PATHOBIOLOGY IN FOCUS

Mechanisms of parenchymal injury and signaling pathways in ectatic ducts of chronic pancreatitis: implications for pancreatic carcinogenesis

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The pathobiology of chronic pancreatitis (CP) remains enigmatic despite remarkable progress made recently in uncovering key mechanisms involved in the initiation and progression of the disease. CP is increasingly thought of as a multifactorial disorder. Apoptosis plays a role in parenchymal destruction, the pathological hallmark of CP. The apoptotic mechanisms preferentially target the exocrine compartment, leaving endocrine islets relatively intact for a prolonged period. Exocrine cells shed their 'immunoprivileged' status, express death receptors, and are rendered susceptible to apoptosis induced by death ligands on infiltrating lymphocytes, and released locally by activated pancreatic stellate cells. Islet cells retain their 'immunoprivileged' status and activate anti-apoptotic programs through NF-κB. Ductal changes, including distortion, dilatation, and pancreatic ductal hypertension in the setting of CP, induce genomic damage and increased cell turnover. In addition, signaling mechanisms that play a role in the development of embryonic pancreas are reinstated, thus, playing a role in repair, regeneration, and transformation. This, in turn, leads to acino-ductal metaplasia (ADM) and pancreatic intraepithelial neoplasia (PanIN). Some of these pathways are activated in pancreatic cancer. We attempt to integrate the current knowledge and major concepts in the pathogenesis of CP and to explain the mechanism of differential cell loss. We also discuss the possible implications of signaling pathway activation in pancreatic inflammation, relevant to the cellular transformation that leads to pancreatic neoplasia.

Chronic pancreatitis (CP) is a progressive, destructive, and inflammatory process of multifactorial etiology that leads to irreversible obliteration of the exocrine and endocrine pancreatic tissues and to its replacement by fibrous tissue, which ultimately results in the clinical manifestations typical of an 'end-stage' disorder of pancreatic function. The disease has an overall bleak long-term outlook and predisposes to the develoment of pancreatic cancer. In developed countries, 60–70% of patients with CP have a long history of heavy alcohol consumption. Other etiologies of CP include auto-immune disease, hypertriglyceridemia, hyperparathyroidism, tropical pancreatitis, pancreas divisum, obstruction of pancreatic duct by tumor, and genetic abnormalities.[1] In a significant number of patients (10–30%), CP is not associated with any of the known processes and is, therefore, termed idiopathic. There is accumulating evidence that many of these 'idiopathic' cases have a genetic basis. This has caused the idiopathic category to shrink in recent years.

Using the TIGAR-O system, major predisposing risk factors for CP have been categorized as toxic-metabolic (T), idiopathic (I), genetic (G), autoimmune (A), recurrent acute pancreatitis (R), or obstructive (O).[2,3] However, none of these risk factors alone consistently result in CP. For example, alcohol is used by a far greater number of people than those who actually go on to develop CP, indicating variable genetic susceptibility. Recent studies suggest that CP has a strong genetic basis, and increasing knowledge of gene–environment interactions has provided new insights into the pathophysiology of CP[4] that is increasingly being thought of as a multifactorial disorder, in which multiple risk factors operate together during disease initiation and its progression.[3] This is an important conceptual change in the understanding of CP.

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In the past decades, four major theories have emerged to explain the pathogenesis of CP.[5] In brief, these are:

(1) *Oxidative stress.* Alcohol-induced oxidative stress may generate free radicals in acinar cells, leading to membrane lipid oxidation and to the activation of transcription factors, including activator protein 1 and NF-κB, which, in turn, induce the expression of chemokines that attract mononuclear cells. Oxidative stress thereby promotes the fusion of lysosomes and zymogen granules, acinar cell necrosis, inflammation, and fibrosis.

(2) *Toxic-metabolic.* Toxins, including alcohol and its metabolites, can exert a direct toxic effect on acinar cells. This may lead to the accumulation of lipids in acinar cells, acinar cell loss, and eventually parenchymal fibrosis.

(3) *Ductal obstruction by concretions.* Some of the inciting agents responsible for the development of CP, such as alcohol, are believed to increase protein concentrations in the pancreatic juice. These proteins form proteinaceous ductal plugs that are observed in most forms of CP, but are particularly prominent in alcoholic CP. The ductal plugs may calcify, forming calculi comprising calcium carbonate precipitates that can further obstruct the pancreatic ducts and contribute to the development of CP.

