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Pancreatic fibrosis

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Abstract

Pancreatic fibrosis is a characteristic feature of chronic pancreatitis. The fibrosis develops as a result of abnormal activation of stromal cells and deposition of extracellular matrix (ECM) proteins. Pancreatic fibrosis impairs exocrine and endocrine functions of pancreas, leading to the severe impairment of patient's quality of life. Identification of important regulators of pancreatic fibrosis, especially pancreatic stellate cells (PSCs), greatly contributed to the understanding of cellular and molecular basis of these pathogenic processes. Various kinds of external stimuli activate PSCs, resulting in the increased proliferation, migration, ECM protein production and cytokine secretion. Recruitment of other inflammatory cells further exacerbates pancreatic inflammation, leading to destruction of acinar cells and islet cells, which is then replaced by massive fibrosis. Dissection of signaling pathways involved in pancreatic inflammation enabled therapeutic intervention using specific inhibitors or antioxidants, and some of them already contributed to the resolution of chronic pancreatitis-related symptoms. However, recovery of pancreatic tissue damage in human chronic pancreatitis has not yet been achieved, despite the favorable effects of therapeutic agents in animal model of chronic pancreatitis. This discrepancy might be attributed to the timing of intervention, since those animal models generally receive therapeutic intervention at the same time of pancreatic injury. Lack of the recognition of early stage chronic pancreatitis in humans hinders for the establishment of organ function-preserving therapy.

Introduction

Characteristic features of advanced chronic pancreatitis are destruction of acinar and islet cells, increased number of stromal cells and prominent fibrosis. These histological changes involve activation of multiple signaling pathways, leading to the remodeling of tissue structure in the pancreas. Perpetuated inflammation produces irreversible damage to exocrine and endocrine pancreatic functions, resulting in the severe impairment of quality of life due to the malabsorption of nutrients and pancreatic diabetes. Even though pancreatic enzyme supplementation or insulin therapy is now available, treating end-stage pancreatic insufficiency is still problematic. Therefore, therapeutic intervention should be made before the establishment of irreversible fibrosis. Unfortunately, this has not yet been achieved.

Numerous studies have described complex mechanisms of pancreatic fibrosis, with regard to aspects of cellular function or molecular regulation. Identification of the specific cell contributing the fibrosis has enabled clarification of fibrosis-promoting mechanisms, which consist of a multicellular inflammatory response. Research has uncovered essential inflammatory signaling pathways and their downstream targets.

Several experimental approaches that could attenuate fibrosis-promoting processes using inhibitors of specific signaling pathways and oxidative stress have been proposed, but their clinical application needs further validation.

This chapter reviews the basic characteristics of pancreatic fibrosis, cellular origin of fibrosis, fibrosis-promoting signaling pathways or cell-to-cell interactions and therapeutic intervention against fibrosis. Current knowledge of the mechanisms of fibrogenesis, fibrosis-promoting cell types and their functions, involved signaling pathways and effects of their inhibition will be discussed.

Pancreatic Fibrosis and Symptoms of Chronic Pancreatitis

Exocrine pancreas insufficiency is a typical symptom of advanced chronic pancreatitis. Destruction of acinar cells lead to the reduced secretion of digestive enzymes, which causes maldigestion. Deposition of type I collagen is a characteristic feature of advanced chronic pancreatitis. Sparse stromal cells are seen around acinar cells in normal pancreas, but prominent extracellular matrix and stromal cells surround acinar cells in chronic pancreatitis. Along with the massive fibrosis, there is evidence of tissue damage to pancreatic acini. This was confirmed, by immunohistochemistry of 4-hydroxynonenal–protein, adducts a lipid peroxidation-derived product (8). This suggests increased oxidative stress within the inflamed pancreas. In addition to this tissue injury marker, the study also confirmed expression of transforming growth factor-β1(TGF-β1) a wound healing and fibrosis-related cytokine. Based on these findings, pancreatic fibrosis is thought to be an active inflammatory process, accompanied by dynamic signals and cell-to-cell interactions. Pancreatic fibrosis also affects the macroscopic ductal structure of the pancreas, typically results in the dilatation of main pancreatic duct or formation of pancreatic stones, derived from inadequate drainage of pancreatic juice (7). Obstruction of the main pancreatic duct could be a cause of acute exacerbation of chronic pancreatitis, which further promotes the necrosis-fibrosis sequence.

