Fibrogenesis in the pancreas

Ellenrieder V, Schneiderhan W, Bachem M, Adler G

Department of Internal Medicine I and Department of Clinical Chemistry, University of Ulm, Germany

Abstract

In recent years, numerous studies have provided novel insights into the pathomechanisms of pancreatic fibrogenesis. This includes in particular the identification and characterization of the pancreatic stellate cells (PSCs) and their role in the synthesis of extracellular matrix (ECM) proteins. It has become clear that pancreatic stellate cell activation is regulated by a complex network of growth factors and cytokines and results in increased expression and release of collagens I and II, fibronectin and other components of ECM. Among the cytokines involved in PSC activation and other fundamental mechanisms of pancreatic fibrosis, transforming growth factor beta (TGF β) is of particular relevance. TGF β stimulates PSC activation and induces transcription of ECM proteins mainly via activation of the Smad proteins which regulate gene expression through functional interaction with co-operating partner proteins such as the zinc finger transcription factor Sp1. Recent progress in understanding of the biochemical and molecular mechanisms of pancreatic fibrosis, is reviewed here.

Key words: pancreas, fibrosis, pancreatic stellate cells, TGF ß, extracellular matrix.

ADDRESS FOR CORRESPONDENCE:
Professor Dr Guido Adler
Department of Internal Medicine I
University of Ulm
Robert-Koch-Str. 8
89081 Ulm, Germany
Tel: + 49 731 500 24300 Fax: + 49 731 500 24302
e-mail: guido.adler@medizin.uni-ulm.de

Received 5.05.2004 Accepted 5.06.2004

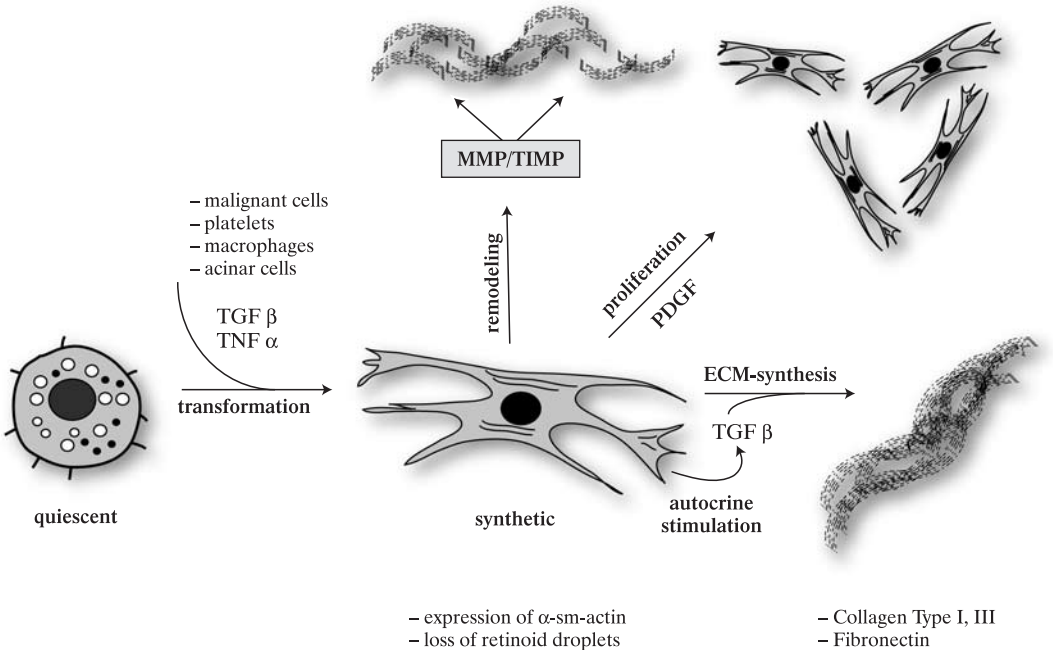
Introduction

Pancreatic cancer, chronic pancreatitis and acute pancreatitis are characterized by profound alterations of extracellular matrix (ECM) formation and composition [1-3]. In these pancreatic diseases the appearance of fibrotic tissue is a result of increased deposition and reduced degradation of extracellular matrix.

The extracellular matrix (ECM) is comprised of four major classes of macromolecules – the collagens, proteoglycans, structural glycoproteins, and elastin [4-6]. Individual members of each class and family of ECM molecules were found to exhibit a degree of tissue-specific distribution implicating the matrix in development and tissue function [7,8]. Cell surface receptors for individual ECM components were identified, which provided a rational basis for linking the ECM with the cell [9-11]. From these discoveries it is now evident that the extracellular matrix is composed of a number of different macromolecules whose structural integrity and functional composition are important in maintaining normal tissue architecture, in development and in tissue-specific function [4,6,8]. On the other hand, it has been recognized that dysfunctional matrix components and abnormalities in ECM biosynthesis and catabolism are of importance in both inherited and acquired diseases and in normal wound healing [4-6].

