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Role of Extracellular Matrix in Pancreatic Diseases

Key Words

Pancreatic cancer · Pancreatitis · Extracellular matrix · Fibrosis ·
Desmoplasia

The extracellular matrix (ECM) has been traditionally thought of as the structurally stable material that provides support for cells and tissues. However, a number of discoveries over the past decades have changed this view. First, it has been shown that four major classes of macromolecules – the collagens, proteoglycans, structural glycoproteins, and elastin – collectively comprise the ECM of animal cells [1–6]. Furthermore, with the exception of elastin, each class of matrix macromolecules has been found to contain families of related proteins with each member being a unique gene product. Second, 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]. Third, specific cell-surface receptors for 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 [2, 4, 6, 8, 12]. Finally, 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 [2, 3, 5, 6].

In particular pancreatic diseases as, e.g., pancreatic cancer and chronic pancreatitis are characterized by profound alterations of ECM formation and composition. The following review summarizes some of the major aspects that have emerged in the recent years concerning composition, formation and regulation of the ECM in human pancreatic diseases and in experimental models of pancreatic fibrosis.

Fig. 1. Northern blot hybridizations representative for chronic pancreatitis (n = 8) and control (n = 7) tissue RNA [14]. 3 lanes of each, chronic pancreatitis and control pancreatic tissue RNA are shown for: **A** Collagen I; **B** collagen III; **C** collagen IV; **D** laminin; **E** fibronectin; **F** MMP-2; **G** TIMP-2; **H** 18 s rRNA. 30 μ g of total RNA were loaded per lane. **H** The 18 s rRNA hybridization used as a control to normalize for varying amounts of RNA loaded into each lane [14].

Implication of the ECM in Pancreatic Diseases

Chronic Pancreatitis

Chronic inflammatory diseases of the pancreas are characterized by destruction of acinar cells and islet cells and replacement by connective tissue. This connective tissue appears to result from an increased deposition and disorganization of extracellular matrix proteins including fibronectin, laminin, and collagens type I, III and IV [13]. Recently, we 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 (fig. 1) [14]. In situ hybridization clearly localized connective tissue cells as the main site of collagen type I and III mRNA transcription (fig. 2).

Pancreatic Cancer

Pancreatic cancer tissue shows a strong desmoplastic reaction, characterized by a remarkable increase of interstitial connective tissue [15, 16]. This desmoplastic reaction in pancreatic cancer tissue samples is accompanied by increased steady state levels of the mRNAs for collagens type I, III and IV, fibronectin and laminin (fig. 3) [15] and of collagen protein (1.8- to 2-fold over healthy controls) (fig. 4). 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

Fig. 2. Collagen histochemistry and in situ hybridization. The panels indicate observations found in four different specimens of paraffin embedded tissue from patients with chronic pancreatitis [14]. The calibration bars indicate 100 μ m. **A** This section was stained with sirius red which binds selectively to collagenous protein. Note the wide, dark bands of collagen fibers separating islands of acinar tissue. **B** This section was hybridized with antisense probe for collagen type III mRNA. After three weeks of exposure the silver grain label is predominantly located over connective tissue fibroblasts which separate the islands of acinar tissue in the lower left and upper right corner [14].

Fig. 3. Northern blot hybridizations representative for pancreatic cancer (n = 8) and control (n = 8) tissue RNA [15]. 4 lanes of each, cancer and control pancreatic tissue RNA are shown for ³²P-labelled probes for MMP-1 (interstitial collagenase), MMP-2 (72 kD type IV collagenase), MMP-3 (stromelysin 1), MMP-9 (92 kD type IV collagenase), TIMP-1 (tissue inhibitor of metalloproteinase type 1), TIMP-2 (tissue inhibitor of metalloproteinase type 2), collagen (α1)I (Col I), collagen (α1) III (Col III), collagen IV (Col IV), laminin (Lam) and fibronectin (fibro), 28s rRNA = 28s ribosomal RNA [15].

hybridization (fig. 4). Interestingly, it appeared that connective tissue cells neighboring the tumor cells contained larger amounts of collagen transcripts than stromal cells distant from the tumor.

Though, in vivo in human tissues stromal cells appear to be the main site of ECM production, pancreatic cancer cell lines were also shown to be able to transcribe and synthesize components of the ECM [17]. Thus, the tumor cells themselves may contribute to some extent to ECM production.