In addition to the exocrine pancreas, islet cells are also affected by pancreatic fibrosis. However, pancreatic diabetes only becomes evident at later stages of the disease (27), which reflects the difference in vulnerability and reserve function. Assessment of pancreatic volume, apoptosis of acinar cells and islet cells in chronic pancreatitis patients and controls showed a decrease of beta-cell content by 29% in chronic pancreatitis, but apoptosis of islet cells was not significantly different from controls. In contrast, acinar cell apoptosis in chronic pancreatitis increased by 10-fold compared with controls, suggesting acinar cells are more vulnerable to inflammatory insults (57). A recent report described that β-cell dysfunction in chronic pancreatitis correlates with the decreased expression of pancreatic duodenal homeobox protein 1 (PDX-1) (44), an essential transcriptional factor for the maintenance of normal islet function (5). Isolated islets revealed impaired glucose-stimulated insulin secretion, indicating pancreatic diabetes in chronic pancreatitis might result from both quantitative and qualitative changes of endocrine pancreas along with fibrosis.

Pancreatic fibrosis is also related to the abdominal pain of chronic pancreatitis. Stricture of main pancreatic duct or pancreatic stones can increase the pressure in the pancreatic duct, leading to the abdominal pain (13). Treatments for the ductal mechanical obstruction are performed using several strategies, such as drainage surgery (Frey’s operation and Berger’s operation), endoscopic placement of stent and extracorporeal shock wave lithotripsy (14, 45). These treatments are generally effective for pain reduction in patients with chronic pancreatitis, but some patients still suffer from persisting abdominal pain. Besides the mechanical obstruction, pancreatic fibrosis also causes abdominal pain by affecting

nerves in the pancreas. Friess et al. reported that the number of nerves and area of neural tissue were increased in chronic pancreatitis subjected to surgical treatment compared with normal pancreas, and was accompanied by neuronal alterations (18). These morphological changes were not different between etiologies of pancreatitis, suggesting that they are a universal phenomenon. These increased nerve tissues contained inflammatory cell infiltration, possibly causing neuronal pain. Following this observation, detailed mechanisms of neuronal damage have been uncovered. Immunohistochemical analysis of intrapancreatic nerve fibers to distinguish sympathetic (tyrosine hydroxylase positive) and parasympathetic (choline acetyltransferase positive) innervation confirmed a marked decrease of sympathetic innervation (9). Since reduction in sympathetic nerve fibers was correlated with the severity of abdominal pain, this neural remodeling is assumed to be an additional cause of pain in chronic pancreatitis. Expression levels of growth factors and chemokines affecting neuronal functions and inflammation are also altered in chronic pancreatitis tissue, in conjunction with the degree of pancreatic fibrosis. For example, fractalkine, a chemokine that induces migration and extravasation of immune cells, is highly expressed in chronic pancreatitis tissues. Its expression level is correlated with the degree of pancreatic fibrosis and severity of pain (10). Another report demonstrated the therapeutic effect of anti-nerve growth factor antibody administration in a rat model of chronic pancreatitis (70). Elevated expression of nerve growth factor and its receptor tyrosine kinase receptor A in human chronic pancreatitis has been described previously (19), and these neural pain-causing molecules might become candidates of alternative therapy against pain of chronic pancreatitis.

In summary, pancreatic fibrosis plays fundamental roles in the pathogenesis of chronic pancreatitis, which could be an attractive therapeutic target for the improvement of clinical outcome and maintenance of pancreatic functions. Schematic view of the relationship between pancreatic fibrosis and symptoms of chronic pancreatitis is summarized in **Figure 1**. Cellular and molecular mechanisms of pancreatic fibrosis were extensively studied thereafter for the establishment of radical therapy for chronic pancreatitis.

