# New aspects of formation and progression of pancreatic fibrosis in pancreatitis

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**Key words:** pancreatitis, pancreatic stellate cells, pancreatic fibrogenesis, signal pathways, extracellular matrix

Chronic pancreatitis is characterized by the gradual formation of fibrous tissue in the pancreas and is a leading risk factor for pancreatic cancer [27]. According to recently published data, every year 48960 newly diagnosed cases of pancreatic cancer are detected in the United States and 40,560 people die from pancreatic cancer each year [24].

The development of pancreatic fibrosis in chronic pancreatitis significantly complicates the treatment of patients. At present, it has been convincingly shown that various types of effector cells, such as fibroblasts, myofibroblasts and fibrocytes [22], play the greatest role in the formation of fibrosis of pancreatic tissue in chronic pancreatitis [22], while fibroblasts and myofibroblasts are the key fibrosis cells and are responsible for the secretion of secretion [5].

The present review presents modern views on the formation of fibrous tissue in the pancreas in patients with chronic pancreatitis.

The formation of fibrosis is a dynamic phenomenon, during which the formation of extracellular matrix occurs in the interstitial spaces and in areas where the main components of the exocrine pancreatic function, acinar cells, are damaged [12]. In the case of pancreatic cancer, macrophages and neutrophils are activated, which, in turn, trigger a "cascade" of activation of proinflammatory cytokines (IL-1, IL-6, and IL-8), chemokines (chemoattractant monocyte-1 protein, macrophage inflammatory protein-1) and growth factors that additionally activate pancreatic stellate cells at rest [12]. In turn, activated pancreatic stellate cells are the main components of fibrosis development in patients with chronic pancreatitis,

synthesizing transforming growth factor-β, fibroblast growth factor and COX-2, which leads to enhanced synthesis of extracellular matrix [3].

In recent years, it has been proven that activated pancreatic stellate cells begin to secrete matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases, thereby performing a dual function — synthesizing and degrading the extracellular matrix [20]. Consequently, pancreatic stellate cells control the balance between fibrogenesis and matrix degradation and are thus able to regulate the state of the pancreatic tissue, maintaining the normal architecture of the tissue or the development of progressive fibrosis with intensive activation.

Currently, several molecular signaling pathways that play a key role in the activation of pancreatic stellate cells are described — Rho-kinase, mitogen-activating protein kinase (MAPk), transforming growth factor-β associated with the protein encoded by the human SMAD gene (TGF-β/SMAD), phosphatidylinositol-3-kinase (PI3K), JAK-STAT-kinase signaling pathway (JAK — Janus-kinase; STAT — signal transducer and transcription activator) [11, 19, 21].

**Rho-kinase way**

The Rho family of proteins includes RhoA, Rac, and Cdc42, which belong to the nucleus molecules and cause stress-induced formation of fibrous fibers, as well as regulation of cell adhesion, remodeling of the cytoskeleton in response to external signals [11]. In patients with chronic pancreatitis, activation of pancreatic stellate cells triggers the formation of stress-induced formation of fibrous fibers with remodeling of cytoskeleton proteins in the course of the disease [16]. Experimental studies have shown that activation of Rho kinase regulates the activation of trypsinogen and its release from pancreatic acini in acute pancreatitis, followed by the formation of chemokines, neutrophil infiltration with the development of pancreatic tissue damage [4]. The use of Rho-kinase inhibitors, such as Y-27632 and HA-1077 (fazudil) is accompanied by inhibition of the activity of pancreatic stellate cells, by reducing the proliferation of α-SMA (smooth muscle actin-α), chemotaxis and the production of collagen type I in

activated pancreatic star-like cells [17]. Consequently, the available experimental data show that Rho-kinase may serve as a new molecular target in the future in the treatment of patients with acute pancreatitis.

**JAK-STAT-kinase signal way**

The JAK/STAT transmission pathway is involved in the regulation of several cellular functions, such as cell proliferation, differentiation, and inflammatory responses [29]. IL-6 is a well-studied pro-inflammatory cytokine that plays a crucial role in the progression of pancreatitis, which is mediated through JAK/STAT [13]. There are studies showing higher serum IL-6 levels in patients with pancreatitis compared with healthy people [10]. In addition, in vitro studies also indicate increased production of IL-6 from a culture of human pancreatic periacinic cells of myofibroblasts under the influence of a number of inflammatory mediators, such as TNF-α, IL-17, IL-1β, and FGF-2, which further confirms the crucial role IL-6 in the pathogenesis of acute pancreatitis [1]. There is evidence that suppressing JAK-1/STAT-1 reduces the severity of cerulein-stimulated experimental pancreatitis by suppressing the activation of NFκB, which further confirms that activation of JAK-1/STAT-1 is involved in the pathogenesis of early changes in pancreatic tissue during the formation of pancreatitis [6].

