ORIGINAL PAPER

Immunohistochemical expression of growth factors in the exocrine pancreas of patients with chronic liver diseases

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Abstract
Acute viral hepatitis has been reported to cause acute pancreatitis. It was also reported that exocrine pancreatic function is damaged in chronic liver disease (CLD). Growth factors stored in the extracellular matrix and released in the course of pancreatic degradation are major mediators of inductive processes. The immunostaining technique was used to evidence the changes of the expression of the growth factors in different pancreatic cells. VEGF and FGF-beta are involved in the angiogenesis processes and in the evolution of the pancreatic interstitial tissue in case of chronic pancreatitis. Theses markers can also be used for the diagnosis of pancreatitis, but their value is variable. They stimulate the pancreatic star cells, the myofibroblasts and play an important role in the genesis of the extracellular matrix and in the repairing of the tissue after the aggression. TGF beta is important for its role in cellular differentiation and growth and in the development of the fibrosis in liver and other organs. The present paper studies the immunohistochemical expression of these growth factors in pancreatic cells.

Keywords: FGF, VEGF, TGF beta, immunohistochemistry, exocrine pancreas.

Introduction
Anatomically, the pancreas and the liver are in close proximity; many of the blood vessels and ducts associated with these organs anatons with each other. Also, pancreatic diseases which affect bile flow may result in concomitant liver damage. However, whether or not diseases affect pancreatic functions has not been clearly resolved. A number of studies reported that patients diagnosed with acute or fulminant hepatitis also suffer from acute pancreatitis. Acute viral hepatitis has been reported to cause acute pancreatitis. Furthermore, a case with acute exacerbation of chronic hepatitis (CH) B complicated by acute pancreatitis was reported, which suggests that pancreatitis may be an extrahepatic manifestation of CH. It was reported that exocrine pancreatic function is damaged in chronic liver disease (CLD). This report has shown that pancreatic amylase output increases in the patients with nonalcoholic, non-cirrhotic CLD, which suggests that pancreatic enzymes may elevate in chronic non-cirrhotic viral diseases [1].

A growing body of evidence supports the concept that growth factors stored in the extracellular matrix and released in the course of pancreatic degradation are major mediators of inductive processes. Numerous studies have documented the interaction of growth factors with cell surface or ECM-bound proteoglycans. The binding may serve to target growth factors, store them in an inactive form, or aid in their binding to specific receptors. All of these possibilities appear to be true for one angiogenic factor, basic FGF. Similarly, it has been postulated that cell surface heparan sulfates are required for the binding of VEGF to its receptor(s). In addition, VEGF is expected to interact with heparin-containing proteoglycans in the ECM. In fact, the VEGF isoforms are known to bind to heparin with VEGF189 and VEGF206 having the highest affinity [2].

Therefore, this paper aims to study the immunohistochemical expression of growth factors in the exocrine pancreatic cells of the patients with CLD.

Material and Methods
We applied the immunohistochemical technique to 40 of the 67 necropsy cases, which presented a history of chronic hepatitis. The 40 pancreatic samples were fixed in 4% formalin solution, paraffin wax embedded and sectioned at a thickness of 5 µm. The sections were
processed using poly-L-Lysine slides respecting the following sequence: deparaffinize, rehydration, endogenous peroxidase inhibition, PBS (pH 7.4) rinses, microwave oven pre-treatment with citrate buffer pH 6.0 at 75 W, tap water rinses, PBS rinse, non-immune serum blocking, over night incubation with the primary antibody (at optimal dilution). For the immunohistochemical study, the following FGF-beta and VEGF markers were used. The technique consisted in: PBS wash, incubation with the secondary biotinilated antibody, PBS wash, Avidin–Biotin complex incubation, tap water rinses; DAB-staining, counterstaining with Mayer’s Hematoxylin, tap water rinses, lithium carbonate treatment; dehydration, clear in three changes of xylol, mount with xylene based medium.

The particularities of the technique we used were: the Avidin–Biotin (Vectastain–ABC kit; Vector, Burlingame, CA) complex technique was used for observing the immunoreaction of the sections treated with polyclonal rabbit antibodies PDGF-BB and bFGF. The sections were stained for 20 minutes with 3,3’-diaminobenzidine in peroxide solution and were counterstained with Mayer’s Hematoxylin, using the dilution of 1:50 for FGF (DAKO).

Results

The studied samples evidenced that FGF-beta was positive in all cases at 100% in pancreatic acinar cells. Compared with these, the stromal cells and the vascular endothelium ones did not have any immunoreactivity. Only for 24 cases, 60% positive immunoreaction was observed in acinar and endothelial cells, stromal components being immuno-negative. Ten of the studied cases had a positive immunoreaction in 25% of the acinar and stromal cells (Table 1). Thus, we can observe the variability of the immune response of the endothelial cells.