Years ago, we and others identified "fibroblast-like" cells in the pancreas that show characteristics of myofibroblasts, e.g. expression of α-smooth muscle actin, synthesis of collagens and fibronectin, and the formation of dense bodies [12,13]. Only recently, these cells were identified as pancreatic stellate cells, formerly named fat-storing cells, which show similarities in their retinoic metabolism and morphology to hepatic stellate cells and are considered as key players in the fibrogenesis in different pancreatic diseases [14-16]. Activation of pancreatic stellate cells is orchestrated by cytokines and components of the ECM as well [21,22]. Among the cytokines involved in this process, transforming growth factor beta (TGF β) is of particular relevance [17].

Figure 1. **Transformation of pancreatic stellate cells is characterized by cellular enlargement, loss of retinoid droplets and the expression of smooth-muscle alpha actin. Initiating stimuli include TGF β and TNF α from platelets, macrophages or acinar cells. The activated pancreatic stellate cell is sensitive to proliferative and fibrogenic cytokines leading to enhanced proliferation and extracellular matrix synthesis. Modulation of extracellular matrix degradation through MMP and TIMP secretion is also a feature of the active pancreatic stellate cell. TGF β, transforming growth factor beta; PDGF, platelet derived growth factor; TNF α, tumor necrosis factor alpha; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases; ECM, extracellular matrix**



Pancreatic stellate cells in fibrogenesis of the pancreas

Cells producing extracellular matrix in the pancreas were described as "fibroblast-like" cells showing characteristics of myofibroblasts, e.g. expression of α-smooth muscle actin, synthesis of collagens and fibronectin, and the formation of dense bodies (microfilaments) [16,18]. The presence of retinoid containing fat-storing cells in the pancreas of mice, rats, and humans was already demonstrated in 1982 [19]. While a potential role of these cells in pancreas remodelling and fibrosis was already discussed by Ikejiri et al. [20], it took another 8 years until Bachem and coworkers were able to isolate and characterize these cells [14]. Their data on description of this cell type have been supported in the same year by Apte and coworkers [15]. Because of their similarity in morphology and retinoid metabolism to hepatic stellate cells, Bachem et al., named these cells pancreatic stellate cells (PSC). PSC's are located in the interlobular and interacinar region of the human pancreas with characteristics of myofibroblasts, e.g. expression of α-smooth muscle actin (α-sm-actin), the formation of dense bodies (microfilaments) and the presence of retinoid-containing fat droplets [14,16] (*Fig. 1*). During primary culture of PSC, however, the number and size of retinoid fat droplets decrease in parallel to the increase in α-sm-actin expression and extracellular matrix synthesis. This switch from a quiescent "fat-storing" to a highly proliferative "myofibroblast-like" phenotype is associated with loss of cellular retinol content, the development of a prominent endoplasmatic reticulum, and increased synthesis of extracellular matrix proteins. In contrast to the "fat-storing" cell-type, the "synthetic" phenotype of PSC synthesizes and secretes high amounts of collagen type I and fibronectin [14]. By quantitative measurement of the procollagen peptides, we have demonstrated that the "synthetic" phenotype of human PSC synthesizes 25-40 fold more collagen type I than collagen type III esters [14]. Together, these studies identified the pancreatic stellate cells as a major source of extracellular matrix proteins.

Fibrogenesis in acute pancreatitis

In acute pancreatitis significant amounts of extracellular matrix are deposited in interlobular and interacinar regions of the pancreas. This has been extensively studied in the model of cerulein pancreatitis [23-25]. Pancreatic regeneration from acute cerulein-induced pancreatitis in rats is characterized by proliferation of acinar and centroacinar cells, an increase in mitotic activity of fibroblasts and by stimulation of transcription, synthesis and deposition of extracellular matrix components, in particular collagens I/III and fibronectin [23-25]. However, approximately two weeks after induction of pancreatitis the histology, organ weight and collagen content of the pancreas returned to control values indicating complete regeneration

[23]. Interestingly, even repetitive induction of cerulein pancreatitis failed to induce fibrosis in the rat pancreas [24]. Thus, the cerulein pancreatitis as a model of acute pancreatitis is characterized by a dynamic process of ECM-formation and removal, making it ideally suited for functional studies of the ECM in the pancreas.

Fibrogenesis in chronic pancreatitis

In chronic pancreatitis fibrosis is the most impressive morphological finding. The current model of molecular pathogenesis of fibrosis demonstrates the central role of activated pancreatic stellate cells. Chronic pancreatitis is characterized by destruction of acinar cells and islet cells, activation of pancreatic stellate cells and replacement by connective tissue. This connective tissue results from an increased deposition and disorganization of extracellular matrix proteins including fibronectin, laminin, and collagens type I, III and IV [26]. We and others could show that these alterations of ECM-proteins are accompanied by an increase of the transcript levels of genes coding for collagens I/III and IV, fibronectin and laminin in human chronic pancreatitis [27]. Ongoing studies are focusing on the identification and characterization of signaling regulated transcription factors, such as the Smads and Sp1 that might play important roles in pancreatic stellate cell activation and expression of other extracellular matrix components [29].