(4) *Necrosis–fibrosis.* Acute pancreatitis results from auto-digestion of the pancreatic tissue by an inappropriate activation of pancreatic enzymes, causing necrosis of acinar cells and the subsequent immune responses as evidenced by the inflammatory infiltrates. It has been proposed that acute pancreatitis initiates a sequence of perilobular fibrosis, duct distortion, and altered pancreatic secretions. Over time and with multiple episodes, this can lead to loss of pancreatic parenchyma and its replacement by fibrosis.[5]

New Developments

Novel concepts integrating the cellular, genetic, and molecular mechanisms have been put forth for explaining the pathogenesis of CP. This definitely promotes a better understanding of the complex pathobiology of CP. According to the Sentinal Acute Pancreatitis Event (SAPE) hypothesis, unregulated trypsin activation initiates the first episode of acute pancreatitis (sentinal event) (Figure 1).[3] An isolated episode may not be enough for sustaining inflammation and its harmful sequels, and the tissue damage may be minor. With complete resolution of the tissue damage, the episode may go unobserved (Figure 2). However, further recurrence or persistent exposure to injurious factors, sets into motion a chain of events characterized by a well-sustained inflammatory response. Cytokines liberated during the early inflammatory phase attract a distinct cellular infiltrate, whereas profibrotic cells including stellate cells are activated in the later phase of acute pancreatitis. The attraction and

activation of pancreatic stellate cells (PSCs) sets the stage for the development of pancreatic fibrosis (Figure 1). The resultant scarring leads to the development of strictures that block the pancreatic ducts, which predispose to recurrent attacks of acute pancreatitis.

These observations together with the recently discovered genetic mechanism of hereditary pancreatitis[6,7] and the pathological case series,[8] lend credence to a close relationship between acute pancreatitis, recurrent acute pancreatitis, and in the development of CP. According to Fukumura et al,[9] small duct epithelia may be the main source of the fibrogenic cytokine, TGF-β, which has been suggested as the main mediator of pathological extracellular matrix accumulation in pancreatic fibrosis.

Genetic Causes of CP

The genetic alterations associated with CP are described below.

PRSS1

Whitcomb et al identified the third exon of the cationic trypsinogen gene on chromosome 7q35. Hereditary pancreatitis is caused by germ-line mutations in the cationic trypsinogen gene, also known as *PRSS1* (protease, serine1). The most common *PRSS1* mutation results in an arginine-to-histidine substitution, thereby eliminating the key site essential for the rapid self-destruction of trypsin in solutions. Therefore, trypsin becomes resistant to inactivation, and the abnormally active trypsin results in the development of acute pancreatitis and its recurrence can lead to CP.[7]

SPINK-1

The serine protease inhibitor Kazal type 1 (*SPINK-1*), as the name suggests, inhibits trypsin activity. The mutation of the *SPINK1* gene causing loss of its function predisposes to an increased risk of recurrent acute pancreatitis and CP.[10]

CFTR

Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. CFTR is a key molecule expressed in the pancreatic ducts. Some *CFTR* gene mutations may be pancreas-specific. *CFTR* mutations associated with a loss/decrease of bicarbonate secretion cause recurrent acute pancreatitis and CP.[11]