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>% Acinar cells</th>
<th>Endothelium</th>
<th>Stromal cells</th>
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<tbody>
<tr>
<td>40</td>
<td>100</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>60</td>
<td>+</td>
<td>+</td>
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<td>10</td>
<td>25</td>
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The above-mentioned aspects lead us to the idea of an “induction” of the immunohistochemical response for the three cell types: acinar, stromal and endothelial cells, because of some lesional change, which involved the stroma, the parenchyma and the vascular structure.

This affirmation was based on “individual cell changes” as well as on the modifications that involved large areas of parenchyma. The alterations and the dystrophies, the necrobiotic lesions were the basis of the low positive or even negative immunohistochemical response to FGF-beta. Compared with FGF-beta, in our cases VEGF was poorly represented in only five samples, with a focal expression in acinar cells.

The examination of the samples evidenced a “mosaic” of the acinar cells expression of FGF. Thus, the immunostaining was highly positive, especially cytoplasmatic, perinuclear, for the acins near conjunctive–vascular septum, compared with the acins in the center of the lobule. Around the necrobiotic lesions, the immunostaining was low positive or even negative (Figure 1).

Table 1 – The distribution of FGF-beta in the chronic pancreatitis cases

Figure 1 – Highly positive FGF-immunostaining, ×100.

In the areas with intense acinar expressions, we noticed the presence of FGF in the normal or discontinuous endothelium (Figure 2, a and d). The acins around the Langerhans’ islands were highly positive for FGF, its expression being observed in island cells around a capillary in the interior of the island (Figure 2b). The stromal cells were moderate positive to FGF (Figure 2c). FGF-beta was positive in acinar cells depending on the pancreatic lesion, being highly positive when the fibrosis was in its early stages and with decreased expression while fibrosis increased.

Figure 2 – (a) FGF-positive in acinar cells and endothelium, ×200; (b) Positive FGF-immunostaining in acinar cells, ×100.
Acinar cells express VEGF zonal, highly positive in lobule’s periphery (Figure 3). TGF-beta was positive in the cytoplasm of the canalicular epithelium cells and with weak expression in the atrophic acinar cells near the fibrosis areas. It also had intense expression in the endothelial cells (Figure 4).

**Discussion**

The diagnostic value of the immunohistochemical markers is certain, but it is variable in every case. These markers play an important role in the collagen creation process, some by activation and stimulation of pancreatic star shaped cells, others by myofibroblasts stimulation. They also participate in the tissue reconstruction after aggression and they can generate the extra cellular matrix.

FGF-2 and VEGF are strong angiogenesis inducers in vivo and in vitro. FGF-2 induces the VEGF-expression in the endothelial cells by autocrine and paracrine mechanisms. The neutralized monoclonal anti-VEGF antibody inhibits the proliferation of the endothelial cells induced by FGF-2. The endogenous production of FGF-2, 18 kD, regulates the VEGF-expression by extra cellular interaction with the receptor of the cell membrane; high-M FGF-2 (22–24 kD) actions throughout the intra cellular mechanisms pathway.

FGF-2 is the prototype member of a family of 13 growth factors with similar structure, which bind heparin. It is expressed by mesoderm and neuroectodermal origin cells, in a variety of tumors. In vitro, FGF-2 is highly mitogen for different cell types like endothelial cells and fibroblasts. Its biologic activity is mediated by a dual receptor system, composed of four tyrosine kinases with high affinity and of low affinity proteoglycans located on the surface of the cell [3–8].

FGF-2 does not have a signaling peptide to direct it toward the classic secretory pathway [8]. Although the mechanisms which liberate FGF-2 out of the cells are yet unknown [10, 11], FGF-2 is found extra cellular and modulates a variety of cell functions in an autocrine way [12–16].

The FGF family members bind heparin and have an intense mitogen and angiogenic activity. FGF is involved in different biological processes like the development of members and of the nervous system, wound healing and tumor growth.

The ARNm of FGF contains multiple sites for polyadenilation and it is translated out of the initiation codons AUG and non-AUG (CUG) into five isoforms with different proprieties. The CUG initiated forms are located in the nucleus and they are responsible of the intracrine effect. Most of the AUG initiated forms are
cytoplasmatic and responsible of the auto and paracrine effects of FGF.

The FGF-beta immunostaining was positive in all cases in the acinar cells, in 24 cases it was observed in acinar and endothelial cells, and 10 cases presented positive staining in acinar and stromal cells.