Experimental Models for the Study of Pancreatic Fibrosis

Morphological and biochemical processes leading to pancreatic fibrosis are still poorly understood and almost impossible to study in the human system. Studies in humans mostly remain purely descriptive, and in vitro models or animal models are required to study functional and regulatory aspects. In the following paragraph, we describe the most commonly used and best characterized animal models for pancreatic fibrogenesis.

Fig. 4. Collagen histochemistry and in situ hybridization. The panels indicate observations found in four different specimens of paraffin-embedded tissue from patients with pancreatic cancer. The calibration bars indicate 100 μm. **A** This section was stained with sirius red which binds selectively to collagenous protein. Note the wide, dark bands of collagen fibers separating islands of acinar tissue. **B** This section was hybridized with antisense probe for collagen type III mRNA. After two weeks of exposure the silver grain label is predominantly located over connective tissue fibroblasts neighboring tumor cells [15].

Fig. 5. Hematoxylin-eosin-stained histological section of the pancreas of a 180-day-old transgenic TGF α mouse (provided by M. Wagner and R.M. Schmid). The section shows typical tubular complexes and pronounced pancreatic fibrosis. Original magnification $\times 50$.

Cerulein Pancreatitis

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 [18–22]. However, approximately 2 weeks after induction of pancreatitis histology, organ weight and collagen content of the pancreas returned to control values indicating complete regeneration [19]. Repetitive induction of cerulein pancreatitis failed to induce fibrosis in the rat pancreas [20]. Thus, the cerulein model is characterized by a dynamic process of ECM formation and removal, making it ideally suited for functional studies of the ECM in the pancreas.

Transgenic Mice Overexpressing Transforming Growth Factor- α (TGF α) in the Pancreas

Overexpression of TGF α in the pancreas of transgenic mice has been reported to cause a progressive fibrosis and the structural transition from acinar cells to tubular complexes [23, 24]. Using this transgenic model Wagner and collaborators recently showed an aggregation of interlobular fibroblasts and a decrease in acinar cell height starting at day 14 after birth (fig. 5) [25]. In older animals these acinar cells appear to transdifferentiate to duct-like cells forming tubular complexes expressing ductal markers.

Transgenic Mice Overexpressing TGF β in the Pancreas

Recently, transgenic mice overexpressing TGF β 1 under the control of the insulin promoter in pancreatic β cells have been generated to study the effect of this growth factor on the pancreas in an *in vivo* situation [26, 27]. Both studies reported cellular infiltration comprising macrophages and neutrophils, fibroblast proliferation and abnormal deposition of extracellular matrix in the pancreas.

Transgenic Mice with Dominant-Negative Mutant TGF β Type II Receptors

Böttinger et al. [28] recently 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.

Pancreatic Fibrosis in Experimental Pancreatitis Induced by Dibutyltin Dichloride

Experimental pancreatitis induced by intravenous application of 8 mg/kg dibutyltin dichloride in rats leads to an acute edematous pancreatitis within 24 h. Extensive infiltration with mononuclear cells occurs after day 7 followed by the development of pancreatic fibrosis. Parallel to cellular infiltration an upregulation of TGF β 1 and collagen type I mRNA was detected [29].

Source of Extracellular Matrix in the Pancreas

The predominant amount of ECM transcripts and proteins in pancreatic cancer and chronic pancreatitis is produced by stromal cells, and in particular fibroblasts [14, 15]. Recently, we have identified and characterized fat-storing cells in the pancreas of mice, rats, and humans [29a]. Because of their similarity to hepatic stellate cells ('HSC', Ito cells, see Gressner and Bachem [30] for an overview) we named these cells 'pancreatic stellate cells' (PSC). PSCs contained numerous fat droplets and were located in the interlobular and interacinar region. The number of these cells increased with the age of the ani-

Fig. 6. Transmission electron micrographs (**A**, **B**) and fluorescence micrographs (**C**, **D**) of cultured rat pancreatic stellate cells. **A** Primary cultured PSC 24 h after seeding showing numerous perinuclear fat droplets (F). **B** In a secondary cultured PSC 48 h after passage the endoplasmic reticulum (RER) is prominent and the fat droplets almost disappeared. **C** Collagen type I immunostaining in PSC after the 5th passage. The cells received 10% fetal calf serum and 100 μ g/ml ascorbic acid. Collagen type I is localized intracellularly and as fibrils between the cells. **D** Immunofluorescence of fibronectin in cultured PSC after the 2nd passage. Fibronectin is located primarily in fibrillar structures on cell surface and between the cells. N = Nucleus; F = fat droplets; RER = rough endoplasmic reticulum. **A**, **B** $\times$ 3,000; **C**, **D** $\times$ 400.

mals. In addition, PSC were abundant in alcoholic chronic pancreatitis in man. Another group previously described a 'fibroblast-like' cell located in periapinar areas of the human pancreas with characteristics of myofibroblast, e.g. expression of α -smooth muscle actin (α sm-actin), synthesis of collagens and fibronectin, and the formation of dense bodies (microfilaments) [31]. We assume that at least part of these cells represent activated PSC displaying the 'synthetic' myofibroblast (mfb)-like phenotype.