Pancreatic Stellate Cells

The identification and characterization of resident PSCs have initiated numerous studies, in recent years, that focused on the determination of their exact role in pancreatic fibrogenesis.[12–16] In their quiescent state, PSCs are periacinar in location and store vitamin A. They can also be found in perivascular and periductal regions.[16] On activation through pro- and anti-inflammatory cytokines, growth factors (TGF-β and PDGF), angiotensin II, and reactive oxygen species that are released by damaged neighboring cells and recruited
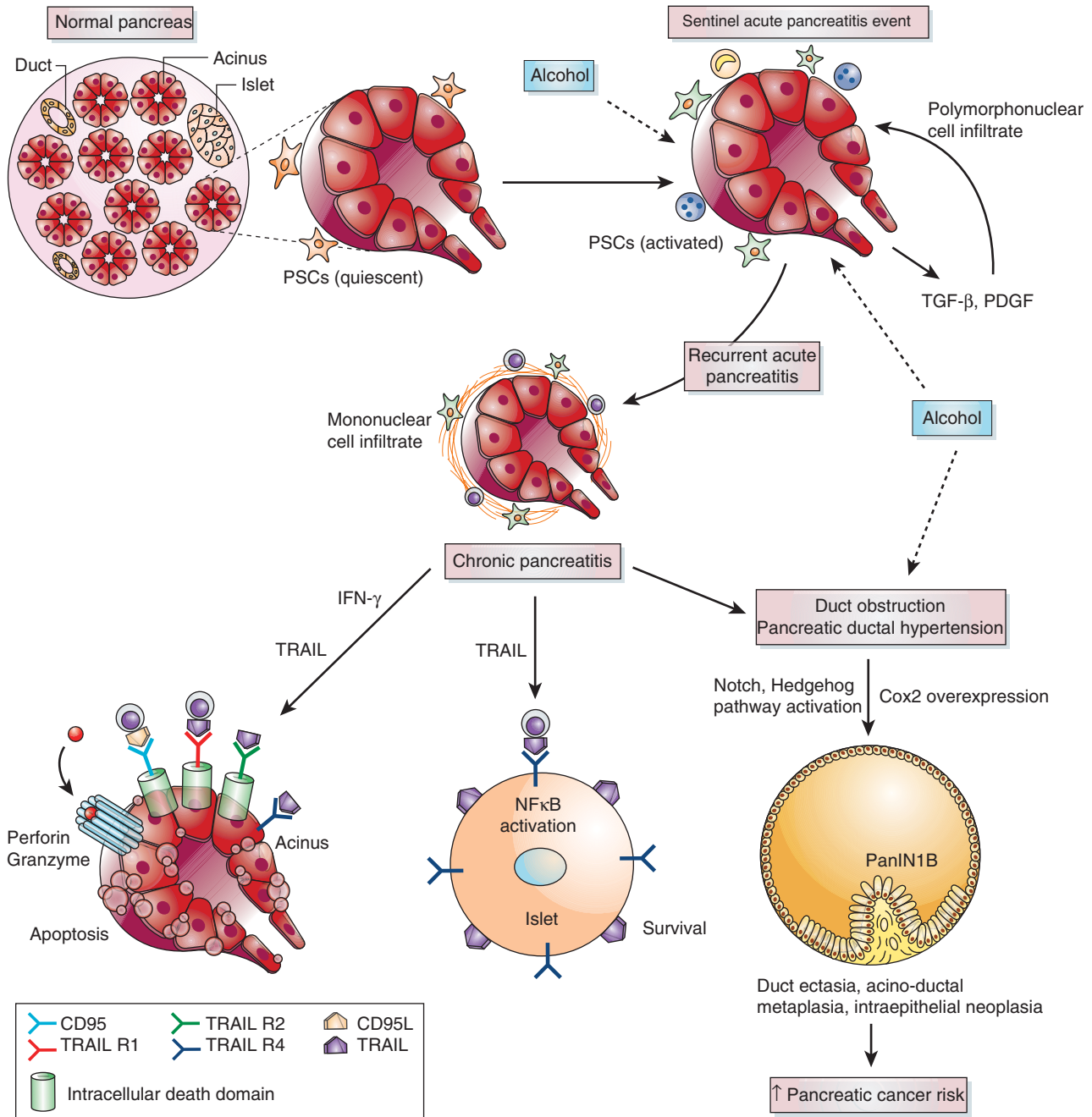
Figure 1 Normal pancreas consists of an exocrine component formed by acini and ducts, and an endocrine portion composed of islets. Alcohol and/or other injurious stimuli initiate the first episode of acute pancreatitis, described as the Sentinel Acute Pancreatitis Event (SAPE) by Whitcomb *et al* and characterized by an acute inflammatory infiltrate. Continued exposure to the injurious factor(s) leads to recurrent episodes of acute pancreatitis, which activate pancreatic stellate cells (PSCs) and initiating pancreatic fibrogenesis that leads to chronic pancreatitis (CP). Under the influence of IFN-γ released by CD4- and CD8-positive T lymphocytes, pancreatic the acini in CP neo-express CD95, TRAIL R1, and TRAIL R2 with intracellular death domains. Therefore, the acini in CP are rendered vulnerable to apoptosis by CD95L-expressing T cells and soluble TRAIL, produced locally by PSCs. A part of acinar cell death in CP is also attributed to perforin–granzyme B pathway. In contrast, the pancreatic islets retain their CD95L-positive and death-receptor-negative status and neoexpress TRAILR4, the latter lacking an intracellular death domain. NF-κB is expressed in islets, which in turn activates inhibitor of apoptosis proteins (IAPs), helping to preserve islets. Finally, in CP, the pancreatic ducts are obstructed, distorted, dilated, and have elevated intraductal pressure. Long-standing ductal changes activate Notch and Hedgehog pathways, which lead to acinoductal metaplasia (ADM) and pancreatic intraepithelial neoplasia (PanIN), thereby predisposing to the development of pancreatic cancer. (The SAPE concept is used here with the permission of Dr Whitcomb.)

leucocytes, assume a myofibroblast-like phenotype.[17] Activated PSCs can produce autocrine factors, such as TGF-β, PDGF, TRAIL, and proinflammatory molecules (COX-2)