Fibrogenesis in pancreatic cancer

Pancreatic cancer tissues shows a strong desmoplastic reaction, characterized by a remarkable increase of interstitial connective tissue [28,29]. This desmoplastic reaction in pancreatic cancer tissue is accompanied by increased steady state levels of the mRNA's for collagens type I, III and IV, fibronectin and laminin [28] and of collagen protein. Connective tissue cells in the stroma of pancreatic carcinomas were shown to be the main site of collagen type I and III mRNA transcription by in-situ hybridization. Interestingly it appeared that connective tissue cells neighbouring the tumor cells contained larger amounts of collagen transcripts than stromal cells distant from the tumor, suggesting that stromal cells appear to be the main site of ECM production in pancreatic cancer [30].

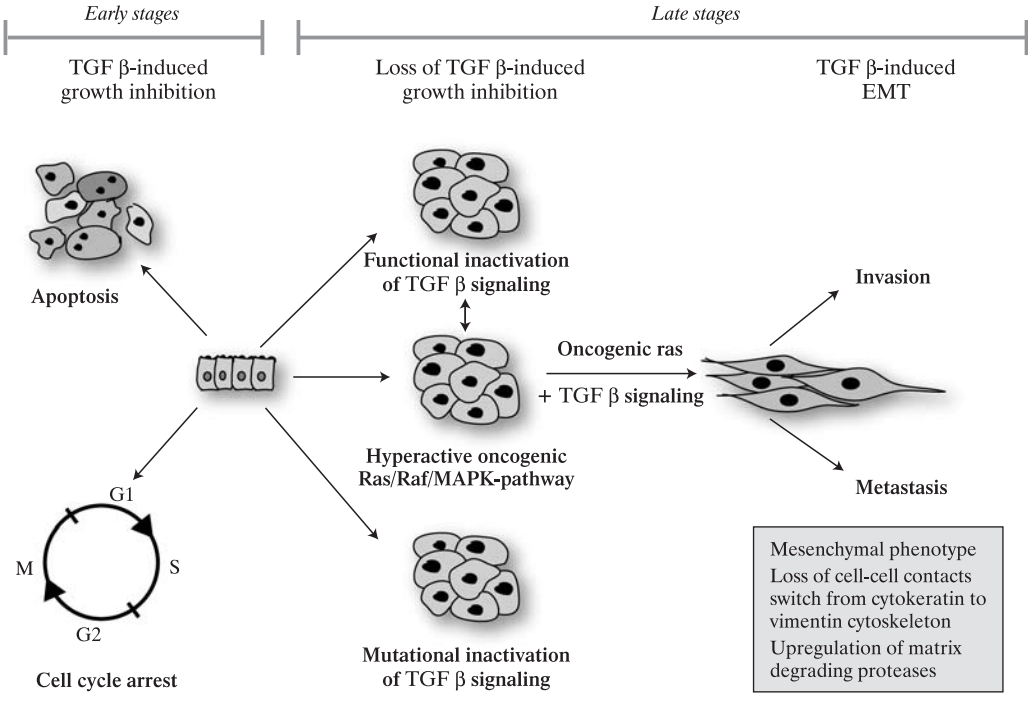
The role of TGF β and other growth factors in the regulation of pancreatic fibrogenesis

TGF β has been shown to be of particular importance in biosynthesis and turnover of extracellular matrix [31,32]. TGF β1 stimulates transcription and biosynthesis of different extracellular matrix proteins and in addition functions as an inhibitor of epithelial cell proliferation [33-35]. We could show that during regeneration from cerulein-induced pancreatitis in rats, alterations of collagen gene expression were accompanied by parallel alterations of TGF β1 gene expression [36]. A maximal increase of TGF β-mRNA (3-fold over controls) was found two days after the end of maximal cerulein infusion, and in-situ hybridization showed that these transcripts were mainly produced by both, stromal cells and acinar cells. TGF β1 protein content in total pancreas reached peak values after one day and protein levels remained high throughout the second day.

Additonal experiments using neutralizing antibodies against TGF β1 demonstrated that inhibition of TGF β during regeneration from cerulein-induced pancreatitis is associated with decreased levels of both collagen type I and TGF β, suggesting that TGF β plays an important role in the regulation of extracellular matrix proteins during regeneration from acute pancreatitis. The latter effect has been attributed to an autocrine loop in which TGF β induces its own synthesis (35,36).

