PANCREATIC STELLATE CELLS

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ABSTRACT

In the human body, the stellate cells consist of vitamin-A storing cells in the various organs. One of these cells located in the pancreas. Pancreatic stellate cells are found in the periacinar, periductal and perivascular regions of exocrine pancreas. There are two forms of these cells to be quiescent and active. Stellate cells are quiescent in normal pancreas, and can be identified with the vitamin-A containing lipid droplets in the cytoplasm. Quiescent pancreatic stellate cells maintain normal tissue architecture by regulating of synthesis and degradation of the extracellular matrix proteins. In response to pancreatic damage, quiescent stellate cells transform into active state, myofibroblast-like phenotype, which lose their vitamin-A stores. It is known that pancreatic stellate cell responsible for the development of fibrosis in pancreas diseases such as chronic pancreatitis and pancreatic cancer. Understanding the process of pancreatic stellate cell activation suggests new drugs for the treatment and prevention of these diseases.

Keywords: Stellate cell, Pancreas, fibrosis.

INTRODUCTION

The pancreas consists of two parts; the exocrine part creates assistive secretions to the digestive system, while the endocrine part produces the hormones that regulate carbohydrate metabolism. The pancreas, surrounded by a thin capsule, is divided into connective tissue and lobes extending from this capsule. The endocrine function of the body is provided by cells in the islets of Langerhans that make up 1% of the area. In the exocrine part; cells that secrete in serous character come together to form an acinus. The digestive enzymes synthesized in the parenchyma-forming acinar cells are released by the parenchyma channel through the duodenum (1). Pancreatic stellate cells (PSC) are one of the cells with exocrine pancreas. They are found around the acini (peri-acinar), periductal and perivascular regions (2). In pancreatic injury, PSCs are differentiated from quiescent to active. Morphological and functional changes are seen in the active PSC. Paracrine and autocrine signals cause PSC activation to continue. Extracellular matrix (ECM) synthesis, which is increased in active cells, is effective in the development of pancreatic fibrosis. Pancreatic stellate cells resemble hepatic stellate cells found in the liver in many ways (3). 70-90% of chronic pancreatitis emerges as a result of alcohol use and the other part is genetic causes such as cystic fibrosis or idiopathic pancreatitis. The risk of pancreatic cancer increases in patients with chronic pancreatitis. The most frequent tumor of the pancreas is fibrotic tissue increase in the pancreatic adenocarcinoma (4). The results of performed studies make us think that active PSC may have a role in chronic pancreatitis and pancreatic cancer.

Discovery of Stellate Cells Stellat

Stellate cells were first defined, in the liver, in 1876 by anatomist Karl Wilhelm von Kupffer. Kupffer and his colleagues examined the liver with gold chloride staining and named these cells in the perisinusoidal area as star-stained cells. However, these cells did not differentiate

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from liver macrophages (Kupffer cell) (5). The first study of stellate cells distinguishing Kupffer cells was made by the Japanese researcher İto in 1951, and these cells, which are located in the perisinusoidal area of the liver, are described as İto cells. Wake et al. (1971) indicated that these cells store vitamin A with electron microscopy and lipid staining. These cells, made with different denominations, defined as hepatic stellate cells with common naming in 1996 (6).

PSCs in the pancreas were first recognized by Watari et al. In 1982 with electron-microscope in rodent and human pancreatic as endothelial cells. The same researchers have pointed out that these cells store vitamins by fluorescence microscopy in the mouse pancreas tissue. Watari reported that the histological features and localization of these cells resemble hepatic stellate cells found in the liver (7). Ikejiri and colleagues reported vitamin-A autofluorescence in humans and rats pancreatic tissue in 1990 (8). Examination of the properties of these cells began with the development of cell culture studies 16 years after the first discovery. The first isolation of PSC from rat and human pancreas and the examination of its properties in cell culture area were performed in 1998 by two different researchers, Apte and Bachem (2, 9).