The FGF-beta positive staining in acinar cells depended of the degree of pancreatic disease, being highly positive when the fibrosis was in early stages, the number of positive acinar cells decreasing while the fibrosis process increased.

VEGF is a glycoprotein that increases the mitosis and the permeability of the endothelial cells. This cytokine is produced by the endothelial cells in low oxygen concentration conditions and it exercises its effect binding two types of tyrosine kinase receptors on the endothelial cell.

The VEGF receptor 1 is responsible with the tubular organization of the endothelial cells, and VEGF receptor 2 induces the migration and proliferation of the endothelial cells. Both receptors are stimulated in case of ischemia [10]. VEGF is a prototype member of a family of four factors with similar structure and it is a strong mitogen for endothelial cells but it does not have significant mitogenic activity on other cell types [17, 18].

In our study, the VEGF-immunostaining was positive in only five cases (12.5%).

There are five VEGF molecules, with 121, 145, 165, 189 and 206 amino acids (VEGF121, VEGF145, VEGF165, VEGF189, and VEGF206), which are coded by the same gene [19–23]. VEGF165, a 46-kD homodimeric glycoprotein, is the dominant isoform produced by a variety of normal and transformed cells. VEGF121 is soluble protein.

The other VEGF-isofroms have a high affinity for heparan sulfate proteoglycans. VEGF165 is soluble, although a fraction may remain binded by the extra cellular matrix. VEGF189 and VEGF206 are almost exclusively found in the extra cellular matrix [17, 19].

VEGF-A, named “the vascular permeability factor” is a homodymeric glycoprotein bound with heparin [17, 24].

TGF-beta (Transformer Growth Factor) is a member of a family of polypeptidic dimeric growth factors with three isoforms described in mammalians: TGF-beta 1, TGF-beta 2, and TGF-beta 3. The TGF family has a vaster role in the regulation of cellular growth and differentiation. TGF-beta regulates these processes by its three surface receptors (type I, II and III) with high affinity. Type I and II receptors are bound to the serine-rheonin protein kinases and after ligand stimulation intracellular signaling is initiated by the SMAD transcription factors pathway. TGF-beta is a marker important in the fibrosis process.

TGF-beta plays a central role in liver and other organs fibrosis development. TGF-beta 1 increases collagen, TIMP-1 and MMP-2 and decreases TIMP-2 and interstitial colagenases.

More and more proves suggest that PSC may also be activated by the paracrine fibrogenetic cytokines like the trombocyte-derived growth factor and TGF-beta derived from macrophages. In addition, PSC produce ECM components as a response to TGF-beta, suggesting that this cytokine plays a role in pancreatic fibrosis [25].

In 11 cases, it was positive in the star shaped cells. On our sections, TGF-beta was positive with low intensity in the acinar atrophic cells near the fibrosis area, suggesting its paracrine secretion. Although PSC produce TGF-beta, the main question is if it has an autocrine effect on the regulation of the matrix homeostasis. If autocrine expression exists, this can be important for the expression of the TGF-beta regulated gene because of the high local concentration of cytokine in the extra cellular medium around the secretory cells.

TGF-beta was positive in the cytoplasm of the canalicular epithelium cells and had low intensity in the acinar epithelium. As Slater SD et al. [26] show, only 38 of acinar cells are positive to TGF-beta in chronic pancreatitis.

Other authors [27], analyzing the MMP-2, MTI-MMP, TIMP-1 and TIMP-2 expression in vivo in chronic pancreatitis, demonstrate that PSC produce TGF-beta and that TGF-beta 1 influences the PSC proliferation, collagen 1 expression and MMP-2, MMP-3, MMP-9 in an autocrine way.

Conclusions

The samples examination evidenced a “mosaic” of the acinar cells FGF immunostaining. The acinar cells near the conjunctive-vascular septum were highly positive, which suggest the involvement of FGF-2 in fibrosis processes.

The FGF-2 immunostaining was concordant with the localization of the acinar cells inside the pancreatic lobules, lesions observed in the liver at intralobular level. For this reason, we confirm the common embryonic origin of liver and pancreas.

The presence of FGF-2 in vascular endothelium, regardless its caliber and integrity confirms its participation in angiogenesis.

The presence of VEGF in acinar cells confirms their participation in angiogenesis.

We consider that FGF-2 and VEGF are inducers of angiogenesis and that they have an important role in the stromal evolution in chronic pancreatitis.

The remaining acins, after the intense fibrosis (with parenchyma replacement) were atrophic and TGF-beta positive.

References


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