Further evidence for the role of TGF β in pancreatic fibrogenesis was provided by two studies using transgenic mice models overexpressing TGF β. TGF β was expressed in pancreatic cells under the control of the insulin promoter to study the effect of this growth factor on the pancreas in an in-vivo situation [35,37]. Both studies reported cellular infiltration comprising macrophages and neutrophils, fibroblast proliferation and abnormal deposition of extracellular matrix in the pancreas. Böttinger and collaborators [38] have used an elegant approach based on the expression of a dominant-negative mutant TGF β type II receptor in transgenic mice to functionally inactivate TGF β signalling in epithelial cells. The dominant-negative mutant type II TGF β receptor blocked signaling by all three TGF β isoforms in primary hepatocyte and pancreatic acinar cell cultures generated from the transgenic mice. Acinar cells in the pancreas of these transgenic mice showed increased proliferation and severely perturbed acinar differentiation. Additional abnormalities in the pancreas included fibrosis, neoangiogenesis and mild macrophage infiltration. Data obtained in the experimental models described above clearly identify TGF β as one of the central regulators of ECM-formation in the pancreas. Moreover it becames clear, that TGF β which is released from various cell types including activated macrophages and platelets induces pancreatic fibrogenesis mainly through its effects on pancreatic stellate cells. It has been shown, for instance, that TGF β promotes PSC activation and increases expression of collagen and other ECM components through induction of an autocrine feedback mechanism [39]. The autocrine feedback loop is initiated through binding of TGF β to a heteromeric complexe of specific type II (TβR-II) and type I (TβR-I) kinase receptors. Receptor activation leads to phosphorylation of the Smad transcription factors, which then translocate to the nucleus and regulate the transcription of various target genes including TGF β itself, and genes involved in PSC activation and ECM synthesis. Interestingly, however, TGF β not only has the capacity to activate transcription of extracellular matrix forming proteins such as collagen I, collagen III and fibronectin but also induces the expression of matrix degrading proteinases such as MMP-2, MMP-9, and MMP-13 [40]. Although abundant data clearly demonstrate the critical role of the Smads in TGF β signaling and transcription, growing evidence suggest that TGF β can also induce the expression or activation of other transcription factors that do not belong to the Smad family but yet might play significant roles in TGF β-induced stellate cell

***Figure 2*. The dual role of TGF β in pancreatic cancer. In early tumor stages, TGF β acts as a tumor suppressor through transcriptional regulation of genes involved in cell cycle control or induction of apoptosis. In later stages of the disease, however, the tumor cells escape from TGF β-growth inhibition and respond to TGF β with increased invasion and metastasis**



activation. Though most of the data on the regulation of Smad signaling were obtained from studies on hepatic stellate cells, it appears most likely that Smad signaling activity in pancreatic stellate cells is also tightly controlled temporally and spatially through multiple mechanisms at the extracellular, membrane, cytoplasmic and nuclear levels. For example, Smad2 was found to be phosphorylated and activated in PSC's depending on the transdifferentiation grade and is negatively regulated through the inhibitory feedback loop imposed by Smad7. On the other hand, positive regulation of the Smads in PSC's occurs through activation of ERK MAP kinase signaling which appears to play a crucial role in Smad-mediated induction of the autocrine TGF β loop [41].

Taken together, the discovery of PSC's and its regulation through TGF β-induced signaling and transcription revealed new insights into the pathogenesis of pancreatic fibrosis and identified these highly active cells as the main cellular source of extracellular matrix in chronic pancreatitis. Thus, recent data propose a new model of the molecular pathogenesis of pancreatic fibrosis which suggests that acinar cell injury is followed by monocyte/macrophage invasion and activation, as well as platelet aggregation in areas of inflammation. This causes release of several polypeptide growth factors and in particular TGF β, which, in turn, stimulate PSC to synthesize extracellular matrix proteins in the pancreas.

This is also true for pancreatic cancer, in which TGF β has been demonstrated to play a key role in tumor cell invasion and in the development of the desmoplastic reaction. Based on studies using cultured cells, transgenic mice, and human tumors, an emerging model suggests a dual role for TGF β-signaling in tumorigenesis [42,43]. In early tumor stages, TGF β inhibits cell proliferation, at least in part, through transcriptional regulation of target genes involved in cell cycle control. During tumorigenesis, however, many tumor cells lose their ability to respond to TGF β with growth inhibition, and instead, respond with increased invasion and metastasis. Thus, loss of sensitivity to inhibition of growth by TGF β by most tumor cells is not synonymous with complete loss of TGF β signaling, but rather suggests that tumor cells gain advantage by selective inactivation of the tumor suppressor activities of TGF β while retaining its tumor promoting activities. We and others have shown that TGF β promotes tumor progression via stimulation of tumor cell invasion and metastasis, increased angiogenesis, and depression of local immune responses [44-46]. A hallmark of TGF β mediated tumor progression is the transdifferentiation of epithelial tumor cells into a fibroblast-like phenotype. We have shown that this so-called EMT (epithelial-mesenchymal transition) is strongly induced by TGF β, and is accompanied by upregulation of mesenchymal markers (e.g. collagen I and vimentin) and down-regulation of cytokeratins [46]. TGF β mediated EMT is further characterized by strong induction of uPA and MMP-2 expression and activation and results in increased motility, matrix degradation and tumor cell invasion [45]. Together, TGF β plays a multifunctional role in various pancreatic diseases ranging from modulator of tissue regeneration to stimulator of tumor cell progression.