Biological Characteristics

The PSCs, which are seen more intensely in the periductal and perivascular areas of the pancreas peri-acinar region, are called "stellate" because of their star appearance with their cytoplasmic extensions. PSCs constitute 4-8% of the pancreas cells (10). Localizations in the parietal region suggest that the localization in the periduktal and perivascular regions is involved in the regulation of vessel and canal tone (11). Cells that resemble pancreatic stellate cells are also found in the liver, kidney, lung, and pituitary (12-14). Pancreatic stellate cells contain different cell-specific interstitial filaments such as vimentin found in muscle cells, glial fibrillar acidic protein (GFAP) found in astrocytes, endothelium, fibroblasts and leukocytes, as well as nestin in neuroepithelial cells. These various types of filaments have been associated with different functions of these cells, such as proliferation, contractility, and ECM synthesis (3, 10). Since the presence of dense lipid droplets in their cytoplasm, PSC can be easily distinguished from other pancreatic cells with centrifugation. In the cell culture area, it was observed that these cells were polygonal and that the lipid droplets were more concentrated around the centrally located nucleus (2). The albumin is synthesized endogenously in PSC, and is found in the same region as the fat droplets containing vitamin-A. While the PSCs in the cell culture area are active, the loss of the contained vitamin A, the decrease in albumin, suggests that albumin may play a role in the formation of lipid droplets (15). There are studies indicating that Vitamin-A storing has a role in the continuation of quiescent state in PSC. Inhibition of vitamin A and its metabolites in the cell culture area leads to the start of alpha-smooth muscle actin (α-SMA) synthesis, which has been shown to activate quiescent in PSC. It is thought that Vitamin-A connects to nuclear receptors and regulates gene expression, thus it is effective in maintaining quiescent phenotype (16). Pancreatic stellate cells synthesize ECM components even if there is a small amount. They are also involved in the maintenance of the normal ECM cycle by the synthesis of matrix metalloproteinases (MMPs-2, MMPs-9, MMPs-13) and MMP tissue inhibitors (TIMPs-1, TIMPs-2). The functions of extracellular matrix synthesis resemble hepatic stellate cells in the liver (17). Pancreatic stellate cells have cholecystokinin 1 and 2 receptors and can produce acetylcholine synthesis-secretion (18). Philips et al reported that cholecystokinin produced acetylcholine with the induction of cholecystokinin as well as the presence of the receptor. These results support that PSC has an indirect role in the formation of exocrine secretion of the pancreas (19).

Toll-like receptors (TLRs) involved in the stimulation of natural immunity are located on the membrane of the PSC. These receptors recognize TLR2 gram positive bacteria, TLR4 gram negative bacteria. TLR 3 recognizing double-stranded RNA produced during viral replication and TLR 5 receptors recognizing flagellin protein, a key component of bacterial flagellum, are also found in PSC (20).

Besides, PSCs phagocytose foreign body and necrotic structures. These features support the role of pancreatic immunoreactivity (21). Pancreatic stellate cells have limited proliferation and migration ability. Pancreatic stellate cells also synthesize cytokines and growth factors. These factors play an important role in the migration of inflammatory cells to the site and in the activation of PSC in damaged cases (11).

Recently performed studies have shown that PSCs produce vascular endothelial growth factor (VEGF). This suggests that production PSC has a role in angiogenesis. At the same time, PSCs ex- press angiogenesis regulatory factors such as VEGF receptors (Flt-1 and Flk-2), angiopoietin-1. Hypoxia increases VEGF production (22). The observed high VEGF levels in chronic pancreatitis and pancreatic cancer suggests that PSCs may be effective in the development of these diseases (23).

Activation of Pancreatic Stellate Cells and Pancreas Fibrosis

Quiescent PSCs show morphological and functional changes when pancreas is damaged and inflamed. The amount of nuclei increases and the amount of endoplasmic reticulum increases. α-SMA synthesis, which is not seen in quiescent PSCs, begins to occur in active cells. With this change, active PSCs are turned into myofibroblast-like cells (3).