During primary culture PSC changes from a quiescent retinoid loaded phenotype to a highly 'synthetic' and proliferating myofibroblast-like cell. It was observed that the retinoid containing fat droplets decreased during culture in size and number in parallel to the increase in α sm-actin-expression and the development of a prominent endoplasmic reticulum (fig. 6a, b).

The 'synthetic' phenotype of PSC synthesizes and secretes high amounts of collagen type I and fibronectin

(fig. 6c, d) [29a]. By quantitative measurement of the procollagen peptides we showed that the 'synthetic' phenotype of human PSC synthesizes 25- to 40-fold more collagen type I than collagen type III esters [29a]. bFGF, PDGF, TGF α and in particular TGF β 1 were identified as the responsible factors acting as fibrogenic mediators by stimulating extracellular matrix synthesis of HSC and PSC [29a, 32, 33].

From our data and previously reported results we propose a cascade of events leading to pancreatic fibrosis. The cascade is initiated by acinar cell injury followed by monocyte/macrophage invasion and activation, as well as platelet aggregation in areas of inflammation causing a release of several polypeptide growth factors including TGF β , TGF α /EGF, bFGF and PDGF. These polypeptide growth factors stimulate extracellular matrix synthesis and proliferation of PSC. Thus, PSCs may be a major matrix producing cell type in pancreas.

Fig. 7. mRNA levels of TGF β 1, - β 2 and - β 3 in acute pancreatitis in response to treatment with antibodies against TGF β 1 (aTGF β) [22]. Two days after induction of pancreatitis, a significant reduction of transcript levels for TGF β 1 (down to one-tenth) and for TGF β 2 (a quarter) was noted. The mRNA of TGF β 3 was readily detectable in pancreata of all experimental groups. Transcript levels of TGF β 3 were neither influenced by pancreatitis nor the antiserum.

Role of Growth Factors for the Regulation of Extracellular Matrix Formation in the Pancreas

Biosynthesis and turnover of extracellular matrix is regulated by different cytokines and growth factors [30]. In this context, members of the TGF β superfamily have been shown to be of particular importance. TGF β 1 is the best studied of a set of five homologous, probably functionally similar peptides (TGF β 1–5), each a 25-kD homodimer in its biologically active form [34, 35]. It stimulates transcription and biosynthesis of different extracellular matrix proteins and in addition functions as an inhibitor of epithelial cell proliferation [26, 30, 36]. It is overexpressed in human chronic pancreatitis [37], and suggested to be involved in the pathogenesis of this chronic inflammatory disease of the pancreas.

Recently, we could show that during regeneration from cerulein-induced pancreatitis in rats alterations of collagen gene expression were accompanied by parallel alterations of TGF β 1 gene expression (fig. 7) [21]. A maximal increase of TGF β 1 mRNA (3-fold over controls) was found 2 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 β 1 protein content in total pancreas reached peak values after 1 day and protein levels remained high throughout the second day.

Additional experiments were designed to inhibit the effect of TGF β 1 during regeneration from cerulein-induced pancreatitis by injection of neutralizing antibodies against TGF β 1 [22]. Inhibition of TGF β 1 by use of this approach was observed to induce a highly significant decrease of the collagen type I and III protein content (up to 10-fold) and of the steady state levels of the corresponding mRNAs (50% decrease for type III, 70% decrease for type I) in rat pancreata (fig. 8). In parallel this treatment was shown to inhibit the rise of steady state levels of TGF β 1 and - β 2 transcripts which is usually observed during regeneration from cerulein pancreatitis (fig. 7). This phenomenon has been attributed to an autocrine loop in which TGF β induces its own synthesis in an autoregulatory way [38–40]. We could show that the TGF β protein is increased during regeneration from acute cerulein-induced pancreatitis before the appearance of the TGF β 1 mRNA, and that platelets or inflammatory cells may be the source of the active TGF β . This protein may contribute to the upregulation of TGF β 1 *de novo* synthesis in the regenerating pancreas. Treatment with TGF β 1 antibodies before and during induction of pancreatitis may block this early effect of TGF β 1 and thus inhibit upregulation of *de novo* transcription.