The role of matrix metalloproteinases and their inhibitors in fibrogenesis

Matrix metalloproteinases (MMP) comprise a growing family of proteolytic enzymes that contain tightly bound zinc [45,46]. According to their substrate specificity MMP's can broadly be classified as collagenases, gelatinases, stromelysins and the membrane-type metalloproteinases. MMP's are secreted as proenzymes which are activated by proteolytic cleavage of an aminoterminal propeptide e.g. by plasmin, cathepsin G, trypsin, α-chymotrypsin and MMP-3 [47,49]. Activity is further controlled by various proteinase inhibitors such as α2-microglobulin and more importantly the family of tissue inhibitors of metalloproteinases, TIMP-1-4. Active MMP's display proteolytic activity for at least one component of the ECM. There is considerable evidence that MMP's have a major role in physiological ECM resorption as in development or postnatal remodeling and in pathological ECM-resorption e.g. associated with local invasiveness or metastasis of malignant tumors and the destruction of joints in rheumatoid arthritis [45-49].

Alterations of the balance of expression between MMPs and TIMPs has been described in several inflammatory diseases. In chronic pancreatitis, increased levels of MMP-2 (72 kDa collagenase IV), TIMP-1 and TIMP-2 have been detected, although the magnitude of MMP/TIMP transcript levels do not correlate to the degree of fibrosis and inflammation [50,51]. Interestingly, transcripts of genes encoding extracellular matrix degrading proteinases with a substrate specificity for interstitial extracellular matrix components such as MMP-1 and MMP-3 are not elevated in chronic pancreatitis. Possibly, the lack of MMP-1 and MMP-3 expression contributes to the deposition of ECM components in the interstitial space.

For a more functional approach to the role of MMP's and their inhibitors in the development of pancreatic fibrosis we used the model of cerulein-induced pancreatitis in rats [52]. Surprisingly increased expression during days 2-4 after induction of pancreatitis could only be demonstrated for MMP-2 and MMP-3, whereas transcript levels of MMP-1 and MMP-9 did not change throughout the regeneration period.

The role of MMPs and TIMPs has been extensively studied in tumorous diseases. Mostly, expression of MMPs and TIMPs was shown to correlate with an increased metastatic and invasive potential of tumor cells [53-55]. High expression and activation levels of MMP-2 have been found in various human cancer tissues, including breast and pancreatic cancer and correlated with tumor stage and grade in several cases [56,57]. We have recently demonstrated that increased expression and activation levels of MMP-2 are strongly associated with elevated expression levels of its activators MT1-MMP and MT2-MMP and that it plays a significant role in TGF β-mediated pancreatic tumor cell invasion [51]. A good correlation was also seen between MMP-2 expression and levels of transcripts coding for extracellular matrix proteins, the amount of collagen protein and the severity of the desmoplastic reaction. In-situ hybridization studies localized transcripts coding for MMP-2 in both, stromal and tumor cells, although it appeared to be more abundant in stromal cells.

Besides the family of metalloproteinases the plasminogen activator/plasmin system is considered as a key player in tumor invasion/metastasis. Tissue-type plasminogen activator (tPA) [58], urokinase-plasminogen activator (uPA) and the uPA-receptor (uPAR) [59] were found to be overexpressed in a variety of epithelial tumor tissues including pancreatic cancer in correlation with decreased postoperative survival [59]. Recently we identified a novel, membrane bound Kunitz-type serine proteinase inhibitor highly overexpressed in pancreatic cancer, which was named "kop" (for Kunitz-type proteinase inhibitor overexpressed in pancreatic cancer) [60]. The same proteinase inhibitor was cloned in human placenta and was called "bikunin". Placenta as pancreatic cancer is a tissue characterized by extensive ECM remodelling requiring the balanced activity of proteinases and proteinase inhibitors [61]. Recently published data suggest that kop/ /bikunin is a strong inhibitor of human plasmin, human tissue kallikrein and human plasma kallikrein [62] and might therefore be involved in the regulation of MMP-activation or directly counteract uPA.

Conclusions

Deposition of extracellular matrix in inflammatory and tumorous diseases of the pancreas is the result of a dynamic process of mechanisms like acinar cell injury and necrosis, inflammation, activation of macrophages, aggregation of platelets, release of growth factors, activation of pancreatic stellate cells, and stimulated synthesis of extracellular matrix and reduced matrix degradation. Pancreatic stellate cells represent the main cellular source of extracellular matrix in chronic pancreatitis and pancreatic cancer. Pancreatic stellate cells share homologies with hepatic stellate cells including storage of retinyl palmitate, retinal esterification, expression of the cytofilaments vimentin and desmin and phenotypic transition to an active matrix producing myofibroblast-like cell.

Among other growth factors, TGF β has been shown to be of paramount importance for the regulation of the dynamic process of extracellular matrix turnover in the pancreas. Proteinases as e.g. metalloproteinases or serine proteinases are TGF β-regulated effector proteins that are responsible for the coordinated removal of ECM components. Imbalances of the ratio between proteinases and their natural inhibitors may be one the major pathogenetic factors leading to fibrosis.