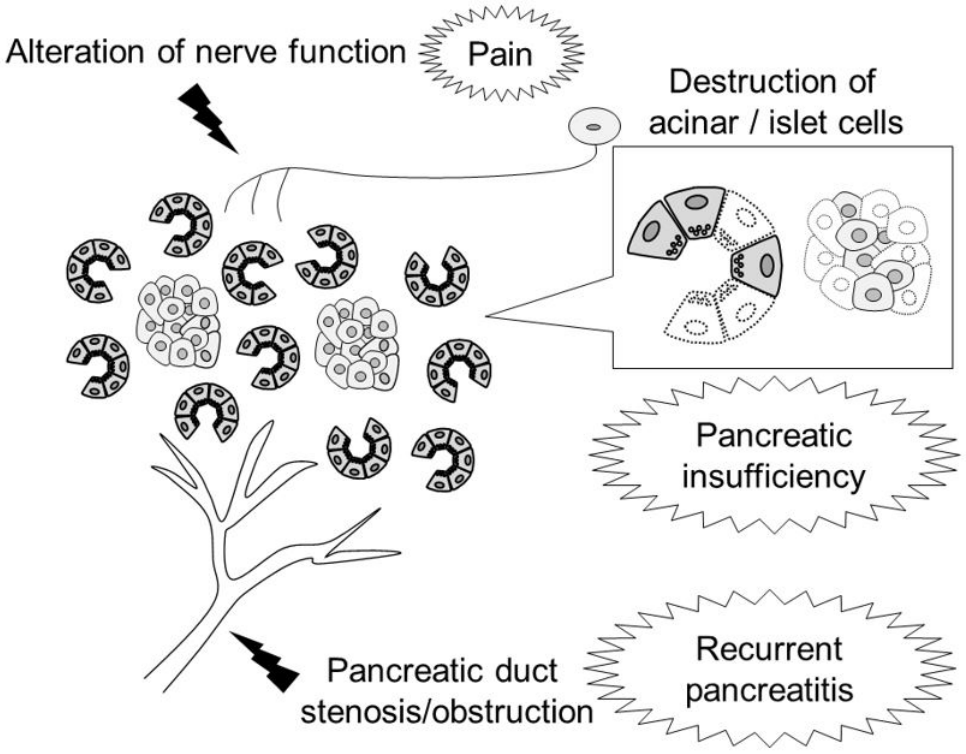

Figure 1. Relation between pancreatic fibrosis and symptoms of chronic pancreatitis

Pancreatic Stellate Cells and Fibrogenesis

Studies have demonstrated the existence of star shaped cells in the liver, located within the space of Disse (28). These hepatic stellate cells (HSCs) are in close contact with hepatocytes and endothelial cells, contributing to the formation of liver fibrosis due to wide variety of liver injury. HSCs contain lipid droplets and stay in a quiescent state in normal liver. Activation of HSCs leads to the morphological and functional changes, which cause tissue remodeling such as extracellular matrix (ECM) deposition. Similar star-shaped cells residing in periacinar space in the pancreas were reported in 1998 by Apte et al and Bachem et al, (1, 6). These cells were named pancreatic stellate cells (PSCs) and their characteristics have been examined. Quiescent PSCs contain vitamin A-containing lipid droplets. This feature enables isolation of lipid-containing PSCs by density-gradient centrifugation. Inflammation within pancreas activates PSCs, causing loss of lipid droplets, increased cellular proliferation and production of cytokines/ECM proteins. *In vitro* culture also activates PSCs, resulting in the expression of several specific markers such as desmin, glial fibrillary acidic protein (GFAP) and α-smooth muscle actin (α-SMA) (65). These PSC markers were used to identify the cellular components of pancreatic fibrosis, and PSCs were confirmed to play central roles in fibrogenesis (39).

Increased production of ECM proteins is essential for the formation of fibrosis. Activated PSCs secrete ECM proteins, such as type I collagen, fibronectin and periostin (15). Deposition of ECM proteins alters cellular microenvironment, which is closely involved with the pathologic processes in chronic pancreatitis. Like other fibrotic diseases, fibrosis is a result of skewed balance between fibrogenesis and fibrosis resolution, in response to persisting inflammation or abnormal stimuli (22). PSCs also express several enzymes degrading ECM proteins. Among them, a previous report in rat PSCs identified the expression of matrix metalloproteinase (MMP) family and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs) (49). This finding suggested a possible role of PSCs in fibrosis resolution, which is attenuated during the progression of chronic pancreatitis. A study confirmed that an acute phase protein, pancreatitis-associated protein (PAP), was able to diminish the expression of MMP-1, MMP-S, TIMP-1 and TIMP-2 in PSCs, as well as their concentrations in culture supernatants (29). This observation partly explains pathogenic alteration of PSC functions by inflammation, possibly leading to the pancreatic fibrosis. In addition, production of ECM-degrading enzymes from PSCs suggests the possibility of fibrosis resolution by specific attenuation of fibrogenic functions of PSCs. To clarify this mechanism, upstream regulators of PSC activation have been examined.