**Mitogen-activating protein kinase (MAPK) way**

Evidence of the involvement of mitogen-activating protein kinase (MAPK) pathway in pancreatitis formation was shown on the example of alcohol-induced pancreatic damage, when it was proved that ethanol and its metabolite acetaldegite led to activation of protein-1 and mitogen-activating protein kinase (MAPK) in pancreatic stellate cells [19]. There is evidence that protease-activating receptor-2 (PAR-2) is also involved in the pathogenesis of pancreatitis, and the use of PAR-2 agonists leads to an increase in collagen synthesis by activating JNK-Jun N-terminal kinases, stress-activated protein kinases involved in the response on the action of cytokines and p-38 MAP kinase pathways in pancreatic stellate cells [15]. This fact confirms the role of PAR-2 in the induction of the formation of fibrous changes in pancreatic tissue [15].

At the same time, the use of the drug PD98059, an inhibitor of MAP/ERK kinase-1 (MEK-1), in the experiment promoted protection against cerulein-stimulated experimental acute pancreatitis in mice [18].

**Phosphatidylinositol-3-kinase (PI3K) way**

The possible involvement of the phosphatidylinositol-3-kinase (PI3K) pathway was shown in an experimental study that proved that the introduction of wortmannin to the experimental rats of the PI3K inhibitor led to a decrease in intrapancreatic activation of trypsinogen in acinar cells [25], and was accompanied by a decrease in activity of the inflammatory cytokines in the sample. [28]. These results suggest that PI3K is involved in the pathogenesis of acute pancreatitis. Further studies revealed that there is a specific PI3K isoform, PI3Kγ, which is known to regulate the pathological responses of acinar pancreatic cells in pancreatitis [9]. The involvement of the PI3Kγ isoform was studied in two different models of acute pancreatitis, cerulin-induced and choline-deficient diet supplemented with ethionine. At the same time, mice lacking the PI3Kγ gene were protected from damage/necrosis of acinar cells, and they showed a decrease in the severity of acute pancreatitis, which suggests the possibility of using PI3K inhibitors in the treatment of patients with acute pancreatitis in the future [14].

**Transforming growth factor-β associated with the protein encoded by the SMAD gene in humans (TGF-β/SMAD) way**

Early studies have demonstrated the involvement of TGF-β in the pathogenesis of acute, chronic pancreatitis and the development of fibrotic changes in the pancreas [23]. As established in experimental studies, TGF-β regulates the activation and proliferation of pancreatic stellate cells through the participation of SMAD-2, SMAD-3 [2]. In addition to the participation of TGF-β in the formation of pancreatic fibrosis, it was found that an increase in TGF-α proliferation can also contribute to the formation of fibrous changes in the pancreas in chronic pancreatitis by increasing the activity of matrix metalloproteinase-1 [26].

According to a recent study, a decrease in the activity of SMAD-4 with an excessive increase in TGF-α indices led to the development of fibrotic changes in

pancreatic tissue [8]. An increase in TGF-β1 activity in inflammatory changes in the pancreas is accompanied by dysregulation of micro RNA-217-SIRT1 with increased development of fibrosis in the pancreas [7].

Thus, the presented review article highlights the fine molecular mechanisms involved in the pathogenesis of pancreatitis. The role of the main signaling pathways for the activation of pancreatic stellate cells in the subsequent development of pancreatic fibrosis after damage to its tissue is indicated. The presented data opens up prospects for the development of new diagnostic areas for the detection of pancreatitis, and also allows for the development of new therapeutic technologies aimed at the identified pathogenetic mechanisms.

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Fibrosis formation is a dynamic process during which the formation of an extracellular matrix takes place in interstitial spaces and areas where the main components of exocrine pancreatic function (acinar cells) are damaged. According to studies, the biggest role in the formation of pancreatic fibrosis upon chronic pancreatitis is played by various types of effector cells, such as fibroblasts, myofibroblasts and fibrocytes, while fibroblasts and myofibroblasts are the key fibrosis cells responsible for the secretion of extracellular matrix. Activated pancreatic stellate cells become main components of fibrosis formation in patients with chronic pancreatitis, synthesizing transforming growth factor-β, fibroblast growth factor, which leads to enhanced synthesis of extracellular matrix.

The presented review highlights molecular mechanisms (Rho-kinase, mitogen-activating protein kinase, transforming growth factor-β, associated with the protein encoded by SMAD in humans, phosphatidylinositol-3 kinase), which play an important role in the activation of pancreatic stellate cells and launching the phenomenon of pancreatic fibrogenesis.

The presented data opens up prospects for the development of diagnostic areas with the search for new markers for the diagnosis of acute and chronic pancreatitis along with development of new therapeutic options for the pathogenetic therapy of patients with acute and chronic pancreatitis based on the results obtained.