Vitamin A, which is seen in the oil droplets, is lost. Proliferation and migration of activated PSCs also increase (12). Synthesis of ECM proteins such as collagen, fibronectin, and laminin is much greater than quiescent PSC (24). Furthermore, MMP synthesis, which plays a role in matrix degradation in active PSC, is reduced (25). Limited amounts of cytokine production (PDGF, TGFβ, CTGF, IL1, IL6, IL15) in quiescent PSCs are increased in active PSCs (12, 26).

It is also observed that the amount of nestinine is increased from cytoskeletal proteins in active PSC (27). These alterations in damage-induced quiescent cells lead to the onset and progression of pancreatic fibrosis (11).

It is known that acute pancreatitis is the most common intensive alcohol use and gallstone obstruction. Chronic pancreatitis resulting from recurrent episodes of acute pancreatitis is characterized by sudden onset of inflammation of the pancreas (28). Chronic pancreatitis includes; fibrosis, microscopic changes such as acinar atrophy and an inflammatory disease characterized by permanent dysfunction. Fibrosis formation is a progressive process and it may be reversible at an early stage (29).

The molecular mechanism of damage that is developed in chronic pancreatitis is not fully understood yet. Following injury, interstitial edema, parenchymal cell necrosis, intrapancreatic trypsin activation, inflammatory cell migration, PSC activation and proliferation are seen. The amount of cytokines, growth factors and reactive oxygen radicals (ROS) also increase (3). Researches on the isolation and identification of pancreatic stellate cells have provided new data on fibrosis pathogenesis.

Proinflammatory cytokines and growth factors released from cells damaged by factors such as pancreatic alcohol and oxidative stress cause activation of quiescent PSC (24). Acetaldehyde, a consequence of alcohol metabolism by alcohol dehydrogenase, and ROR resulting from necroinflammation are involved in this activation (30).

Besides inflammatory causes, stimuli such as hyperglycemia, endothelin-1, cyclooxygenase (COX) -2, galectin and fibrinogen also cause PSC activation (12). In pancreatic injury, inflammation of cytokines and growth factors released from inflammatory cells, thrombocytes, endothelium, acinar and ductal cells, and activation of PSCs by autocrine effects continue (11). The cytokines (IL-1, IL-6, IL-8 and TNF-alpha), growth factors (PDGF, TGF-β1), angiotensin II, endothelin-1 and ROS play a role in the activation of PSC by paracrine action (31). In addition, mediators such as IL-1, IL-6, TGF-β1, PDGF, endothelin-1, CTGF and COX-2 released from PSC as autocrine play a role in sustaining this activation initiated by paracrine stimulation (3). Chemokines such as TGF-β, CTGF, IL-1, IL-8, IL-15, MCP-1 and RANTES produced by PSC in pancreatic inflammation stimulate localized inflammatory cell migration while ICAM-1 increases inflammatory cell adhesion (12, 32) .

When PDGF, one of the growth factors secreted in pancreatic injury, is added to the cell culture area, the proliferation and migration of PSCs are increased (33). In vitro studies have shown that ethanol and metabolite acetaldehyde induce lipid peroxidation in PSC, leading to activation (34). Antioxidant vitamin E inhibits the activation of PSC starting with ethanol and acetaldehyde, suggesting that oxidative stress plays a role in PSC activation (3). Experimental pancreatic necrosis induced by trinitrobenzene sulfonic acid administration and post-inflammatory PSC activation and pancreatic fibrosis have been observed (35). PSC activation and fibrosis are also observed in pancreatitis models empirically generated by cerulein and dibutyl chloride in mice (36, 37).