A wide variety of growth factors, cytokines, small molecules and environmental changes activate stellate cells. Transforming growth factor-β (TGF-β1) and platelet derived growth factor (PDGF) are well-known growth factors activating PSCs (56, 63). Other inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukins also increase α-SMA expression in PSCs, a hallmark of activation (43). These factors are provided from various kinds of cells including damaged acinar cells, neutrophils, macrophages and PSCs themselves (64). Therefore, these growth factors and cytokines form a feed-forward loop of fibrogenic process during the progression of pancreatitis. In addition to the endogenous factors, exogenous molecules also activate PSCs. Besides the necroinflammatory process following tissue injury, ethanol and its metabolites acetaldehyde directly activate PSCs (3). This activation involves increased oxidative stress within PSCs, which is also caused by respiratory burst of neutrophils in acute inflammation (67). Gram-negative bacteria-derived lipopolysaccharide (LPS) also activates

PSCs, which facilitates ethanol-induced pancreatic fibrosis in a rat model (66). As a result of excess ECM deposition, interstitial pressure in the pancreas increases in chronic pancreatitis, resulting in poor blood perfusion. These environmental factors such as external pressure and hypoxia also cause PSC activation. Mechanical compression of cultured rat PSCs by helium gas increased intracellular reactive oxygen species (ROS) production, leading to the increased expression of α-SMA, α1(I)-procollagen, and TGF-β1 (4). Hypoxic condition also increases the production of ECM protein, periostin and type I collagen, in cultured human PSCs (16). These PSC-activation factors are closely related with each other, leading to the perpetuation of inflammation and fibrosis. Schematic view of these factors is summarized in **Figure 2**. After the identification of PSC-activating factors, their downstream signaling pathways and specific inhibitors were identified.

Pancreatic Fibrogenesis-Related Signaling Pathways

Numerous signaling pathways are involved in the pancreatic fibrosis. Among them, signaling pathways that activate PSCs have been well studied. The mitogen-activated protein kinase (MAPK) pathway activation plays an indispensable role in the PSC activation, including cellular proliferation, migration, cytokine production and ECM production (36). Three kinds of MAPK pathways, extracellular signal regulated kinase (ERK), p38 MAPK, c-jun N-terminal kinase (JNK) have been described and they are activated by various PSC-activating stimuli. For example, ethanol and acetaldehyde activate ERK, p38 and JNK in rat PSCs. McCaroll et al studied the inhibition of each pathway using specific inhibitors (U0126 for ERK pathway inhibition, SB203580 for p38 MAPK pathway inhibition and SP600125 for JNK pathway inhibition); only inhibition of the p38 MAPK pathway could attenuate the ethanol or acetaldehyde-induced α-SMA expression of PSCs (41). Another report confirmed the activation of all three MAPK pathways and activator protein-1 by an ethanol metabolite palmitic acid ethyl ester in PSCs (34). TGF-β1 also activates ERK pathway, whose inhibition led to the attenuation of TGF-β1 autocrine loop in PSCs (48). PDGF-induced cellular migration is mediated by phosphatidylinositol 3-kinase (PI3-kinase) pathway, which also activates ERK pathway, suggesting signaling cross-talk (40).