(Figure 1).[16] This makes them capable of performing numerous biological functions, such as proliferation, migration, synthesis, and secretion of extracellular matrix components,

as well as synthesis and secretion of matrix-degrading enzymes and their inhibitors. Therefore, PSCs possess the dual capacity of synthesizing, as well as degrading ECM components, thus, implying their key role in the maintenance of the normal architecture of the healthy pancreas.[18,19]

Thus, the concept of SAPE incorporates the molecular mechanism of pathogenesis while unifying earlier theories,[3] implying that CP is the result of recurrent and/or sustained immune activation that causes cytotoxic injury to the acinar cells. This immune activation is followed by a stimulation of anti-inflammatory responses that drive fibrosis, in which PSCs play a pivotal role.

A number of reports indicate that apoptosis plays an important role in parenchymal destruction that is observed in CP.[20–23] In the acinar cells of CP, Bateman et al showed a statistically significant increase in apoptotic index compared with that in controls. In a recent report, Schrader et al[24] showed a lack of increased β-cells turnover in CP patients, despite an ~10-fold increase in the number of apoptotic acinar cells, suggesting that the damage to the pancreas somehow specifically targets the exocrine compartment and affects the endocrine islets to a lesser extent.

IMMUNE RESPONSE AND CELL INJURY IN CP

A major area of interest in CP is its cellular response to injury. There are significant portions of the two major T-cell subsets, as well as of NK cells and macrophages, in the cellular infiltrates of CP.[25] In the male Wistar rat model of spontaneously developing CP, CD8 + cytotoxic T cells invade pancreatic lobules and form close associations with acinar cells, some of which showed apoptosis.[26] This provides a circumstantial evidence for an involvement of autoreactive T cells in the parenchymal destruction in CP. This is further substantiated by observing that major histocompatibility complex class-II (MHC-II)-deficient mice developed an immune-based selective loss of exocrine pancreatic parenchyma, caused by CD8 + T lymphocytes.[27] CD8 + cytotoxic T cells can cause the death of target cells through multiple mechanisms,[28] such as contact-dependent cytotoxicity as well as abundant cytokine production. The cytotoxicity has been ascribed to two distinct but complementary pathways, each requiring close contact between the T cells and its target. The first, mainly used by CD8 + T cells, is the perforin–granzyme pathway involving a release of cytotoxic granules from the effector cell toward the target. The other pathway involves an interaction between soluble and/or membrane-bound death ligands (CD95L and TRAIL) and their signal-transducing receptors, CD95, TRAIL-R1 and TRAIL-R2, expressed on target cells. This latter pathway is mainly operative in CD4 + T cells. On the activated T cells, when CD95L/TRAIL binds to CD95, TRAIL-R1 and TRAIL-R2 on the acinar cells, the interaction is sufficient enough to trigger an apoptotic cell death (Figure 1). A minor but significant part of killing is attributed to a MHC-independent destruction through NK cells. In addition, activated CD4 + and CD8 + T cells have

the ability to produce high levels of the proinflammatory cytokine, IFN-γ,[29] which was detected in significantly higher amounts of the CP specimens compared with that of the normal pancreas.[20] IFN-γ upregulates the expression of MHC-II molecules, enhances antigen expression on target cells and, thus, sustains inflammation and stimulates non-specific effector cells, such as macrophages and NK cells.[30] Intriguingly, the endocrine islets remain intact even in the later stages of CP, whereas the exocrine parenchyma is progressively destroyed.[31]

DEATH RECEPTORS AND LIGANDS IN CP

Insights into the differential cell loss of pancreatic epithelia in CP have also been gained. It was shown that normal pancreatic islets express the CD95L but do not express its receptor CD95.[20,21] In CP, this 'immunoprivileged' status is conserved in endocrine cells, whereas the exocrine epithelia shed this status, neoexpress CD95 along with MHC-I, and render sensitive targets for activated T lymphocytes that are equipped with CD95L in the adjacent inflammatory infiltrate.[20] Thus, the binding of CD95L on lymphocytes with CD95 induced on the surface of acinar cells, under the influence of IFN-γ, proves lethal for the acinar cells, whereas the islet cells secure themselves by conserving their CD95L + status (Figure 1).

