Chronic Pancreatitis: Pathogenesis and Molecular Aspects

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Abstract

Chronic pancreatitis (CP) is characterized by irreversible morphological and functional alterations of the pancreas presenting clinically with upper abdominal pain as well as exocrine and endocrine insufficiencies. CP is morphologically characterized by pancreatic head enlargement, calcifications of the parenchyma, cysts, and pancreatic stones. The most common etiological factor of CP in Western industrialized countries is alcohol abuse; less common factors include hereditary pancreatitis, CP due to metabolic disturbances, CP due to pancreas divisum or duodenal wall cysts, and idiopathic CP. The molecular alterations leading to the chronic inflammatory process are nor completely understood. Research during the last years, however, has elucidated that a number of growth factors and their receptors are overexpressed in CP, which is thought to contribute to the high degree of pancreatic fibrosis and to the proliferative potential of ductular cells in this disorder. In addition, gene mutations have been detected in a subgroup of CP samples underscoring the premaligant potential of CP. In this review we will summarize our current knowledge about pathogenic and molecular aspects of CP.

Key words: Chronic pancreatitis, growth factors, gene mutations, CFTR, k-ras.

Riassunto

LA PANCREATITE CRONICA: PATOGENESI E ASPETTI MOLECOLARI

La pancreatite cronica è caratterizzata da alterazioni morfologiche e funzionali irreversibili a carico della ghiandola pancreatica, che si presentano clinicamente con dolore addominale alto così come negli stadi successivi co l'insufficienza esocrina ed endocrina del pancreas. Morfologicamente la pancreatite cronica è caratterizzata da un ingrossamento della testa pancreatica, da calcificazioni del parenchima, cisti e calcoli pancreatici. Il fattore eziologico principale della pancreatite nei paesi occidentali industrializzati, è l'abuso di alcool; altri fattori meno comuni includono la pancreatite ereditaria, la pancreatite dovuta a disturbi metabolici, la pancreatite da pancreas divisum o da cisti della parete duodenale e la pancreatite idiopatica. Le alterazioni molecolari che portano all'infiammazione cronica del pancreas non sono completamente chiarite. Durante gli ultimi anni la ricerca tuttavia ha dimostrato che un numero di fattori di crescita e di loro recettori risultano iperespressi in corso di pancreatite cronica, facendo ipotizzare un loro

Introduction

Chronic pancreatitis (CP) is an inflammatory disease of the pancreas that leads to persistent, irreversible, and progressive morphological and functional alterations of the whole organ resulting in severe exocrine and endocrine insufficiencies in its terminal state (1, 2, 3). Approximately 4/100,000 people are affected by this disease every year, and a rising incidence has been observed over the past 20 years (4). Morphologically, chronic inflammation of the pancreas is associated with pancreatic head enlargement, calcifications of the parenchyma, cysts, necrosis and pancreatic stones (5, 6). The continuous and irreversible tissue destruction and subsequent remodeling causes finally the two major clinical symptoms: upper abdominal pain and maldigestion.

The pathobiological mechanisms of pain in chronic pancreatitis are still controversial: focal acute inflammation of the pancreas, increased intraductal/intraparenchymal pressure, extrapancreatic causes like common bile duct stenosis or duodenal stenosis, and postprandial pancreatic hyperstimulation due to decreased secretion capacity and the insufficient functioning of negative feedback regulation have been proposed to play a role in the generation of pain in CP (7, 8). In addition, recent studies using modern molecular biology techniques have led to the concept that direct alterations of nerves and changes in neurotransmitters might cause pain in patients with CP (9-13).

Maldigestion is the second leading clinical symptom in CP that is caused by the loss of functioning exocrine parenchyma. The mechanisms leading to the destruction of the exocrine pancreas and the replacement of normal pancreatic architecture by degenerating acinar cells, proliferating ductal cells, and most prominently by fibrosis

contributo nello sviluppo dell'alto grado di fibrosi pancreatica e dei processi proliferativi a carico delle cellule duttali. Ancora, mutazioni genetiche sono state dimostrate in un sottogruppo di campioni di pancreatite cronica, sottolineando la potenziale premalignita' di queste lesioni. In questa review viene fatto il punto dell'attuale conoscenza degli aspetti patogenetici e molecolare della pancreatite cronica.
Parole chiave: Pancreatite cronica, fattori di crescita, mutazioni genetiche CTFR, K-ras.

are not completely understood, and a number of pathophysiological concepts were introduced in recent decades (14, 15).

The fast-developing field of molecular biology has enabled researchers and clinicians to gain insight into the histomorphological and pathophysiological changes in CP. Bockman and coworkers reported in 1992 that transgenic mice overexpressing transforming growth factor-α (TGF-α) developed morphological pancreatic changes similar to those found in CP in humans (16). For example, there was a high degree of pancreatic fibrosis and dedifferentiation of pancreatic acinar cells into tubular structures. These findings provided the first evidence that growth factors and growth factor receptors might play a role in the morphological changes that occur in CP. In this review we will summarize some of our current knowledge about the pathogenesis and molecular aspects with special emphasis on growth factors and their receptors in CP.