References

1. Uscanga L, Kennedy RH, Choux R, Druguet M, Grimaud JA, Sarles H. Sequential connective matrix changes in experimental acute pancreatitis. An immunohistochemical and biochemical assessment in the rat. Int J Pancreatol, 1987; 2: 33-45.

2. Kennedy RH, Bockman DE, Uscanga L, Choux R, Grimaud JA, Sarles H. Pancreatic extracellular matrix alterations in chronic pancreatitis. Pancreas, 1987; 2: 61-72.

3. Gress TM, Menke A, Bachem M, et al. Role of extracellular matrix in pancreatic diseases. Digestion, 1998; 59: 625-37.

4. Hay RD. Cell biology of extracellular matrix. Plenum press, New York, 2nd edition, 1991.

5. Royce PM and Steinmann B. Connective tissue and its heritable

disorders. Molecular, genetic and medical aspects. Wiley-Liss Inc, New York, 1993.

6. Zern MA, Reid LM. Extracellular matrix: chemistry, biology and pathobiology with emphasis on the liver. Marcel Decker Inc, New York, 1993.

7. Hay ED. Cell and extracellular matrix: Their organization and mutual dependence. Modern Cell Biology, 1983; 2: 509-48.

8. Trelstad RL. The role of the extracellular matrix in development. Alan R. Liss, New York, 1984.

9. Juliano RL, Haskill S. Signal transduction from the extracellular matrix. J Cell Biol, 1993; 120: 577-85.

10. Ruoslahti E. Intergins. J Clin Invest, 1991; 87: 1-5.

11. Hynes RO. Integrins: a family of cell surface receptors. Cell, 1987; 48: 549-52.

12. Bachem MG, Meyer D, Melchior R, Sell KM, Gressner AM. Activation of rat liver perisinusoidal lipocytes by transforming growth factors derived from myofibroblastlike cells. A potential mechanism of self perpetuation in liver fibrogenesis. J Clin Invest, 1992; 89: 19-27.

13. Gressner AM, Bachem MG. Cellular sources of noncollagenous matrix proteins: role of fat-storing cells in fibrogenesis. Semin Liver Dis, 1990; 10: 30-46.

14. Bachem MG, Schneider E, Gross H, Weidenbach H,Schmid RM, Menke A, Siech M, Beger H, Grunert A, Adler G. Identification, culture, and characterization of pancreatic stellate cells in rats and humans. Gastroenterology, 1998; 115: 421-32.

15. Apte MV, Haber PS, Applegate TL, Norton ID, McCaughan GW, Korsten MA, Pirola RC,Wilson JS. Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. Gut, 1998; 43: 128-33.

16. Saotome T, Inoue H, Fujimiya M, Fujiyama Y, Bamba T. Morphological and immunocytochemical identification of periacinar fibroblast-like cells derived from human pancreatic acini. Pancreas, 1997; 14: 373-82.

17. Schmid-Kotsas A, Gross HJ, Menke A, Weidenbach H, Adler G, Siech M, Beger H, Grunert A, Bachem MG. Lipopolysaccharide-activated macrophages stimulate the synthesis of collagen type I and C-fibronectin in cultured pancreatic stellate cells. Am J Pathol, 1999; 155: 1749-58.

18. Okumura Y, Shintani Y, Kato Y, Tamba J, Inoue H, Fujiyama Y, Bmba T. Proliferative effect of phospholipase A2 on rat periacinar fibroblastoid cells of the pancreas. Pancreas, 1998; 16: 505-10.

19. Watari N, Hotta Y, Mabuchi Y. Morphological studies on a vitamin A-storing cell and its complex with macrophage observed in mouse pancreatic tissues following excess vitamin A administration. Okajimas Folia Anat Jpn, 1982; 58: 837-58.

20. Ikejiri N. The vitamin A-storing cells in the human and rat pancreas. Kurume Med J, 1990; 37: 67-81.

21. Schneider E, Schmid-Kotsas A, Zhao J, Weidenbach H, Schmid RM, Menke A, Adler G, Waltenberger J, Grunert A, Bachem MG. Identification of mediators stimulating proliferation and matrix synthesis of rat pancreatic stellate cells. Am J Physiol Cell Physiol, 2001; 281: 532-43.

22. Luttenberger T, Schmid-Kotsas A, Menke A, Siech M, Beger H, Adler G, Grunert A, Bachem MG. Platelet-derived growth factors stimulate proliferation and extracellular matrix synthesis of pancreatic stellate cells: implications in pathogenesis of pancreas fibrosis. Lab Invest, 2000; 80: 47-55.

23. Elsässer HP, Adler G, Kern HF. Fibroblast structure and function during regeneration from hormone-induced acute pancreatitis in the rat. Pancreas, 1989; 4: 169-78.

24. Elsässer HP, Haake T, Grimmig M, Adler G, Kern HF. Repetitive cerulein-induced pancreatitis and pancreatic fibrosis in the rat. Pancreas, 1992; 7: 385-90.