Further evidence for the role of TGF β for pancreatic fibrogenesis was provided by Van Laethem et al. [40]. This group studied the effect of the application of recombinant TGF β on the development of pancreatic fibrosis after repeated induction of acute pancreatitis in mice by intraperitoneal injections of cerulein. TGF β had no influence on a single course of acute pancreatitis. After six courses of acute pancreatitis mice not treated with recombinant TGF β showed only mild inflammatory changes. However, in the group of mice treated with TGF β prominent areas of perilobular and intralobular fibrosis were observed adjacent to inflammatory and necrotic foci.

Data obtained in the experimental models described above clearly identify TGF β as one of the central regulators of ECM-formation in the pancreas. However, evidence is available indicating that additional growth factors collaborate with TGF β in regulating the development of pancreatic fibrosis. Inhibition of TGF β during regeneration from cerulein-induced pancreatitis in the rat did not completely abolish the formation of ECM, indicating the involvement of other factors [22]. In the same model, TGF β did not appear to significantly influence expression and activity of matrix metalloproteinases which participate in the removal of ECM during regeneration from pancreatitis [41] (see below). In addition, enhanced expression of a large number of growth factors and their

Fig. 8. Western blot analyses of different extracellular matrix proteins during cerulein-induced pancreatitis in rats. The left hand side of each panel shows the results obtained with proteins of pancreata from rats treated with TGF β antibody (aTGF β) sacrificed at different time points (0 days–7 days) after induction of pancreatitis. The right hand side of each panel shows the results obtained at the same time points with saline-treated control rats. Treatment with TGF β antibodies led to a clear reduction of collagens I/III and fibronectin whereas actin concentrations in the rat pancreas were not influenced by treatment with neutralizing antibody [21, 22].

receptors has been observed in both, pancreatic cancer and chronic pancreatitis (e.g. FGFa/b [43, 42], PDGF a/b [44].

Role of Proteinases and Proteinase Inhibitors for the Breakdown of Extracellular Matrix

Matrix metalloproteinases (MMP) comprise a family of 19 proteolytic enzymes that contain tightly bound zinc [45, 46]. According to their substrate specificity (table 1). MMPs can broadly be classified as collagenases (MMP1, MMP8, MMP13), gelatinases (MMP2, MMP9), stromelysins (MMP3, MMP10, MMP11) and the membrane-type metalloproteinases (MMP14, MMP15, MMP16, MMP17). On the basis of sequence homology MMP7 (formerly known as Pump) and MMP12 (metalloelastase) do not belong to these subgroups and most likely represent distinct entities. MMPs are secreted as proenzymes which are activated by proteolytic cleavage of an amino-terminal propeptide, e.g. by plasmin, cathepsin G, trypsin, α -chymotrypsin and MMP3 [47, 48]. Activity is further controlled by various proteinase inhibitors such as α_2 -microglobulin and more importantly the family of tissue inhibitors of metalloproteinases, TIMP1-4 [46, 49 for overviews]. Active MMPs display proteolytic activity for at least one component of the ECM (table 1). There is considerable evidence that MMPs 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 malig-

nant tumors and the destruction of joints in rheumatoid arthritis [45, 46, 49].

Pancreatic Cancer

The balance between gene expression 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 [50–52]. In pancreatic cancer and control pancreatic tissue transcripts for MMP-1 (interstitial collagenase) and MMP-3 (stromelysin-1) were not detectable [15]. Steady-state levels of transcripts encoding extracellular matrix proteins, MMP-2 (72-kD collagenase IV), MMP-9 (92-kD collagenase type IV), TIMP-1 and TIMP-2 were elevated in the majority of pancreatic cancer tissue samples as compared to control pancreatic tissue (fig. 3). A good correlation was seen between overexpression of the named MMPs and TIMPs and the steady state 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, MMP-9, TIMP-1 and TIMP-2 in both stromal and tumor cells. However, MMP-2 transcripts appeared to be more abundant in stromal cells, TIMP-1 and TIMP-2 transcripts were evenly distributed over tumor and stromal cells and relatively more MMP-9 transcripts were found in tumor cells (fig. 9). Similar results were obtained by Bramhall and colleagues [53, 54].