Pathogenic concepts in CP

Chronic pancreatitis can be subdivided into different etiologic groups: alcoholic chronic pancreatitis, which is the most common form in Western industrialized countries, will be discussed in detail in this review. Tropical pancreatitis is prevalent in India and Indonesia, effects both sexes equally, and is thought to be caused by dietary factors. The exact pathogenesis of this clinical entity, however, is not known (17, 18). Other forms of CP include hereditary pancreatitis due to gene mutations (e.g. the cystic fibrosis gene), CP due to metabolic disturbances such as hypercalcemia or hyperlipoproteinemia, CP due to pancreas divisum or duodenal wall cysts, and idiopathic CP in whom all known causes of CP have been excluded.

Alcoholic CP is by far the most prevalent form of CP in Western industrialized countries. Estimates of the prevalence of alcohol-induced CP range from 38% to 94% in this population (4). The most favored pathophysiologic concept suggests that alcohol overconsumption results in reduced secretion of lithostatin, a protein which inhibits the formation of protein plugs by keeping the pancreatic juice soluble. It is hypothesized that protein plugs are formed in CP leading to obstruction of the pancreatic ductal system and subsequently to the induction of a chronic inflammatory process in the pancreas (19). Other proposed mechanisms include recurrent attacks of acute pancreatitis with subsequent necrosis, periductular fibrosis, ductal obstruction and continuous fibrosis (20). In addition, the direct toxic effects of alcohol and its metabolites on pancreatic acinar and ductal cells were hypothezised to be involved in the pathophysiology of CP (15). Furthermore, direct damage of the pancreatic parenchyma by increased levels of free radicals caused by reduced hepatic detoxification has been suggested as an important cause of pancreatic injury in CP (21). However, none of these concepts can explain the morphological, functional and clinical picture of chronic pancreatitis completely (15). Therefore, additional mechanisms must be involved in the pathogenesis of chronic pancreatitis, such as inflammatory destruction of the healthy tissue, which causes pain and exocrine/endocrine failure. Supporting the later hypothesis, it has been shown that there is a relationship between inflammatory cell infiltration, pain, and changes in and around pancreatic nerves (12). Thus, expression of the growth-associated protein 43, a marker of neuronal plasticity, is significantly increased in pancreatic nerve fibers and neurons in CP, and its expression correlates with pain. In addition, infiltration of pancreatic nerves by immune cells also correlates with pain intensity (12). Exocrine and endocrine pancreatic destruction and fibrotic replacement seems to be additionally influenced by activated cytotoxic lymphocytes and by activation of non-pancreatic proteolytic systems (13, 22). Thus, perforin, a specific marker for activated cytotoxic cells, is significantly elevated in the pancreas of patients with CP, suggesting an involvement of cell-mediated cytotoxicity in the pathogenesis of this disorder (13). In addition, molecular research over the past decades has identified a number of alterations, which have the potential to contribute to the pathogenesis of CP.

The Epidermal Growth Factor (EGF) family of Receptors and Ligands

The EGF receptor, also known as human EGF receptor I (HER-1), is the prototype of a family of transmembrane tyrosine-kinase receptors (23-25). This family also includes c-erbB-2 (HER-2) (26, 27), c-erbB-3 (HER-3) (28), and c-erbB-4 (HER-4) (29). The EGF receptors are activated by binding of specific ligands to the extracellular domain of the receptors. Upon ligand binding, the EGF receptors form hetero- and/or homodimers, which subsequently leads to auto- and/or transphosphorylation of the receptors, and activation of intracellular signaling cascades such as the ras-raf-MAP kinase or the PI-3 kinase pathways (23-25). Several ligands have been identified during the past decades, which bind and activate EGF receptors. These ligands include EGF, transforming growth factor-α (TGF-α), amphiregulin, betacellulin and heparin-binding EGF,

cripto, epiregulin, and the neuregulin family of ligands (30-34). These polypeptides are generated by proteolytic cleavage of the extracellular domains of precursor molecules that possess a hydrophobic transmembrane domain and an intracellular domain.

The exact physiological and pathophysiological role of the EGF system of receptors and ligands in the human pancreas is not completely understood. However, using Northern blot analysis, it could be shown that expression of the EGF receptor, EGF, and TGF-α mRNA is present at low levels in the normal pancreas (35-37). *In situ* hybridization localized EGF receptor, EGF, and TGF-α mRNA transcription in acinar and ductal cells in the normal pancreas. TGF-α mRNA *in situ* hybridization signals were present at relatively high levels in the normal pancreas. All three mRNA moieties preferentially localized at the apical portion of ductal cells. In contrast, in acinar cells, *in situ* hybridization signals of the three mRNA moieties were present in the basal portion (35-37). In tissue samples obtained from patients with CP, expression of EGF receptor, EGF and TGF-α was increased. In contrast, amylase mRNA expression, which served as a control, was considerably lower in comparison with the normal pancreas (37). Overall, 70% and 74% of the CP samples exhibited increased EGF-receptor and TGF-α expression, respectively. *In situ* hybridization analysis also demonstrated an increase in EGF receptor, EGF and TGF-α mRNA expression in both the remaining acinar cells and ductal cells in the CP samples. Quantitative video image analysis of the *in situ* hybridization data revealed that there was a 23-fold and 4-fold increase of EGF receptor and TGF-α mRNA levels, respectively, in the CP tissues in comparison with the normal pancreas (37). Interestingly, EGF receptor and TGF-α *in situ* hybridization signals were localized in exocrine pancreatic cells, but not in stromal cells or in the fibroblasts.