Furthermore, Hasel et al showed that the normal pancreas lack TRAIL receptors. In CP, there is a strong induction of the death signal transducing TRAIL-R1 and TRAIL-R2 in exocrine cells (Figure 1). In contrast, islet cells are strongly TRAIL-R4-positive and are essentially devoid of TRAIL-R1 and TRAIL-R2. TRAIL produced locally by activated PSCs binds to TRAIL-R1 and TRAIL-R2 on acinar cells inducing their apoptosis (Figure 1).[22] However, an interesting question remains: Does TRAIL-R4 expression offer survival advantage to islet cells? TRAIL-R4 lacks the cytoplasmic death domain and was, therefore, regarded as a decoy receptor. Moreover, studies carried out in T lymphocytes hint at an alternative functional role of TRAIL-R4; which does not transmit a direct death signal, but increasing evidence suggests that binding with TRAIL activates NF-κB,[32] which may prove beneficial for the islets cells. In two independent experimental studies, TRAIL has been shown to protect islet cells in experimental type I diabetes in mice.[33,34] Mi et al[34] reported TRAIL gene upregulation in islet cells during the development of diabetes in non-obese diabetic (NOD) mice. The fact that freshly isolated islet cells are TRAIL-resistant and that TRAIL blockade exacerbated the onset of type I diabetes in NOD.Scid recipients of transferred diabetogenic T cells and in cyclophosphamide-treated NOD mice, strongly points to a protective role of TRAIL during the onset of autoimmune diabetes. Furthermore, Lamhamedi-Cherradi et al[33] treated normal and TRAIL-deficient mice with multiple low-dose streptozotocin to induce diabetes. They found that both the incidence and the degree of inflammation were significantly enhanced in TRAIL-deficient animals. Both groups attribute

the protective TRAIL effect to the inhibition of proliferation of diabetogenic T cells and not to the direct interaction with endocrine cells. In the setting of CP, TRAIL may exert protective influence on islets cells by activating NF-κB through its binding with TRAIL-R4. Another recently reported mechanism by which NF-κB may be activated in islets cells, is the CD40 receptor expressed in mouse and human β-cells. Its expression is regulated by proinflammatory stimuli.[35] The consequences of the NF-κB activation on the two epithelial compartments of the pancreas are completely converse and are discussed next.

NF-κB: SAVIOR OR DESTROYER?
NF-κB is an inducible dimeric transcription factor that comprises one or two of the five Rel family members namely, RelA, c-Rel, RelB, NF-κB, and NF-κB2. NF-κB is expressed ubiquitously, recognizes a common sequence motif, and regulates an exceptionally large number of genes in response to infections, inflammation, and other stress-related stimuli.[36] We found that the normal pancreas are characterized by low expression levels of NF-κB subunit transcripts in both the exocrine and the endocrine compartments. In CP, we found an induction of IKK-γ and RelA protein with maximum levels in islets. This increase in islets goes along with the degree of fibrosis. We further showed that there is a fibrosis-associated increase in mRNA for NF-κB1, NF-κB2, RelA, RelB, and c-Rel in islet cells, whereas the only transcripts that showed an increase in the acini were those of NF-κB2. TRAIL, through TRAIL-R4 broadly expressed in CP, may be one of the factors responsible for the above scenario.[37]

At present, there is a wealth of data regarding the defensive and protective role that NF-κB plays in the acute phase response in inflammation.[38,39] NF-κB was shown to limit the tissue damage in the cerulein model of acute pancreatitis, whereas blocking the NF-κB activation had an adverse effect.[40] Conversely, intraductally administered *in vivo* gene transfer of RelA led to NF-κB activation, acinar damage, and acute pancreatitis in rats.[41] It has also been shown that the inhibition of NF-κB activation or translocation may be useful in preventing islet-cell dysfunction and death.[42–45] Thus, it remains unclear whether the NF-κB activation in the pancreas is protective or deleterious.[46] It has been suggested that RelA and c-Rel are functional antagonists with respect to apoptosis induction; RelA overexpression inhibits apoptosis, whereas c-Rel acts in a proapototic way by enhancing the expression pf TRAIL-R1, TRAIL-R2, or suppressing the inhibitor of apoptosis proteins (IAPs).[47] It was also suggested that NF-κB2 might be a direct activator of programmed cell death.[48,49] Remarkably, in the acini of CP with severe fibrosis, it seems that elevated NF-κB2 mRNA expression eventually tilts the balance in favor of apoptotic cell death in the exocrine compartment, whereas islets resist the insult.[37] It is likely that, in CP, a process smoldering for years, endocrine cells undergo a reprogramming and, less so, exocrine cells for an altered state of NF-κB-regulated transcriptional activity,

the functional consequences of which, at the single cell level, remain to be determined. An important mechanism by which NF-κB can inhibit apoptosis is through the regulation of IAPs.[50,51]