Besides the family of metalloproteinases, the plasminogen activator/plasmin system has also been implicated

Table 1. Matrix metalloproteinases

Enzyme	Number	Principal substrates
Interstitial collagenase	MMP-1	fibrillar collagens, types I, II, III
Neutrophil collagenase	MMP-8	fibrillar collagens, types I, II, III
Collagenase 3	MMP-13	type I collagen
MMP-18(*)	MMP-18	fibrillar collagens, types I, II, III
Gelatinase A	MMP-2	non-fibrillar collagens, fibronectin, laminin
Gelatinase B	MMP-9	non-fibrillar collagens
Stromelysin-1	MMP-3	non-fibrillar collagens, proteoglycans, laminin, collagen III, fibronectin
Stromelysin-2	MMP-10	non-fibrillar collagens, proteoglycans, laminin
Stromelysin-3	MMP-11	Serine proteinase inhibitors (α 1-antitrypsin)
Matrilysin	MMP-7	non-fibrillar collagens, fibronectin, laminin
Metalloelastase	MMP-12	Elastin, non-fibrillar collagens
MT1-MMP	MMP-14	Pro-MMP-2
MT2-MMP	MMP-15	Pro-MMP-2
MT3-MMP	MMP-16	Pro-MMP-2
MT4-MMP ¹	MMP-17	?
MMP-19 ¹	MMP-19	?

¹ MMP-17 [71], MMP-18 [68, 69] and MMP-19 [70] have recently been cloned and very little functional data is available to date. MMP-18 appears to have an essentially identical substrate specificity as MMP1 [68], MMP-17 has been classified as membrane-type MMP based on structural characteristics [71] and MMP-19 appears to belong to a new subfamily of MMPs [70].

with tumor invasion/metastasis. Pro-uPA (urokinase-plasminogen activator) is secreted as a soluble protein by tumor cells or tumor stroma, which binds to a specific receptor found on tumor cells [55]. Activity of uPA is thus focused to the plasma membrane. In experimental models binding of uPA to the cell membrane has been shown to increase experimental metastasis and invasion [56]. This may in part be due to the ability of plasmin to activate MMPs by proteolytic cleavage of the propeptide from the inactive proforms [55, 56]. The serine proteinases tissue-type plasminogen activator (tPA) [57], urokinase-plasminogen activator (uPA) and the uPA receptor (uPAR) [58] were found to be overexpressed in pancreatic cancer tissues. The concomitant overexpression of uPA and uPAR was shown to correlate to a decreased postoperative survival [58]. 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) [59]. 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 bal-

anced activity of proteinases and proteinase inhibitors [60]. The same group could show that *kop/bikunin* is a strong inhibitor of human plasmin, human tissue kallikrein and human plasma kallikrein [61]. The overexpression of a membrane bound inhibitor of plasmin in pancreatic cancer cells is highly interesting. It may e.g. participate in regulating the activation of MMPs or directly counteract uPA.

Acute and Chronic Pancreatitis

Alterations of the balance of expression between MMPs and TIMPs in inflammatory diseases has been described, e.g. in active rheumatoid arthritis [62] and Hashimoto's thyroiditis [63]. In chronic pancreatitis steady state levels of transcripts encoding MMP-2 (72 kD collagenase IV), TIMP-1 and TIMP-2 were elevated in most tissue samples (fig. 1). The magnitude of MMP/TIMP transcript levels could not be correlated to the degree of fibrosis and inflammation detectable by histological analysis or to the total amount of deposited collagen protein. Similar to pancreatic cancer, it appears that transcripts of genes encoding extracellular matrix degrading proteinases with a substrate specificity for interstitial

Fig. 9. In situ hybridizations MMP-2 and MMP-9. Representative observations found in four different specimens of each tissue are shown as dark-field micrographs (left panels) and corresponding bright field micrographs (right panels). The calibration bars indicate 100 μm [15]. **A** MMP-2 expression is found in tumor and in stromal cells. However, a larger amount of silver grains is found in stromal cells. **B** MMP-9, carcinoma cells in their duct like formation show a higher decoration with silver grains than the neighboring connective tissue.