c-erbB-2 mRNA expression is low in the normal pancreas (38,39). In contrast, some of the CP samples exhibited markedly increased c-erbB-2 mRNA expression, whereas others expressed levels comparable to those of the normal controls. Analysis of the clinical data of the CP patients revealed that patients with pancreatic head enlargement had increased c-erbB-2 mRNA levels, whereas patients without pancreatic head enlargement did not exhibit enhanced c-erbB-2 mRNA expression (38). Interestingly, linear regression analysis of c-erbB-2 mRNA expression levels with the vertical pancreatic head diameter showed a significant positive relationship. When expression analysis of the EGF receptor was performed in the same CP samples, only 54% of the CP samples with pancreatic head enlargement exhibited enhanced expression of the EGF receptor (38). In addition, 42% of the CP patients with no enlargement of the vertical pancreatic head diameter showed marked overexpression of the EGF receptor. These results imply an important role of c-erbB-2 in the pathological entity of CP with pancreatic head enlargement. *In situ* hybridization and immunohistochemistry in the normal pancreas and in CP samples without pancreatic head enlargement demonstrated c-erbB-2 mRNA expression in acinar and ductal cells (40). In contrast, patients with pancreatic head enlargement expressed consistently higher levels of c-erbB-2 in the remaining pancreatic acinar and ductal cells. Southern blot analysis revealed that overexpression of c-erbB-2 was not caused by gene amplification, as has been reported in many mammary cancers (38).

c-erbB-3, the third member of the EGF receptor family, was present at relatively low levels in the normal pancreas by Northen blot analysis. In contrast, 24% of the CP samples exhibited enhanced c-erbB-3 mRNA levels. By immunohistochemistry, c-erbB-3 expression was found only in a few ductal cells in the normal pancreas (40). Centroacinar cells and most ductal cells in the intralobular and interlobular ducts showed faint to moderate c-erbB-3 immunostaining. In CP samples, faint to moderate c-erbB-3 immunoreactivity is present in the remaining acinar and ductal cells, and regions with pseudoductal metaplasia exhibited moderate to intense c-erbB-3 immunostaining, whereas in fibrotic tissues no immunostaining for c-erbB-3 was detectable (40).

Cripto is also a member of the EGF family of ligands (41). In contrast to EGF and TGF-alpha, cripto does not bind to the EGF receptor and at present its high affinity receptor and exact function is not known. Functional studies with teratocarcinoma cell lines suggested that cripto expression is associated with de-differentiation (41). Northern blot analysis revealed an increase of the cripto mRNA levels in the CP samples in comparison with normal controls that was not caused by gene amplification or gene rearrangement (42). In the normal pancreas, cripto immunostaining was present in ductal cells and only faintly in a few acinar cells. In contrast, in CP tissues, the intensity of cripto immunostaining was closely related to the histomorphological damage of the exocrine pancreatic parenchyma. Thus, areas with minor histomorphological damage exhibited cripto immunostaining comparable to that of normal controls, but atrophic acinar cells and ductal cells in regions with ductal metaplasia exhibited intense cripto immunoreactivity (42).

The Fibroblast Growth Factor (FGF) family of Receptors and Ligands

Acidic FGF (FGF-1) and basic FGF (FGF-2) belong to a family of polypeptide growth factors, which influence various biological functions such as cell differentiation, cell migration, and angiogenesis (43-49). Both growth factors also exert chemotactic effects on fibroblasts and stimulate collagen production (43, 48, 50). The fibroblast growth factor gene family currently includes 14 members (51), which display between 30% and 70% amino acid homology. The prototypes of this family FGF-1 and FGF-2 are found in abundance in the extracellular matrix in

various organs, which seems to serve as a reservoir for FGFs. It is hypothesized that through this mechanism, FGFs can quickly be mobilized in response to requirements such as cell migration, wound healing and angiogenesis. As other growth factors, fibroblast growth factors transmit their messages via binding to specific transmembrane tyrosine-kinase receptors. Four FGF receptors (FGFRs) have been identified: FGFR-1 (flg-1), FGFR-2 (bek), FGFR-3 (cek-2), and FGFR-4 (flg-2). FGFRs consist of two or three immunoglobulin (Ig)-like regions in the extracellular domain, a short transmembraneous region and an intracellular domain with tyrosine kinase activity, which is separated into two regions. A number of receptor isoforms are generated through different mRNA splicing (43, 45, 47, 52, 53).
FGF-1, FGF-2, FGF-5, and FGF-7 and the four high-affinity FGF receptors are present in the normal human pancreas (54-59). Only faint FGF-1 and FGF-2 immunoreactivity was found in the cytoplasm of acinar and ductal cells in the normal pancreas. However, FGF-1 was present preferentially in ductal cells, whereas FGF-2 was more frequently found in acinar cells (56, 57). In addition, FGF-5 was present in ductal and islet cells as well as in fibroblasts in the normal pancreas (58). FGF-7 expression was observed in acinar and ductal cells in these tissues (59). In contrast, in CP samples, there was an increase of FGF-1, FGF-2, and FGF-5 mRNA expression in comparison to the normal pancreas. *In situ* hybridization analysis localized FGF-1 and FGF-2 mRNA expression in acinar and ductal cells, especially when these cells were located in areas with atrophic changes. In regions of CP samples exhibiting less damage of the parenchyma, the intensity and the frequency of FGF-1 and FGF-2 mRNA expression were only slightly above those of normal controls. Furthermore, in the surrounding stroma and in the fibrotic regions, expression of both mRNA moieties was slightly elevated (57). In contrast, FGF-5 mRNA was predominantly localized in the periductal fibroblasts and endocrine islet cells, as well as in the atrophic acinar and ductal cells (58). Immunohistochemical analysis revealed FGF-1 and FGF-2 immunoreactivity in the normal pancreas only in some acinar cells and ductal cells of small ductules or larger interlobular ducts. In contrast, in the CP tissue samples, FGF-1 and FGF-2 immunoreactivity was intense in degenerating acinar and ductal cells, and in areas exhibiting pseudoductular metaplasia. Areas of CP tissues with minor damage showed immunostaining in acinar and ductal cells that was only slightly increased compared to normal controls (57).