INHIBITOR OF APOPTOSIS PROTEINS
The IAPs are a family of caspase inhibitors that specifically inhibit caspases 3, 7, and 9 and thereby, prevent apoptosis.[50,52] We showed that a fibrosis-associated accentuation of cIAP1 and survivin protein in parallel with RelA emerges in islets in CP, thereby suggesting that a sustained enhanced expression of NF-κB subunits and, consequently, of IAPs act as lifeguards for endocrine cells in the adverse microenvironment of CP.[37,50] In a recent report, it was shown that survivin induced global changes in β-cells that could contribute to a generalized escape from apoptosis by the inhibition of multiple apoptotic pathways.[53] Conversely, the pattern of NF-κB activation proves to be deleterious for the exocrine parenchyma. We believe that one of the key regulators of this reprogramming might be TRAIL. Therefore, the two major epithelial components of the pancreas respond differently to the immune attack in different chronic inflammatory diseases. In CP, in which the exocrine parenchyma is progressively lost, the endocrine islets remain intact and functional, even in advanced stages of fibrous replacement. Vice versa in type I diabetes, the islet cells disappear in the course of insulitis, whereas the exocrine parenchyma is unaffected. In both the processes, apoptotic cell death, which is prevented by CD95L and TRAIL, is the key event[54] supporting the current understanding that immune attacks are cell-type-specific. Nevertheless, chronic inflammation functions through the local release of chemokines, cytokines, and soluble death receptor ligands that lack the specificity of cognate T cell–target cell interaction. These short-range-acting molecules not only fuel the directed attack but also inevitably inflict collateral damage,[55] particularly when the targets and innocent bystanders are so intimately assembled. Therefore, survival programs that are run by exocrine and endocrine cells regulating their propensity to be apoptosis-resistant or apoptosis-susceptible, differ fundamentally in CP and type I diabetes; although the death pathways and their interfering inhibitors are very likely just the same in both cell types. Against this background, we proposed a hypothetical model of CP leading to an enforced death of exocrine cells and concurrently to an armoured survival of endocrine islets (Figure 1).[37,56]

CP AND PANCREATIC CANCER LINK
CP predisposes to the development of pancreatic cancer and is, therefore, considered to be a pre-cancerous condition.[57,58] Chronic inflammation produces alterations in the microenvironment of the ductal epithelium that may increase the risk of neoplastic transformation by increased genomic damage and cellular proliferation. During repair, after paren-

chymal injury to the pancreas, genetic programs that operate during the development of the pancreas are reinstated and may contribute to neoplastic transformation.[59] In the setting of CP, inflammatory cells, cytokines, chemokines, and activated signaling pathways play a role in the transformation of normal ductal epithelium to metaplastic (acino ductal metaplasia (ADM)) and early neoplastic lesions (pancreatic intraepithelial neoplasia (PanIN)), which eventually give rise to pancreatic cancer.[57,58] (Figures 1 and 2). Some of the key molecules and signaling pathways that may play a role in cellular transformation and pancreatic cancer development are discussed next.