25. Gress T, Müller-Pillasch F, Elsässer HP, Bachem M, Ferrara C, Weidenbach H, Lerch M, Adler G. Enhancement of transforming growth factor 1 expression in the rat pancreas during regeneration from cerulein-induced pancreatitis. Eur J Clin Invest, 1994; 24: 679-85.

26. Kennedy RH, Bockman DE, Uscanga L, Choux R, Grimaud JA, Sarles H. Pancreatic extracellular matrix alterations in chronic pancreatitis. Pancreas, 1987; 2: 61-72.

27. Gress TM, Müller-Pillasch F, Lerch MM, Friess H, Büchler M, Beger HG, Adler G. Balance of expression of genes coding for extracellular matrix proteins and extracellular matrix degrading proteases in chronic pancreatitis. Z Gastroenterol, 1994; 32: 221-5.

28. Gress TM, Müller-Pillasch F, Lerch MM, Friess H, Büchler M, Adler G. Expression and in-situ localization of genes coding for extracellular matrix proteins and extracellular matrix degrading proteases in pancreatic cancer. Int J Cancer, 1995; 62: 407-13.

29. Garcia-Ruiz I, de la Torre P, Diaz T, Esteban E, Fernandez I, Munoz-Yague T, Solis-Herruzo JA. Sp1 and Sp3 transcription factors mediate malondialdehyde-induced collagen alpha 1(I) gene expression in cultured hepatic stellate cells. J Biol Chem, 2002; 277: 30551-8.

30. Hernandez I, de la Torre P, Rey-Campos J, Garcia I, Sanchez JA, Munoz R, Rippe RA, Munoz-Yague T, Solis-Herruzo JA. Collagen alpha1(I) gene contains an element responsive to tumor necrosis factor-alpha located in the 5' untranslated region of its first exon. DNA Cell Biol, 2000; 19: 341-52.

31. Border WA, Noble NA, Yamamoto T, Harper JR, Yamaguchi Y, Pierschbacher MD, Ruoshlati E. Natural inhibitor of transforming growth factor beta protects against scaring in experimental kidney disease. Nature, 1992; 360: 361-4.

32. Van Laethem JL, Robberecht P, Résibois A, Devière J. Transforming growth factor β promotes development of fibrosis after repeated courses of acute pancreatitis. Gastroenterolgy 1996; 110: 576-82.

33. Löhr M, Trautmann B, Göttler M, Peters S, Zauner I, Maillet B, Klöppel G. Human ductal adenocarcinomas of the pancreas express extracellular matrix proteins. Br J Cancer, 1994, 49: 144-51.

34. Siegel PM, Massague J. Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. Nat Rev Cancer, 2003; 3(11): 807-21.

35. Lee MS, Gu D, Feng L, Curriden S, Arnush M, Krahl T, Gurushanthaiah D, Wilson C, Loskutoff DL, Fox H. Accumulation of extracellular matrix and developmental dysregulation in the pancreas by transgenic production of transforming growth factor beta1. Am J Pathol, 1995; 147: 42-52.

36. Menke A, Yamaguchi H, Gress TM, Adler G. Extracellular matrix is reduced by inhibition of transforming growth factor β1 in pancreatitis in the rat. Gastroenterology, 1997; 113: 295-303.

37. Sanvito F, Nichols A, Herrera PL, Huarte J, Wohlwend A, Vassalli JD, Orci L. TGF-β1 overexpression in murine pancreas induces chronic pancreatitis and, together with TNFalpha triggers insulin-dependent diabetes. Biochem Biophys Res Comm, 1995; 217: 1279-86.

38. Böttinger EP, Jakubczak, JL, Roberts IS, Mumy M, Hemmati P, Bagnall K, Merlino G, Wakefield LM. Expression of a dominant-negative mutant TGFβ type II receptor in transgenic mice revealy essential roles for TGF-β in regulation of growth and differentiation in the exocrine pancreas. EMBO J, 1997; 16: 2621-33.

39. Kruse ML, Hildebrand PB, Timke C, Folsch UR, Schmidt WE. TGFbeta1 autocrine growth control in isolated pancreatic fibroblastoid cells/stellate cells in vitro. Regul Pept, 2000; 90: 47-52.

40. Phillips PA, McCarroll JA, Park S, Wu MJ, Pirola R, Korsten M, Wilson JS, Apte MV. Rat pancreatic stellate cells secrete matrix metalloproteinases: implications for extracellular matrix turnover. Gut, 2003; 52: 275-82.

41. Ohnishi H, Miyata T, Yasuda H, Satoh Y, Hanatsuka K, Kita H, Ohashi A, Tamada K, Makita N, Iiri T, Ueda N, Mashima H, Sugano K. Distinct roles of Smad2-, Smad3-, and ERK-dependent pathways in transforming growth factor-beta1 regulation of pancreatic stellate cellular functions. J Biol Chem, 2004; 279: 8873-8.

42. Piek E, Roberts AB. Suppressor and oncogenic roles of TGF-beta and its signaling pathways in tumorigenesis. Adv Cancer Res, 2001; 83: 1-54.