The Transforming Growth Factor-β (TGF-β) family of Receptors and Ligands

Transforming growth factor betas (TGF-ßs) and their homologues form another important family of growth factors. TGF-ß superfamily members are multifunctional polypeptide growth factors, which influence growth, angiogenesis, and extracellular matrix composition (60). TGF-ßs are excreted as inactive molecules, which have to be activated before binding to specific cell surface receptors. In mammalian cells three TGF-β isoforms have been identified: TGF-ß1, TGF-ß2 and TGF-ß3 (61-64). TGF-ßs have been demonstrated to be potent inhibitors of growth in many cell types, including epithelial, endothelial, neuronal, hemopoetic and lymphoid cells (60, 64). In addition, TGF-ßs stimulate the expression of extracellular matrix-forming proteins and of several proteases which degrade extracellular matrix proteins, thereby controlling wound healing, cellular adhesion and extracellular matrix deposition (60, 64-66).
TGF-ß signaling occurs via specific cell surface receptors with serine-threonine-kinase activity. Two major TGF-ß signaling receptors have been characterized (60, 67-69). The type II TGF-β receptor (TβRII) binds TGF-βs in the absence of the type I TGF-β receptor (TβRI). Upon ligand binding TbRII forms a heteromeric complex with TβRI, which cannot bind ligand in the absence of the TβRII (60). Following heterodimerization of type I and type II TGF-β receptors, which most likely consists of two type I and type II receptors, activated TβRII then trans-phosphorylates the glycine and serine (GS) rich domain of the type I receptor kinase, thereby activating TβRI (60). Activated TβRI then transiently associates with Smad2 and/or Smad3. These proteins belong to a recently discovered family of intracellular signalling molecules (60). Phosphorylated Smad2 and/or Smad3 then form separately heteromeric complexes with Smad4, the common mediator of the TGFβ superfamily signalling pathway. These complexes then translocate to the nucleus where they can act as transcriptional activators (60).
In the normal human pancreas, low levels of TGF-ß1, TGF-ß2 and TGF-ß3 mRNA are present. *In situ* hybridization localized TGF-ß1, TGF-ß2 and TGF-ß3 mRNA expression in islet cells, acinar cells, and ductules, but rarely in larger ducts in the normal pancreas. In addition, TßR-I and TßR-II mRNA expression were present in a few acinar and ductal cells within the normal pancreas. Immunostaining for TGF-ß1, TGF-ß2, TGF-ß3, TßR-I and TßR-II demonstrated a distribution pattern similar to that observed by *in situ* hybridization. Analysis of CP samples indicated strong immunostaining in the majority of ductal cells for TGF-ß1. TGF-ß1 immunoreactivity was also present in mononuclear cells and some fibroblasts in areas with inflammation and fibrosis (70, 71). Remnant islet cells showed diffuse TGF-ß1 immunoreactivity in chronic pancreatitis tissues in a pattern similar to that seen in the normal pancreatic samples. In addition, CP tissues also markedly overexpress TβRI, TβRII, and connective tissue growth factor (CTGF), which has been implicated in fibrotic processes, and which is regulated by TGF-β. Using *in situ* hybridization, TβRI and TβRII are found to be located

in ductal cells and atrophic acinar cells in CP, whereas CTGF mRNA expression is observed in degenerating acinar cells and fibroblasts sourounding these areas (72). The importance of TGF-ß signaling in the formation of fibrosis is underlined by experiments in transgenic mice overexpressing TGF-ß1 in the pancreas (73). These animals exhibit an increase in extracellular matrix content in the pancreas, which is histologically identical to chronic pancreatitis in humans. Therefore, upregulation of TGF-ßs in the human pancreas might influence fibrogenesis and contribute indirectly to the ongoing destruction of the exocrine and endocrine parenchyma.