COX-2 OVEREXPRESSION IN CP

Cyclooxygenases (Cox-1 and Cox-2) are rate-limiting enzymes used in the production of prostaglandins (PGs), which are short-lived lipid-signaling molecules involved in a host of biologic functions. Cox-1 is expressed constitutively, whereas Cox-2 is induced as a response to inflammatory stimuli and is upregulated in CP. Cox-2 overexpression is a well-established factor linking chronic inflammation with metaplastic and neoplastic changes in various tissues. This is substantiated by Colby et al, through their BK5-Cox-2 mice in which Cox-2 overexpression causes the progression of ADM to severe dysplasia that are suggestive of pancreatic
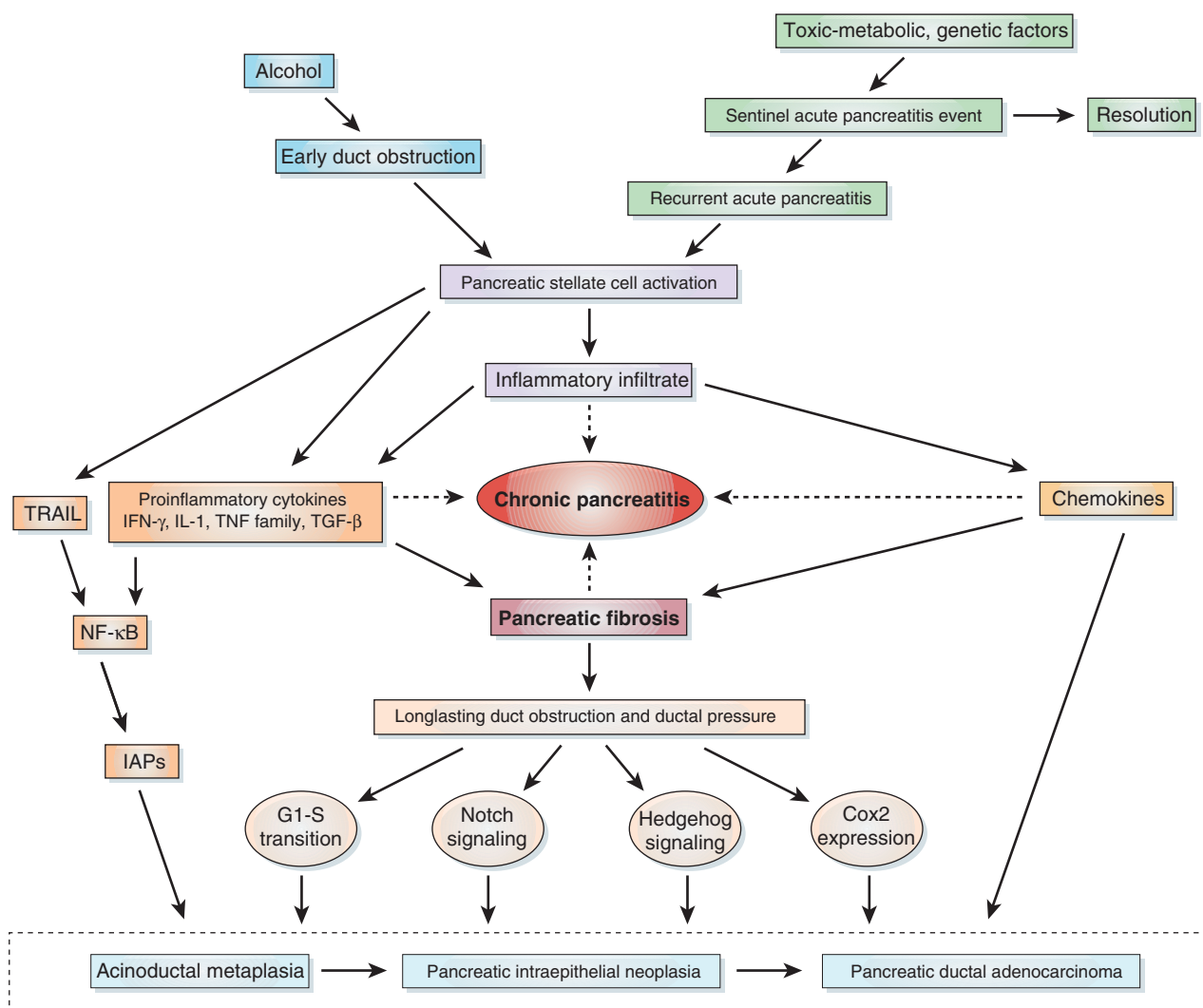

Figure 2 Scheme representing the sequence of events leading to the activation of pancreatic stellate cells (PSCs) resulting in chronic pancreatitis. Inciting factors (eg, alcohol and toxic-metabolic stress) initiate the first episode of acute pancreatitis (SAPE). The inflammation gets resolved if the stress is short-lived. However, continued exposure to or persistence of the injurious agent causes recurrent acute pancreatitis (RAP), which activates PSCs. TRAIL, proinflammatory cytokines, and chemokines produced by inflammatory infiltrate and PSCs cause parenchymal destruction and drive fibrosis, thus causing ductal distortion, obstruction, dilation, and elevated intraductal pressure. Long-standing ductal changes activate signaling pathways (eg, Notch and Hedgehog) leading to cellular transformation resulting in acinoductal metaplasia (ADM), pancreatic intraepithelial neoplasia (PanIN), and pancreatic ductal adenocarcinoma.

ductal adenocarcinoma (PDA). Prostaglandin (PGE$_2$), a primary metabolite of Cox-2, is shown to promote cell survival, proliferation, and angiogenesis and inhibit apoptosis, all favoring cancer development.[60]

SIGNALING PATHWAY ALTERATIONS IN ECTATIC DUCTS OF CP

The theory of ductal obstruction by concretions is one of the four major theories that explain the pathogenesis of CP.[5] Some of the inciting agents, such as alcohol, are believed to increase protein concentrations in the pancreatic juice. The proteins form ductal plugs that are observed in most forms of CP and are particularly prominent in alcoholic CP. Duct obstruction due to any cause plays an important role in the pathogenesis of CP. Recently, Yamamoto et al,[61] in their experimental rat model of CP, showed a persistent pancreatic ductal hypertension (PDH) for 2-week-induced morphologic changes similar to that in human CP specimens. The formation of strictures and ductal concretions in the early stages of the disease frequently cause duct obstruction, leading to PDH and duct ectasia. Moreover, metaplastic and early neoplastic lesions are frequently observed during the course of CP. ADM was observed in male Wistar rats after pancreatic injury induced by duct ligation.[62]