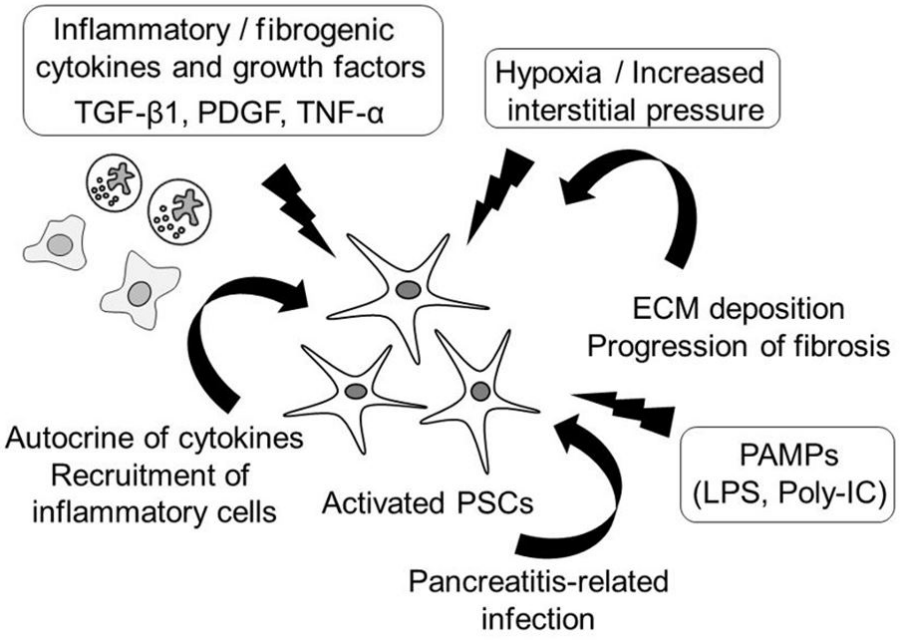

Figure 2. Activators of PSCs and feed-forward loop of PSC activation

Besides the small molecule-activated or growth factor-activated MAPK pathways, additional signaling pathways are involved in PSC activation. PSCs express Toll-like receptors (TLRs), which recognize pathogen-associated molecular patterns molecules (PAMPs) (33). PSCs express TLR2, 3, 4, 5 and associated molecules CD14 and MD2. These TLRs recognize various molecules derived from pathogens. TLR ligands, lipoteichoic acid, polyinosinic–polycytidylic acid, LPS and flagellin increase production of monocyte chemoattractant protein-1 (MCP-1) and cytokine-induced neutrophil chemoattractant-1 (CINC-1) from PSCs. These processes were accompanied with the activation of nuclear factor-κB (NF-κB) pathway, a typical inflammatory signaling pathway, as well as the activation of three MAPK pathways. However, the MAPK inhibitors failed to show uniform inhibition of MCP-1 and CINC-1 production, as the NF-κB inhibitor did, suggesting a central role of NF-κB in TLR-mediated chemokine production. This observation suggests that PSCs are not only activated by inflammatory signals, but also act as an amplifier of inflammatory response within pancreas. Another extracellular ligand affecting the function of PSCs is Indian hedgehog (Ihh), a family member of hedgehog peptides, which are involved in the developmental process and tissue patterning (47). PSCs were found to express hedgehog receptor components, patched (Ptc-1) and smoothened (Smo) (60). Binding of hedgehog ligand to Ptc-1 abrogates its inhibitory effect on Smo, leading to the downstream signal activation represented by the nuclear accumulation of Gli transcriptional factor (24). Ihh-treated PSCs revealed increased cellular migration, without alteration of proliferation or ECM protein production. Instead, Ihh increased the amount of membrane-type I MMP in PSCs, which was attenuated by TIMP-2, an inhibitor of metalloproteinase. This observation indicates that ECM-degrading enzymes are regulated by external stimuli, which affects the cellular function of PSCs themselves.

There are several signaling pathways involved in the maintenance of the quiescent state of PSCs. Quiescent PSCs have intracellular lipid droplet containing Vitamin A. Vitamin A and its metabolites bind to the nuclear receptors, leading to the alteration of gene expression through retinoic acid responsive elements (11). Vitamin A and its metabolites all-trans retinoic acid (ATRA) and 9-cis retinoic acid (9-RA) suppressed cellular proliferation, α-SMA expression, ECM protein production and MAPK activation (42). Cultured PSCs retained the expression of retinol-converting enzyme and nuclear receptors for ATRA and 9-RA, which possibly contribute to the attenuation of activated PSC functions. This suggests that the morphological changes in PSCs represent the functional alterations in activated PSCs, possibly causing the loss of endogenous factors for quiescence. Similarly, another nuclear receptor, peroxisome proliferator-activated receptor γ (PPAR-γ) was also found to regulate PSC activation. Oxidative metabolites of polyunsaturated fatty acids and prostaglandins bind to this ligand-activated transcriptional factor, regulating inflammatory responses (25). Cultured PSCs expressed PPAR-γ, and its ligands (15d-PGJ_2 and troglitazone) inhibited PDGF-induced proliferation of PSCs (32). Expression of α-SMA and production of type I collagen or MCP-1 were suppressed by 15d-PGJ_2 and troglitazone treatments, indicating PPAR-γ-mediated signal contributes to the inhibition of PSC activation. In addition to these ligands for nuclear receptors, vitamin D also has anti-fibrogenic property. Recent report identified the expression of vitamin D receptor in PSCs, and the potent vitamin D analogue, calcipotriol induced reprogramming of activated PSCs into quiescent state (58).

According to these studies, signaling pathways promoting pancreatic fibrosis are mainly activated by extracellular stimuli toward PSCs, which harbor endogenous quiescence-maintaining machinery in normal pancreas.