Gene Mutations in CP

Since pancreatic lesions of cystic fibrosis resemble those of chronic pancreatitis patients, it has been hypothesized that mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene may also be involved in the pathogenesis of CP. It could be demonstrated that CFTR mutation occur in 13.4 percent of CP patients as compared to 5.3 percent among unrelated partners of persons with a family history of cystic fibrosis, suggesting that mutations of the CFTR gene are associated with a subgroup of CP (74).

Mutations of the K-ras gene are very common in pancreatic cancer. Therefore considerable effort has been made to clarify the role of these mutations in CP, since the risk of pancreatic cancer is significantly elevated in subjects with CP (75). Most studies have concluded that K-ras mutations are rare events in chronic pancreatitis, suggesting that they either do not occur in CP at all, or that the time span between the occurrence of K-ras mutations and malignant transformation is rather short (75-78). However, other groups have reported that K-ras mutations occur in a subgroup of CP patients with ductal hyperplasia with a frequency of up to 18% (79). It seems that -summarizing all reports regarding K-ras mutations in CP- this alteration is absent in the vast majority of CP and its presence most likely represent early pancreatic cancer and misdiagnosis of CP.

Conclusions

Enhanced expression of a variety of growth factors and growth factor receptors are present in the majority of CP samples. These factors are most likely produced by the remaining pancreatic acinar and ductal cells, and thus might directly influence the morphological changes that occur in CP. Interestingly, overexpression of a variety of growth factors and their receptors has also been reported for pancreatic cancer suggesting that molecular alterations in CP might act as precancerous factors under certain circumstances. Supporting this hypothesis, there is clinical evidence that patients with CP have a significantly higher risk of developing pancreatic cancer (78). However, most studies have characterized molecular changes in advanced stages of CP, when surgical resection is required. It is presently not possible to determine whether upregulation of growth factors and their receptors is an early or late event in the development of CP in humans, because patients with early stages of CP do not require surgery and therefore no pancreatic tissue is available for analysis. Future concepts of CP have to consider alteration of growth factors and their receptors as well as other molecular perturbations as important aspects in the pathophysiological process of CP which might influence the morphological and clinical course of this disease.

References

1) Sarles H., Bernard J.P., Johnson C., Chir M.: *Pathogenesis and epidemiology of chronic pancreatitis.* Ann Rev Med, 1989, 40:453-468.

2) Di Magno E.P., Layer P., Clain J.E.: *Chronic pancreatitis. The exocrine pancreas: biology, pathobiology, and diseases.* Edited by Go V.L.W., Di Magno E.P., Gardner J.D., Lebenthal E., Reber H.A., Scheele G.A. New York: Raven Press, 1993, pp. 665-706.

3) Di Magno E.P.: *A short, elective history of exocrine pancreatic insufficiency and chronic pancreatitis.* Gastroenterology, 1993, 104:1255-1262.

4) Worning H.: *Incidence and prevalence of chronic pancreatitis. In: Chronic pancreatitis.* Ed. Beger HG, Büchler M, Ditschuneit H, Malfertheiner P. Berlin: Springer Verlag, 1990, pp. 8-14.

5) Bockman D.E.: *Surgical anatomy of the pancreas and adjacent structures.* In: Beger H.G., Büchler M., Malfertheiner P. (eds.): *Standards in pancreatic surgery.* Heidelberg-New York: Springer, 1993, pp. 1-9.

6) Oertel J.E., Heffess C.S., Oertel Y.C.: *Pancreas.* In: Sternberg S.S. (ed.): D*iagnostic Surgical Pathology*, Chapter 32. New York, Raven, 1989, pp. 1057-1093.

7) Di Magno E.P., Layer P., Clain J.E.: *Chronic pancreatitis.* In: Go V.L.W., Di Mango E.P., Gardner J.D., Lebenthal E., Reber H.A., Scheele G.A. (eds.): *The Pancreas.* New York, Raven, 1993, pp. 707-740.

8) Di Magno E.P.: *Conservative management of chronic pancreatitis.* In: Beger H.G., Büchler M., Malfertheiner P. (eds.): *Standards in pancreatic surgery.* Heidelberg-New York: Springer, 1993, pp. 325-331.

9) Bockman D.E., Büchler M., Malfertheiner P., Beger H.G.: *Analysis of nerves in chronic pancreatitis.* Gastroenterology, 1988, 94:1459-1469.

10) Büchler M., Weihe E., Friess H., Malfertheiner P., Bockman E., Müller S., Nohr D., Beger H.G.: *Changes in peptidergic innervation in chronic pancreatitis.* Pancreas, 1992, 7:183-192.

11) Weihe E., Nohr D., Müller S., Büchler M., Friess H., Zentel H.J.: *The tachykinin neuroimmune connection in inflammatory pain.* Annals of the New York Academy of Sciences, 1991, 632:283-295.

12) Di Sebastiano P., Fink Th., Weihe E., Friess H., Innocenti P., Beger H.G., Büchler M.W.: *Immune cell infiltration and growth-associated protein-43 expression correlate with pain in chronic pancreatitis.* Gastroenterology, 1997, 112:1648-1655.

13) Hunger R., Müller Ch., Zgraggen K., Friess H., Büchler M.W.: *Cytotoxic cells are activated in cellular infiltrates of alcoholic chronic pancreatitis.* Gastroenterology, 1997, 112:1656-1663.