NOTCH SIGNALING IN ECTATIC DUCTS OF CP

An evolutionary conserved pathway, Notch, regulates the various aspects of cell signaling. Four Notch receptors (Notch1–4) and five ligands (Jagged1, Jagged2, Delta like 1(Dll-1), Dll-3, and Dll-4) have been described in mammals.[63,64] Notch signaling is activated by the interaction of adjacent cells through cell-to-cell contact of the membrane-associated Notch receptor and ligand. After ligand binding, two enzymatic cleavages occur for releasing the Notch intracellular domain (NICD) from the plasma membrane. The NICD translocates into the nucleus and binds to members of the CSL transcription factor family, which are thought to mediate most of the downstream effects of Notch signaling. After NICD binding, the CSL family member, CBF-1/RBP-Jκ, which is normally a part of a co-repressor complex with histone deacetylase-1, becomes a transcriptional activator. Downstream targets of CBF-1 include a large family of β-helix loop helix transcription factors known as the hairy/enhancer of split (HES) and HES-related repressor protein. Notch is particularly important during embryonic pancreas development and in phenotypic plasticity of adult exocrine component. Notch is reactivated during pancreatic injury and in the ensuing repair process. Jensen et al[65] observed that Notch pathway components were induced during the exocrine pancreas regeneration. Rooman et al[62] reported Notch activation in their pancreatic duct ligation model of tissue damage.

Using oligonucleotide microarrays, pathway analysis, and real-time PCR, we recently showed a differential regulation of Notch receptors, ligands, and targets in microdissected normal pancreatic ducts vs the ectatic ducts of CP. Furthermore, these alterations were reflected, up to an extent in vitro, in human pancreatic duct epithelial cells that were subjected to sustained elevated hydrostatic pressure.[66] Notch is re-expressed in metaplastic lesions that are thought to be a 'pre-PanIN' lesion. Notch activity is required for TGF-α-induced acinar-to-duct conversion.[67] Murtagh et al showed that Notch enhanced the process and sensitized cells to Kras^{G12D}-driven metaplasia and to the development of mouse PanINs. This provides evidence for a synergistic role of Notch in Kras-induced mPanIN initiation and progression.[67] Ectopic Notch activation is an early event in pancreatic carcinogenesis and Notch is dysregulated in pancreatic cancer.[59,68] Alterations of these functions of Notch have been associated with different types of cancer, with some exceptions suggesting pleiotropism of Notch.[69]

HEDGEHOG SIGNALING IN ECTATIC DUCTS OF CP

The Hedgehog (Hh) signaling pathway is crucial for embryonic development. It plays an important active role in gastrointestinal patterning.[70] Hedgehog ligands bind to their receptors and activate a signaling cascade that leads to a nuclear translocation of Gli transcription factors and to the regulation of target genes. Among the targets of Hedgehog signaling are the genes-encoding cell-cycle regulators, Cyclin D1, N-myc, and p21, and the Wnt proteins. It is interesting that Sonic Hedgehog (Shh) excluded from the development, as well as the mature pancreas gets activated in early neoplastic lesions (PanINs) with increasing levels, as the lesions progress to develop into PDA. The ectopic expression of Shh induces intestinal metaplasia accompanied by Kras mutations in the pancreas.[71] Furthermore, inhibition of Hedgehog signaling blocked proliferation and induced apoptosis in culture and xenografts, providing evidence that Shh activation is an early event in the development of pancreas cancer.[71] Recently, it was shown in a model of cerulein-mediated injury and repair that acinar cell regeneration is associated with the activation of Hedgehog signaling.[72] Using cDNA microarrays, we found an altered Hedgehog pathway in microdissected ectatic ducts of CP (unpublished data). This further indicates that signaling pathways that are activated during long-lasting chronic inlammatory processes may have a crucial role in cellular transformation.

In a recent landmark analysis of 24 cases of pancreatic cancer, various genetic alterations that were found defined a core set of 12 signaling pathways that were altered in nearly 70–100% of the tumors. Interestingly, in this report, Notch and Hedgehog pathways were altered in all pancreatic cancers.[73] These findings indicate a mechanistic link between the activation of signaling pathways during repair/regeneration after inflammatory injury and their role in the development of pancreas cancer. Therefore, it might appear that similar in-depth studies may not only help in better understanding the pathobiology of CP, but may also prove useful in establishing the link between core signaling pathway alterations found in CP and PDA.

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