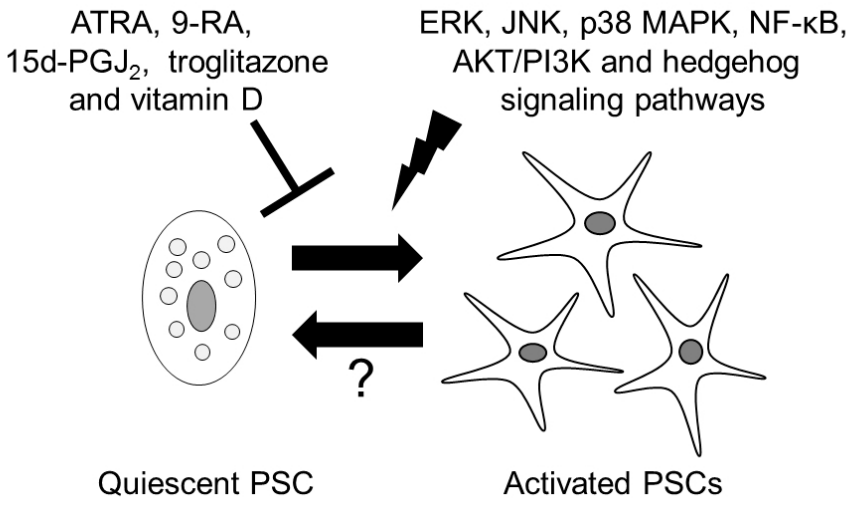

Figure 3. PSC-activating signals and quiescence-maintaining factors

Schematic view of pro- and anti-fibrogenic signaling pathways in PSCs is summarized in **Figure 3**.

Cell-To-Cell Interaction in Pancreatic Fibrosis

Pancreatic fibrosis also involves other types of cells besides PSCs. Activated PSCs secrete a wide variety of inflammatory cytokines such as IL-1β, IL-6, MCP-1 and TNF-α. These factors contribute to the recruitment of inflammatory cells to the pancreas. RelA/p65, a component of the NF-κB pathway in myeloid cells is essential for the establishment of pancreatic fibrosis in mouse model (62). This suggests the importance of infiltrating macrophages for pancreatic fibrosis, through the production of TNF-α and TGF-β1 under the NF-κB regulation. Similar evidence was also reported recently, which confirmed the contribution of alternatively activated macrophages (AAMs, M2) in the pancreatic fibrosis (68). Unlike classical macrophages (M1, induced by interferon gamma (IFNγ) or LPS), AAMs are induced by IL-4 or IL-13, and play an important role in fibrosis and tumorigenesis (21). TheAAM-inducing factors are secreted from PSCs, when IFNγ production is low (68). Suppression of IL-4/IL-13 improved established chronic pancreatitis in mouse model, and this could be a candidate therapeutic target.

Similar cell-to-cell interaction has been also reported in mast cells, an immediate mediator of allergic reaction. Existence of mast cells in chronic pancreatitis tissue was described by Esposito et al, and correlated with the extent of fibrosis and the intensity of inflammation (17). Mast cell infiltration was observed in chronic pancreatitis regardless of the etiology. Zimnoch et al noted an increasing number of degranulated mast cells in chronic pancreatitis parallel to increasing degree of fibrosis, suggesting mast cell-derived factors have some roles in PSC activation (71). Finally, a recent study identified the mutual activation between PSCs and mast cells that plays a pivotal role in pancreatic cancer progression. Mast cell-derived IL-13 and tryptase were found to stimulate PSC proliferation, and PSCs were also found to facilitate cytokine and tryptase release from human mast cell lines (30). T-cell function is also affected by chronic pancreatitis. Comparison of patient-derived T-cells from chronic pancreatitis, pancreatic cancer and healthy individuals revealed that regulatory T-cells recognizing pancreatitis-associated antigens were expanded in chronic pancreatitis, leading to the regulatory cytokine profile represented by IL-

10 production (55). Taken together, these cell-to-cell interactions alter local and systemic inflammatory response in chronic pancreatitis, resulting in the fibrotic tissue remodeling.