Fig. 10. Slot blot hybridization for interstitial collagenase (MMP-1), 72 kD type IV collagenase (MMP-2) and stromelysin (MMP-3) illustrating representative results observed at different time points after induction of pancreatitis ($10 \mu\text{g kg}^{-1} \text{ h}^{-1}$; 1d.–7 days) for 3 animals at each time point. 10 μg total RNA were loaded into each slot. C = Saline infusion; + $\alpha\text{TGF}\beta 1$ = animals treated with three injections (i.v.) of 150 μg neutralizing TGF $\beta 1$; +control Ab-animals treated with three injections of control serum [41].

extracellular matrix components (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 MMPs and their inhibitors in the development of pancreatic fibrosis we used the model of cerulein-induced pancreatitis in rats [41]. Surprisingly, increased expression during days 2–4 after induction of pancreatitis could only be demonstrated for MMP-2 and MMP-3. Transcript levels of MMP-1 and MMP-9 did not change throughout the regeneration period (fig. 10).

12 h after the induction of pancreatitis, a marked increase of gelatinolytic and caseinolytic activity was observed at approximately 92 kD (MMP9) and 45 kD (ac-

tive MMP.3), respectively, which decreased 2 (MMP-9) or 3 (MMP-3) days after the end of cerulein infusion. Simultaneously, an increase of gelatinolytic activity of active MMP2 (62 kD) was noted on day 2 reaching peak values on day 3 after the induction of pancreatitis, accompanied by a parallel decrease of the 72-kD inactive form of MMP2. Proteolytic activities detected by substrate zymographies and transcript levels of MMP-2 and TIMP-2 were not influenced by treatment with neutralizing TGF $\beta 1$ antibodies. Only the transcript levels of MMP-3 showed a minor reduction upon treatment with neutralizing TGF $\beta 1$ antibodies. Thus, MMP-2, MMP-3 and MMP-9 appear to be of importance for the removal of extracellular matrix during regeneration from cerulein pancreatitis in rats. At least in this model TGF β does not

Fig. 11. The ECM in acute and chronic pancreatitis. Summary of the most essential findings/hypotheses reviewed in this article concerning regulation and formation of the ECM in acute and chronic inflammatory diseases of the pancreas. PSC = Pancreatic stellate cells; fibrobl. = fibroblasts.

Fig. 12. The ECM in pancreatic cancer. A working model summarizing the formation of the ECM in pancreatic tumors based on the most essential data/hypothesis reviewed in this article. GF-R = Growth factor receptors.

seem to be of major importance for the regulation of ECM removal by MMPs.

As for pancreatic cancer Friess et al. [64] could recently show a concomitant increased expression of urokinase plasminogen activator (uPA) and urokinase plasminogen activator inhibitor receptor (uPAR) in chronic pancreati-

tis tissues. Expression of both appeared to correlate to TGFβ expression. The authors speculated that increased amounts of plasmin may activate latent TGFβ, which may be one of the mechanisms leading to the accumulation of fibrotic tissue in the pancreas.

Summary and Conclusions

The presented review summarizes some of the most recent aspects of the study of the extracellular matrix in the pancreas. Though the precise role of increased ECM production in inflammatory and malignant diseases of the pancreas is still unknown, essential data concerning composition, formation and regulation of the ECM has emerged in the recent years (fig. 11, 12). New models using transgenic mouse technology or experimental models of pancreatitis have been established for the study of pancreatic fibrosis. Besides fibroblasts, pancreatic stellate cells (PSCs) in analogy to the well-known HSCs (hepatic stellate cells) appear to contribute to extracellular matrix production. TGF β has been shown to be of paramount importance for the regulation of the dynamic process of extracellular matrix turnover in the pancreas. However, other growth factors have been described to be associated with chronic pancreatitis and pancreatic cancer. Proteinases as, e.g., metalloproteinases or serine proteinases may be of particular importance for the removal of ECM in the pancreas. Imbalances of the ratio between proteinases and their natural inhibitors may be one the major pathogenetic factors leading to pancreatic fibrosis. The current knowledge may well contribute to develop clinical thera-

peutic approaches using pharmacological compounds, as has been the case, e.g. for synthetic inhibitors of MMPs. In vitro and animal experiments have shown that these inhibitors and native tissue inhibitors of MMPs can prevent the growth and spread of experimental tumors [65, 66]. Recent studies have shown that MMP inhibitors may effectively be combined with cytoreductive cancer treatments. E.g. the combination of the synthetic inhibitor CT1746 (Celltech) and cyclophosphamid was shown to be significantly more effective in inhibiting the growth and metastasis of murine lung carcinoma than either agent used alone [67]. Synthetic matrix metalloproteinase inhibitors have now reached the stage of clinical testing and preliminary results show that the compounds may be effective in slowing tumor growth [49].

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