14) Bordalo O., Bapista A., Dreiling D., Noronha M.: *Early pathomorphological pancreatic changes in chronic alcoholism.* In: Gyr K.E., Singer M.V., Sarles H. (eds.): *Pancreatitis-concepts and classification.* North Holland, Amsterdam: Elsevier, 1984, No. 642.

15) Di Magno E.P.: *A short, eclectic history of exocrine pancreatic insufficiency and chronic pancreatitis.* Gastroenterology, 1993, 104:1255-1262.

16) Bockman D.E., Merlino G.: *Cytological changes in the pancreas of transgenic mice overexpressing transforming growth factor.* Gastroenterology, 1992, 103:1883-1892.

17) Zuidema P.J.: *Cirrhosis and disseminated calcification of the pancreas in patients with malnutrition.* Trop Geog. Med, 1959, 11:70-74.

18) Viswanathan M., Capecomorin S.P.: *Tropical chronic pancreatitis.* In: *The pancreas.* Eds: Beger H.G., Warshaw A.L., Büchler M.W., Carr-Locke D.L., Neoptolemos J.P., Russell C., Sarr M.G. Oxford: Blackwell Sciences, 1998, pp. 688-697.

19) Sarles H., Dagorn J.C., Giorgi D., Bernard J.P.: *Remaining pancreatic stone protein as "lithostatine".* Gastroenterology, 1990, 99:900-905.

20) Klöppel G., Maillet B.: *Pseudocysts in chronic pancreatitis: a morphological analysis of 57 resection specimens and 9 autopsy pancreata.* Pancreas, 1991, 6:266-274.

21) Braganza J.M.: *Pancreatic disease: a casualty of hepatic "detoxification"?* Lancet, 1983, ii 8357:1000-1003.

22) Friess H., Cantero H., Graber H., Tang W.H., Guo X.Z., Kashiwagi M., Zimmermann A., Gold L.I., Korc M., Büchler M.W.: *Enhanced urokinase plasminogen activation in chronic pancreatitis suggests a role in its pathogenesis.* Gastroenterology, 1998, 113:904-913.

23) Ullrich A., Schlessinger J.: *Signal transduction by receptors with tyrosine kinase activity.* Cell, 61:203-212.

24) Schlessinger J., Ullrich A.: *Growth factor signaling by receptor tyrosine kinases.* Neuron, 1992, 9:383-391.

25) Yarden Y., Ullrich A.:. *Molecular analysis of signal transduction by growth factors.* Biochemistry, 1988, 27:3113-3119.

26) Coussens L., Yank-Feng T.L., Liao Y.C., Chen E., Gray A., McGrath J., Seeburg P.H., Libermann T.A., Schlessinger J., Francke U.: *Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene.* Science, 1985, 230:1132-1139.

27) Di Fiore P.P., Pierce J.H., Kraus M.H., Segatto O., King C.R., Aaronson S.A.: *erbB-2 is a potent oncogene when overexpressed in NIH/3T3 cells.* Science, 1987, 237:178-182.

28) Kraus M.H., Issing W., Miki T., Popescu N.C., Aaronson S.A.: *Isolation and characterization of ERBB3, a third member of the ERBB/epidermal growth factor receptor family: evidence for overexpression in a subset of human mammary tumors.* Procedures of the National Academy of Sciences USA, 1989, 86:9193-9197.

29) Plowman G.D., Green J.M., McDonald V.L., Neubauer M.G., Disteche C.M., Todaro G.J., Shoyab M.: *The amphiregulin gene encodes a novel epidermal growth factor-related protein with tumor-inhibitory activity.* Mol Cell Biol, 1981, 10:1969-1981.

30) Barton C.M., Hall P.A., Hughes C.M., Gullick W.J., Lemoine N.R.: *Transforming growth factor alpha and epidermal growth factor in human pancreatic cancer.* Journal of Pathology, 1991, 163:111-116.

31) Ciccodicola A,. Dono R., Obici S., Simeone A., Zollo M., Persico M.G.: *Molecular characterization of a gene of the 'EGF family' expressed in undifferentiated human NTERA2 teratocarcinoma cells.* EMBO J, 1989, 8:1987-1991.

32) Higashiyama S., Abraham J.A., Miller J., Fiddes J.C., Klagsbrun M.: *A heparin-binding growth factor secreted by macrophage-like cells that is related to EGF.* Science, 1991, 251:936-939.

33) Plowman G.D., Culouscou J.M., Whitney G.S., Green J.M., Carlton G.W., Foy L., Neubauer M.G., Shoyab M.: *Ligand specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family.* Procedures of the National Academy of Sciences USA, 1993, 90:1746-1750.

34) Prigent S.A., Lemoine N.R.: *The type 1 (EGFR-related) family of growth factor receptors and their ligands.* Progress in Growth Factor Research, 1992, 4:1-24.

35) Korc M., Chandrasekar B., Yamanaka Y., Friess H., Büchler M., Beger H.G.: *Overexpression of the epidermal growth factor receptor in human pancreatic cancer is associated with concomitant increases in the levels of epidermal growth factor and transforming growth factor alpha.* J Clin Invest, 1992, 90:1352-1360.

