c. injection of BOP (70 mg/kg) as the initiation dose. At 11 days after BOP initiation, the animals received four daily i.p. injections of DL-ethionine (500 mg/kg) combined with a choline-deficient diet. The hamsters were then returned to basal diet and given a single i.p. injection of L-methionine (800 mg/kg)

followed by a single s.c. injection of BOP (20 mg/kg) at day 5 after the beginning of the augmentation pressure cycle. In hamsters receiving BOP initiation followed by three cycles of augmentation pressure, atypical hyperplasia of the ductal epithelium and intraductal carcinomas were evident on day 46 after the beginning of the experiment, and development of intraductal carcinomas and invasive PDACs was observed on day 70. K-*ras* mutations were observed to occur in atypical hyperplasia (56 %) and in intraductal carcinomas (57 %) [247]. The augmentation pressure model is useful for investigating potential modulating factors of pancreatic carcinogenesis because large numbers of lesions can be developed within only 10 weeks.

Increases in ectopic fat and inflammatory factors by high-fat diet

Feeding high-fat diets has been demonstrated to increase PDAC development in several animal models. However, the promoting mechanisms of obesity on pancreatic carcinogenesis have yet to be completely elucidated.

We examined whether aggravated hyperlipidemia with a high-fat diet affects pancreatic carcinogenesis using BOP-treated hamsters. A high-fat diet is shown to increase serum lipid levels and enhance fatty infiltration in the pancreas along with abnormal adipokines production. Acinar cell damage caused by BOP treatment and hyperlipidemia may contribute to fatty infiltration [248]. Pancreatic fatty infiltration has been shown to be associated with a high body mass index, elevated visceral fat weight and serum lipids, and diabetes mellitus in humans [249, 250]. Thus, fatty infiltration may also accelerate and enhance pancreatic cancer through elevation of adipokines and inflammatory factors. Indeed the expression of inflammatory-related genes, including MCP-1, IL-1β, and COX-2, was found to be increased or tended to be increased in the pancreas of hamsters treated with BOP + high-fat diet [248]. The levels of mRNAs encoding growth-related genes such as insulin, IGF-I, and cyclin D1 were also elevated in the pancreas in the BOP + high-fat diet group, indicating enhanced proliferation by the high-fat diet. The enhanced pancreatic fatty infiltration and the elevation of inflammatory- and proliferation-associated genes in the hamster pancreas by the high-fat diet were suggested to be involved in the promotion of PDAC development.

In addition, a high-fat/high-protein diet containing 30 % protein and 30 % fat was shown to increase the prevalence of PDACs in rats implanted with DMBA compared to those in rats fed a normal diet containing 23.4 % protein and 4.5 % fat, the prevalence being 29 vs 17 % at 9 months. [221].

Inflammation-related pancreatic carcinogenesis with a high-fat diet was also investigated in mice with pancreas-specific (p48-Kras) and acinar-specific (Ela-Kras) expression of oncogenic K-*ras* [251]. In 30-week-old p48-Kras mice, the incidences of mPanIN1b, mPanIN2, and mPanIN3 lesions were higher in the high-fat diet group compared to the regular chow diet group, being 75, 50, and 12.5 vs 44, 22, and 0 %, respectively. Feeding with a high-calorie diet accelerated mPanIN development in p48-Kras mice. Along with increased infiltration of inflammatory cells in the pancreas, inflammatory cytokines such as TNF-α, IL-1β, and IL-6 mRNA and circulating levels of TNF-α and IL-6 were significantly elevated in p48-Kras mice. To investigate the role of TNF-α on increased mPanIN development, p48-Kras mice were further crossed with TNF receptor (TNFR) 1-deficient mice. TNFR1 deletion significantly attenuated high-fat-diet-induced PanIN development in p48-Kras mice. $TNFR1^{-/-}$-p48-Kras mice revealed lesions mostly residing at the head of the pancreas. The pancreas contained mostly intact acini and reduced fibrosis. Thus, increases in inflammation and TNF-α played an important role in developing tumor promotion by feeding a high-fat diet in p48-Kras mice.

Inflammation induced by elevated levels of Ras activity

Ras activity is greatly up-regulated in all PDAC cells in mice and humans. In GEM models, transformation of acinar cells by endogenous levels of mutant K-*ras* needs another genetic ablation of the tumor suppressor genes. On the other hand, overexpression of mutant K-*ras* in acinar cells has been shown to develop abundant mPanINs, cystic papillary carcinomas, and PDACs, accompanied with widespread senescence of acinar cells, profound inflammation, and fibrosis. Expression of tumor suppressor genes *p15* and *p16* were found to be elevated in K-*ras*-driven inflammatory tissue but lost in cancer cells [209]. Loss of tumor suppressors is permissive for cells to evade oncogenic K-*ras*-induced senescence, and spontaneous loss of those is accelerated by Ras activity due to the increase in genetic instability. It has also been reported that Twist expression elevated by oncogenic K-*ras* suppresses p16 expression [252].

Anti-inflammatory agents as a good candidate for chemopreventive agents

Importance of prevention against pancreatic cancer

Similar to other solid tumors, the curative potential for pancreatic cancer is dependent on the stage of disease at diagnosis. As the progress of pancreatic cancer is very silent, symptoms do not develop until it is either unresectable or metastatic, and the majority of patients with pancreatic cancer are diagnosed incurable. Moreover, even when patients are diagnosed at an early stage, the likelihood of cure remains very low with currently available therapies. As