43. Wakefield LM, Roberts AB. TGF-beta signaling: positive and negative effects on tumorigenesis. Curr Opin Genet Dev, 2002; 12: 22-9.

44. Ellenrieder V, Adler G, Gress TM. Invasion and metastasis in pancreatic cancer. Ann Oncol, 1999; 10: 46-50.

45. Ellenrieder V, Hendler SF, Ruhland C, Boeck W, Adler G, Gress TM. TGF-beta-induced invasiveness of pancreatic cancer cells is mediated by matrix metalloproteinase-2 and the urokinase plasminogen activator system. Int J Cancer, 2001; 93: 204-11.

46. Ellenrieder V, Hendler SF, Boeck W, Seufferlein T, Menke A, Ruhland C, Adler G, Gress TM. Transforming growth factor beta1 treatment leads to an epithelial-mesenchymal transdifferentiation of pancreatic cancer cells requiring extracellular signal-regulated kinase 2 activation. Cancer Res, 2001; 61: 4222-8.

47. Woessner JF. The family of matrix metallopproteinases. Ann NY Acad Sci, 1994; 732: 11-21.

48. Brown PD. Matrix metalloproteinase inhibitors in the treatment of cancer. Med Oncol, 1997; 14: 1-10.

49. Ellenrieder V, Alber B, Lacher U, et al. Role of MT-MMPs and MMP-2 in pancreatic cancer progression. Int J Cancer, 2000; 85: 14-20.

50. Ishihara T, Hayasaka A, Yamaguchi T, Kondo F, Saisho H. Immunohistochemical study of TGFbeta, matrix metalloproteinase-2,9, tissue inhibitors of metalloproteinase-1,2, and basement membrane components at pancreatic ducts in chronic pancreatitis. Pancreas, 1998; 17: 412-8.

51. Gress TM, Muller-Pillasch F, Lerch MM, Friess H, Buchler M, Beger HG, Adler G. Balance of expression of genes coding for extracellular matrix proteins and extracellular matrix degrading proteases in chronic pancreatitis. Z Gastroenterol, 1994; 32: 221-5.

52. Müller-Pillasch F, Gress TM, Yamaguchi H, Geng M, Adler G, Menke A. The influence of transforming growth factor β1 on the expression of genes coding for matrix metalloproteinases and tissue inhibitors of metalloproteinases during regeneration from cerulein-induced pancreatitis. Pancreas, 1997; 15: 168-75.

53. Levy AT, Cioce V, Sobel ME, Spiridone G, Grigoioni WF, Liotta LA. Increased expression of the Mr 72,000 type IV collagenase in human colonic adenocarcinoma. Cancer Res, 1991; 51: 439-44.

54. Muller D, Wolf C, Abecassis J, Millon R, Engelmann A, Bronner G, Rouyer N, Rio MC, Eber M, Methlin G. Increased stromelysin 3 gene expression is associated with increased local invasiveness in head and neck squamous cell carcinomas. Cancer Res, 1993; 53: 165-9.

55. Urbanski SJ, Edwards DR, Maitland A, Leco KJ, Watson A, Kossakowska AE. Expression of metalloproteinases and their inhibitors in primary pulmonary carcinomas. Br J Cancer, 1992; 66: 1188-94.

56. Bramhall SR, Stamp GWH, Dunn J, Lemoine NR, Neoptolemos JP. Expression of collagenase (MMP2), stromelysin (MMP3) a tissue inhibitor of the metalloproteinase (TIMP1) in pancreatic and ampullary disease. Br J Cancer, 1996; 73: 972-8.

57. Bramhall SR. The Matrix Metalloproteinases and their inhibitors in pancreatic cancer. Int J Pancreatology, 1997; 21: 1-12.

58. Paciucci R, Berrozpe G, Torà M, Navarro E, Garcia de Herreros A, Real FX. Isolation of tissue-type plasminogen activator, cathepsin H, and non-specific cross-reacting antigen from SK-PC-1 pancreas cancer cell lines using subtractive hybridization. FEBS Letters, 1996; 385: 72-6.

59. Cantero D, Friess H, Deflorin J, Zimmermann A, Brundler MA, Riesle E, Korc M, Büchler MW. Enhanced expression of urokinase plasminogen activator and its receptor in pancreatic cancer. Br J Cancer, 1997; 75: 388-95.

60. Müller-Pillasch F, Wallrapp C, Bartels K, Varga G, Friess H, Buchler M, Adler G, Gress TM. Cloning of a new Kunitz-type protease inhibitor overexpressed in pancreatic cancer. Biochim Biophys Acta, 1998; 1395: 88-95.

61. Marlor CW, Delaria KA, Davis G, Muller DK, Greve JM, Tamburini PP. Identification and cloning of human placental bikunin, a novel serine protease inhibitor containing two kunitz domains. J Biol Chem, 1997; 272: 12202-8.

62. Delaria K, Muller DK, Marlor CW, Brown JE, Das RC, Roczniak SO, Tamburini PP. Characterization of placental bikunin, a novel human serine protease inhibitors. J Biol Chem, 1997; 272: 12209-24.