36) Yamanaka Y., Friess H., Kobrin M.S., Büchler M., Beger H.G., Korc M.: *Coexpression of epidermal growth factor receptor and ligands in human pancreatic cancer is associated with enhanced tumor aggressiveness.* Anticancer Research, 1993, 13:565-570.

37) Korc M., Friess H., Yamanaka Y., Kobrin M., Büchler M.W., Beger H.G.: *Chronic pancreatitis is associated with increased concentrations of epidermal growth factor receptor, transforming growth factor alpha and phospholipase C gamma.* GUT, 1994, 35:1468-1473.

38) Friess H., Yamanaka Y., Büchler M.W., Hammer K., Kobrin M., Beger H.G.: *A subgroup of patients with chronic pancreatitis overexpress the c-erbB-2 protooncogene.* Ann Surg, 1994, 220:183-192.

39) Yamanaka Y., Friess H., Kobrin M.S., Büchler M., Kunz J., Beger H.G., Korc M.: *Overexpression of HER2/neu oncogene in human pancreatic cancer.* Human Pathol, 1993, 24:1127-1134.

40) Friess H., Yamanaka Y., Kobrin S.M., Do D.A., Büchler M.W., Korc M.: *Enhanced erbB-3 expression in human pancreatic cancer correlates with tumor progression.* Clinical Cancer Research 1995, 1: 1413-1420.

41) Baldassarre G., Bianco C., Tortora G., Ruggiero A., Moasser M., Dmitrovsky E., Bianco A.R. Ciardiello F.: *Transfection with a cripto anti-sense plasmid suppresses endogenous CRIPTO expression and inhibits transformation in a human embryonal carcinoma cell line.* Int J Cancer, 1996, 66:538-543.

42) Friess H., Yamanaka Y., Büchler M., Kobrin M.S., Tahara E., Korc M.: *Cripto, a member of the epidermal growth factor family, is*

overexpressed in human pancreatic cancer and chronic pancreatitis. Int J Cancer, 1994, 56:668-674.

43) Burgess W.H., Maciag T.: *The heparin-binding (fibroblast) growth factor family of proteins.* Ann Rev Biochem, 1989, 58:575-606.

44) Damon D.H., Lobb R.R., D'Amore P.A., Wagner J.A.: *Heparin potentiates the action of acidic fibroblast growth factor by prolonging its biological half-life.* J Cell Physiol, 1989, 138: 221-226.

45) Folkman J., Klagsbrun M.: *Angiogenic factors.* Science, 1987. 235:442-447.

46) Gospoderowicz D., Neufeld G., Schweigerer L.: *Molecular and biological characterization of fibroblast growth factor, an angiogenic factor which also controls the proliferation and differentiation of mesoderm and neuroectoderm derived cells.* Cell Differentiation, 1986. 19:1-17.

47) Gospoderowicz D., Ferrara N., Schweigerer L., Neufeld G.: *Structural characterization and biological functions of fibroblast growth factor.* Endocrine Review, 1987, 8:95-114.

48) Klagsbrun M.: *The fibroblast growth factor family: structural and biological properties.* Progressive Growth Factor Research, 1989, 1:207-235.

49) Givol D., Yardon A.: *Complexity of FGF receptors: genetic basis for structural diversity and functional specificity.* FASEB J, 1992, 6:3362-3369.

50) Courty J., Loret C., Chevallier B., Moenner M., Barritault D.: *Biochemical comparative studies between eye and brain derived growth factors.* Biochemie, 1987, 69:511-516.

51) Kornmann M., Beger H.G., Korc M.: *Role of fibroblast growth factors and their receptors in pancreatic cancer and chronic panceratitis.* Pancreas, 1998, 17:169-175.

52) Mori H., Maki M., Oishi K., Jaye M., Igarashi K., Yoshida O., Hatanaku M.: *Increased expression of genes for basic fibroblast growth factor and transforming growth factor type 2 in human benign prostatic hyperplasia.* Prostate, 1990, 16:71-80.

53) Schulze-Osthoff K., Risau W., Vollmer E., Sorg C.: *In situ detection of basic fibroblast growth factor by highly specific antibodies.* American Journal of Pathology, 1990, 137:85-92.

54) Kobrin M.S., Yamanaka Y., Friess H., Lopez M.E., Korc M.: *Aberrant expression of the type I fibroblast growth factor receptor in human pancreatic adenocarcinomas.* Cancer Research, 1993, 53:4741-4744.

55) Friess H., Kobrin M.S., Korc M.: *Acidic and basic fibroblast growth factors and their receptors are expressed in the human pancreas.* Pancreas, 1992, 7:737, Abstract.

56) Yamanaka Y., Friess H., Büchler M., Beger H.G., Uchida E., Onda M., Kobrin M.S., Korc M.: *Overexpression of acidic and basic fibroblast growth factors in human pancreatic cancer correlates with advanced tumor stage.* Cancer Research, 1993, 53:5289-5296.

57) Friess H., Yamanaka Y., Büchler M., Beger H.G., Do D.A., Kobrin M.S., Korc M.: *Increased expression of acidic and basic fibroblast growth factors in chronic pancreatitis.* Am J Pathol, 1994, 144:117-128.