Other cellular components of the pancreas are variously affected in chronic pancreatitis based on cell-to-cell interactions. As mentioned earlier, pancreatic endocrine functions are impaired in chronic pancreatitis. Parts of these mechanisms are now being uncovered using experimental methods. Co-culture with PSCs reduced insulin production and induced apoptosis in pancreatic β-cell line RIN-5F (26). The study also confirmed the existence of α-SMA-positive activated PSCs within the islet of chronic pancreatitis, indicating cell-to-cell interaction between PSCs and islet cells contributes to the impaired pancreatic endocrine function. An ECM protein, secreted protein acidic and rich in cysteine (SPARC), has been reported to attenuate growth factor-stimulated signaling, cellular growth and cell survival in INS-1 β-cell line and primary mouse islet cells (53). SPARC inhibited hepatocyte growth factor- and IGF-1-induced activation of ERK and Akt, leading to the reduced cellular growth and cell survival. Since SPARC is predominantly produced from PSCs (31), these results explain part of the mechanisms how PSCs inhibit islet functions. In addition to these interactions, high glucose also affects PSC functions. Treatment of PSCs by high glucose (30 mM) caused increased reactive oxygen species (ROS) production, detected by dichloro-dihydrofluorescein diacetate (DCF) fluorescence. This treatment led to the increased production of α-SMA, IL-6 and collagen, accompanied by cellular proliferation of PSCs (54). This observation means once a diabetic state is established, activation of PSCs would be further promoted, by a feed-forward loop. Another report has proven this concept, using diabetic model rat. Transplantation of PSCs isolated from 8-week-old Wistar rats into Goto-Kakizaki rats resulted in the exacerbation of impaired glucose tolerance, while Wistar rats with PSC transplantation did not reveal any changes. Islet of PSC-transplanted Goto-Kakizaki rats showed increased islet fibrosis compared with Wistar rats with PSC transplantation, suggesting higher blood glucose enhanced PSC function (69). PSC-conditioned medium (PSC-CM) and high glucose additively increased C/EBP Homologous Protein (CHOP) expression, a hallmark of ER stress, in the INS-1cells in this study.

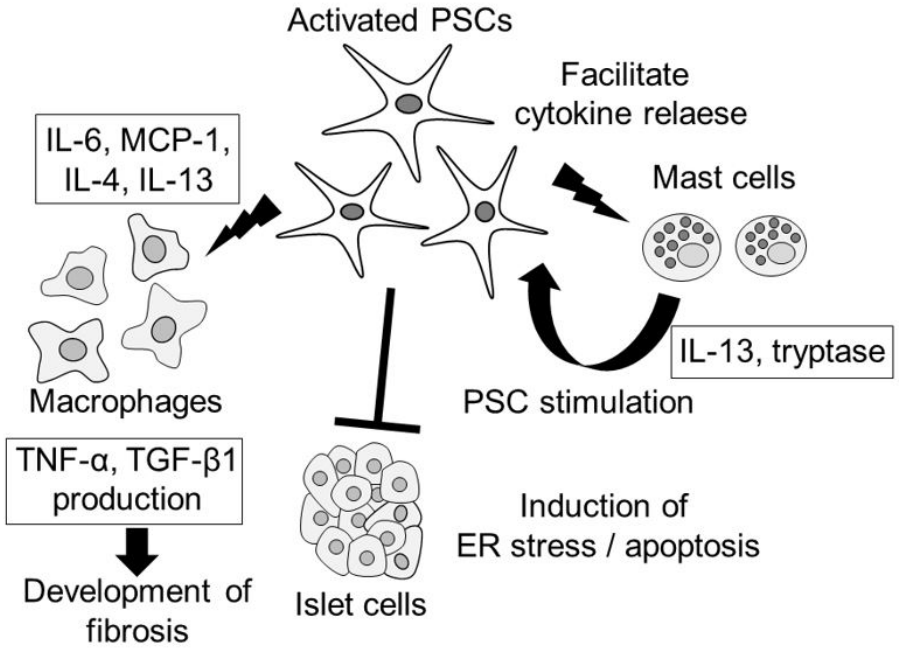

Figure 4. Cell-to-cell interactions and their mediators involved in pancreatic fibrosis

Based on these observations, cell-to-cell-interactions, especially with PSCs, play important roles during the remodeling of tissue structure in chronic pancreatitis. These interactions and their mediators are summarized in **Figure 4**. Together with the pro-fibrogenic signaling pathways, fibrosis-related interactions have been investigated as candidates for the anti-fibrosis therapy.