Quiescent PSCs play an important role in maintaining the ECM structure with MMP and TIMP they secrete. In active PSCs, the synthesis of these proteins is altered and dense ECM synthesis results in degradation of normal structure. TGF-β, a proliferative protein secreted from damaged pancreatic acidic cells, causes increased synthesis of collagen and fibronectin in α-SMA synthesis in PSC (25). In addition, TGF-β1, PDGF, CTGF, and endothelin-1 released from active PSC stimulate cell proliferation (collagen, fibronectin, laminin) by autocrine effect (31). Experimental animal models have shown that PSC is the major source of type I collagen in pancreatic fibrosis in human tissues (10). Collagen and α-SMA increase were detected in the histological staining performed on the fibrosis area in human and rat pancreas. In in situ hybridization studies to investigate where these increases are occurring; It has been observed that the expression of α-SMA and collagen mRNA in PSC is increased (38). Desmin staining intensity, which is a stellate cell marker in the pancreatic fibrosis area, is also increasing (39).

Increased fibrotic tissue is thought to be due to increased proliferation in the affected PSC, decreased apoptosis, and PSC migrating to the region (38). The phagocytosis function of pancreatic stellate cells is regulated by peroxisome proliferator-activated γ ligand. Profibrotic cytokines, which are expected to increase phagocytosis, have a phagocytic capacity-reducing effect on activated PSC when added to TGF-β and TNF-α cell culture area. This suggests that regulating phagocytosis and profibrotic functions of PSCs in the fibrosis region is a complex process (21).

Pancreas Cancer Development

Ductal adenocarcinoma (PDAC), which is the most common cancer of the pancreas, has dense fibrosis known as desmoplastic reaction. Fibrosis development is also an important feature of chronic pancreatitis (40). The increased risk of PDAC in patients with chronic pancreatitis supports the role of fibrotic microenvironment in this tumor pathogenesis (41). The fibrotic tissue formed after chronic inflammation is thought to provide a suitable environment for the growth and migration-stimulating factors of pancreatic cancer cells (22).

MMP-2, one of the factors facilitating the spread of pancreatic cancer, is also secreted by PSC. Similar to pancreatic fibrosis, studies have shown that the main source of collagen in the stromal reaction around the cancerous region is PSC (10).

Masamune and colleagues demonstrated that PSC coexists with pancreatic cancer cells under the skin of pressed immune system (Nude) mice. When tumor mass surface area measurement was performed after four weeks, it was observed that the tumor size in the area to which the PSCs were added was larger than only the cancer cells in the area (11). The interaction between the tumor and PSC is thought to cause the proliferation of tumor cells and the formation of an environment that facilitates metastasis (42).

Experimental studies have been continuing to develop drugs that stimulate the activation of PSCs involved in the development and progression of chronic pancreatitis fibrosis, stimulate apoptosis, and interact with tumor cells and PSC in pancreatic cancer.

Pancreas Islet Stellate Celss

Islet stellate cell (ISH) isolation was also performed in a recent study in Langerhans islets located in the endocrine part of the pancreas. These cells have been shown to have similar surface markers to PSC. Unlike PSC, however, it is observed that quiescent ISHs also have α-SMA, less cytoplasmic lipid droplets than PSC, and less proliferation and migrations. In experimental Type II diabetes models, there is a relationship between fibrosis suppression and increased beta-cell function. This result is vital because it demonstrates that ISH activation may play a role in the development of Type II diabetes (43).

RESULT

Pancreatic stellate cells are cells that are located within the stroma-supporting tissue and whose functions have not yet been fully understood. However, the data acquired from studies point out that these cells are quiescent and active forms. It is monitored that in the quiescent forms, when the conditions to activate when there is more storage function, these cells acquire myofibroblast-like cell characteristics and take an active role in disease development processes. Pancreatic stellate cells, especially pancreatic fibrosis and their role in cancer development, are very important. Stellate cells with similar characteristics to these cells are found in different organs. Evaluating the effects of these cells on disease development processes and studies on regulating their activation will contribute to the determination of new treatment approaches.

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