58) Kornmann M., Ishiwata T., Neger H.G., Korc M.: *Fibroblast growth factor-5 stimulates mitogenic signaling and is overexpressed in human pancreatic cancer: evidence for autocrine and paracrine actions.* Oncogene, 1997, 15:1417-1424.

59) Ishiwata T., Kornmann M., Beger H.G., Korc M.: *Enhanced fibroblast growth factor 5 expression in stromal and exocrine elements of the pancreas in chronic pancreatitis.* Gut, 1998, 43:134-139.

60) Massague J.: *TGF-beta signal transduction.* Ann Rev Biochem, 1998, 67:753-791.

61) Hebda P.A.: *Stimulatory effects of transforming growth factor-beta and epidermal growth factor on epidermal cell outgrowth from porcine skin explant cultures.* J Invest Dermatol, 1988, 91:440-445.

62) Massague J., Cheifetz S., Laiho M., Ralph D.A., Weiss F.M.B., Zentella A.: *Transforming growth factor-beta.* Cancer Surveys, 1992, 12:81-103.

63) Roberts A.B., Sporn M.G., Assoian R.K., Smith J.M., Roche N.S.: *Transforming growth factor type-b: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro:* Procedures of the National Academy of Sciences USA, 1986, 83:4167-4171.

64) Sporn M.B., Roberts A.B.: *Transforming growth factor-beta: Recent progress and new challenges.* J Cell Biol, 1992, 119:1017-1021.

65) Roberts A.B., Sporn M.B.: *Physiological actions and clinical applications of transforming growth factor-beta.* Growth Factors, 1993, 8:1-9.

66) Roberts A.B., Sporn M.B.: *The transforming growth factor-beta.* In: Sporn M.B., Roberts A.B. (eds.): *Peptide growth factors and their receptors. Vol. 95 of Handbook of Experimental Pharmacology.* New York: Springer-Verlag, 1990, pp. 419-472.

67) Lin H.Y., Wang X.-F., Ng-Eaton E., Weinberg R.A., Lodish H.F.: *Expression cloning of the TGF-ß type II receptor, a functional transmembrane serine/threonine kinase.* Cell, 1992, 68:775-785.

68) Lopez-Casillas F., Cheifetz S., Doody J., Andres J.L., Lane W.S., Massague J.: *Structure and expression of the membrane proteoglycan betaglycan, a component of the TGF-ß receptor system.* Cell, 1991, 67:785-795.

69) Wrana J.L., Attisano L., Carcamo J., Zentella A., Doody J., Laiho M., Wang X.-F., Massague J.: *TGF-ß signals through a heteromeric protein kinase receptor complex.* Cell, 1992, 71:1003-1014.

70) Slater S.D., Williamson R.C.N., Foster C.S.: *Expression of transforming growth factor-beta1 in chronic pancreatitis.* Digestion, 1995, 56:237-241.

71) Van Laethem J.L., Deviere J., Resibios A., Rickaert F., Vertongen P., Ohtani H., Cremer M., Miyazono K., Robberecht P.: *Localization of transforming growth factor beta 1 and its latent binding protein in human chronic pancreatitis.* Gastroenterology, 1995, 108:1873-1881.

72) Di Mola F.F., Friess H., Martignoni M., Di Sebastiano P., Zimmermann A., Innocenti P., Graber H., Gold L.I., Korc M., Büchler M.W.: *Connective tissue growth factor (CTGF) is a regulator for fibrosis in human chronic panceratitis.* Ann Surg, 1999, 230:63-71

73) Van Laethem J.L., Robberecht P., Resibios A., Deviere J.: *Transforming growth factor beta promotes development of fibrosis after repeated courses of acute pancreatitis in mice.* Gastroenterology, 1996, 110(2):576-582.

74) Sharer N., Schwarz M., Malone G., Horwarth A., Painter J.,

Super J., Braganza J.: *Mutations of the cystic fibrosis gene in patients with chronic pancreatitis.* N Engl J Med, 1998, 339:645-652.

75) Lowenfels A.B., Maisonneuve P., Cavallini G., Ammann R.W., Lankisch P.G., Andersen J.R., Dimagno E.P., Andren-Sandberg A., Domellof L.: *Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group.* New England Journal of Medicine, 1993, 328:1433-1437.

76) Gansauge S., Schmid R.M., Muller J., Adler G., Mattfeld T., Beger H.G.: *Genetic alterations in chronic pancreatitis: evidence for early occurence of p53 but not K-ras mutations.* Br J Surg, 1998, 85:337-340.

77) Hsiang D., Friess H., Büchler M.W., Ebert M., Butler J., Korc M.: *Absence of K-ras mutations in the pancreatic parenchyma of patients with chronic pancreatitis.* Am J Surg, 1997, 174: 242-246.

78) Orth M., Gansauge F., Gansauge S., Beger H.G., Adler G., Schmid R.M.: *K-ras mutations at codon 12 are rare events in chronic pancreatitis.* Digestion, 1998, 59:120-124.

79) Kondo H., Sugano K., Fukuyama N., Hosokawa K., Ohkura H., Ohtsu A., Mukai K., Yoshida S.: *Detection of K-ras gene mutations at codon 12 in the pancreatic juice of patients with intraductal papillary mucinous tumors of the pancreas.* Cancer, 1997, 79:900-905.

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