Therapeutic Intervention Targeting Fibrogenesis

Since activation of PSCs and their interaction with other cell types contribute to the pathogenesis of chronic pancreatitis, therapeutic interventions against these interactions have been evaluated. A wide variety of growth factors and extracellular stimuli exert activation of multiple signaling pathways such as MAPK, Akt and NF-κB. Effective inhibition of these pathways by single inhibitor presumably has limitation in therapeutic potential, and selection of a certain target on which these stimuli converge would be ideal. One of such candidates is an increased oxidative stress in PSCs. There is evidence as regards the substantial contribution of NADPH oxidase in PSCs (38). A wide variety of cytokines and growth factors such as PDGF, IL-1 and angiotensin II increase ROS production in PSCs, which is efficiently suppressed by treatmentwith NADPH oxidase inhibitors, diphenylene iodonium and apocynin. These attenuate proliferation of PSCs and cytokine production. Other antioxidants such as curcumin or ellagic acid have similar effects (35, 37), suggesting antioxidant agents could be a promising therapeutic strategy against chronic pancreatitis. Recently, two meta-analyses of antioxidant therapy for pain reduction in chronic pancreatitis have been published. Both concluded that antioxidant therapy is safe and has a beneficial role in pain reduction (52, 61). As described earlier, pancreatic fibrosis mechanistically and functionally affects pancreatic nerve fibers, and attenuation of inflammatory processes might contribute to these clinical outcomes. Other food-derived compounds also have inhibitory effects on PSC functions. Tocotrienol and tocopherol are vitamin E compounds, which have been reported to inhibit PSC activation (2, 51). Especially, tocotrienol causes apoptosis of PSCs *in vitro*, while tocopherol did not have a similar effect (51). This process was accompanied by development of autophagy and sustained mitochondrial permeability transition, resulting in the cell death of activated PSCs. Interestingly, tocotrienol did not affect the cell viability of acinar cells or quiescent PSCs, showing favorable profile as a therapeutic agent.

In addition to antioxidants and NADPH oxidase inhibitors, other fibrogenic signaling pathways have been targeted in PSCs. Administration of halofuginone, a plant alkaloid analog, inhibited TGF-β1 signaling, ECM production and activation of MAPK signals (72). Similarly, transgenic expression of Smad7, an inhibitory Smad against TGF-β1 signaling, showed protective role in caerulein-induced pancreatic fibrosis in a mouse model (23). A recent report described an effective anti-fibrosis therapy using the novel prostacyclin analog ONO-1301 in rat pancreatitis model (46). ONO-1301 attenuated experimental pancreatic fibrosis in dibutyltin dichloride-induced pancreatitis, with reduced expression of TGF-β1, TNF-α, IL-1β and MCP-1. These effects were mediated by the induction of hepatocyte growth factor, which repressed cytokine and chemokine production from monocytes. Production of connective tissue growth factor (CTGF) from injured acinar cells plays a pivotal role in the production of inflammatory cytokines such as IL-1β and CCL3 in ethanol and caerulein-induced chronic pancreatitis in mice. An inhibitor of chemokine receptor, BX471, could attenuate the chemotaxis of macrophage cell line toward AR42J acinar cell line (12). Administration of camostat mesilate, an oral protease inhibitor, also reduced severity of pancreatic fibrosis in dibutyltin

dichloride-induced chronic pancreatitis (20). Camostat mesilate reduced MCP-1 and TNF-α production from LPS-stimulated monocytes, together with the inhibition of proliferation and MCP-1 production from PSCs (20). These results indicate that inflammation-triggering signaling pathways and their mediators are attractive targets for anti-fibrosis therapy.

However, these therapeutic interventions have limited benefit for patients with advanced chronic pancreatitis, whose pancreatic parenchyma has already lost functional acinar and islet cells. Since these therapeutic interventions were applied to animal model at the same time of pancreatic injury, their efficacy in human disease is unclear. Lack of a diagnostic method for subclinical chronic pancreatitis hampers early intervention, such as alcohol abstinence or smoking cessation (7). Japanese clinical diagnostic criteria for chronic pancreatitis were revised in 2009, to define a new disease entity, early chronic pancreatitis (59). Diagnosis of early chronic pancreatitis is based on abdominal symptoms, laboratory data and imaging findings characteristic to chronic pancreatitis, intending to categorize patients with early stage disease. Efficacy of anti-fibrosis therapy for these patients needs to be clarified. Similar validation could be performed in patients with hereditary pancreatitis, in whom effective prophylactic therapies have not yet been available (50). In summary, precise identification of patients with early stage disease must be enabled for the establishment of an effective anti-fibrosis therapy.

Conclusion

Progress in the research field of pancreatic fibrosis identified intriguing cellular components, signaling pathways and upstream regulators. Inhibition of fibrogenic processes revealed therapeutic efficacy in animal models, but their clinical application has not yet achieved. For this goal, identification of patients with early stage chronic pancreatitis with novel diagnostic strategy will be necessary.

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