Pancreatic ductal adenocarcinoma is characterized by «tumor desmoplasia», a remarkable increase in connective tissue that penetrates and envelopes the neoplasm. It is becoming clear that this desmoplastic microenvironment of pancreatic cancer — which is forming approximately eighty percent of the tumor mass — is not a passive scaffold for the tumor cells but an active player in carcinogenesis. Several chemotherapeutic agents and novel molecular targeted therapies against epithelial tumor cells — although showing antitumor activity in cell culture and mouse experiments — have failed to show significant effects in the clinic. Thus, targeting pancreatic tumor cells alone seems unlikely to improve the dismal prognosis of pancreatic cancer. It has recently been shown that the activated stroma of pancreatic cancer is an independent prognostic marker with an impact on patient survival as much as the lymph node status of the cancer. Several primarily benign conditions associated with expansion of stromal and inflammatory components, such as chronic pancreatitis or hereditary pancreatitis are believed to increase the risk of pancreatic cancer. Similar observations have been made in other cancer types such as chronic hepatitis-liver cancer, Barrett dyplasia-esophageal cancer, and inflammatory bowel disease-colon cancer. The common denominator of all these conditions is; chronic inflammation leads to increased incidence of cancer. In this review the impact of the activated stroma on pancreatic carcinogenesis is discussed.

**Key Words**: microenvironment, pancreatic cancer, extracellular matrix.

**Components of the pancreatic cancer microenvironment.** The activated stroma of pancreatic cancer comprises several different cell types including stellate cells, endothelial cells, nerve cells, immune cells such as macrophages, lymphocytes, dendritic cells and the extracellular matrix.

**Pancreatic stellate cells.** In 1998 Bachem and Apte isolated and cultured pancreatic stellate cells (PSC) [1, 2]. Morphologic, functional and gene expression studies revealed that PSC resemble HSC characteristics and therefore may possibly share a common origin [3]. However, the origin of stellate cells is still controversially debated. Mesenchymal, endodermal as well as neuroectodermal origins are suggested. Further, it is postulated that in the diseased organ, stellate cells are transformed from their quiescent precursors, or recruited from local fibroblasts, bone marrow derived cells or generated via epithelial-mesenchymal transformation [3]. Pancreatic stellate cells (PSC) are myofibroblast-like cells found in the periacinar spaces in the normal pancreas and have long cytoplasmic processes that encircle the base of the acinus like the pericytes around the breast acini [2]. In their quiescent state, pancreatic stellate cells comprise approximately 4% of the pancreatic cell population. They can also be found in perivascular, periductal and periacinar regions of the pancreas and serve as key participants in the pathobiology of the major disorders of the exocrine pancreas, i.e., chronic pancreatitis and pancreatic cancer [1, 2, 4, 5]. In these disorders, PSC participate in disease pathogenesis after transforming from a quiescent state into an «activated» state, also known as a «myofibroblastic» state. This activation process is accompanied by a loss of the characteristic retinoid containing fat droplets in their cytoplasm with a concomitant expression of alpha smooth muscle actin [1, 2] (Fig. 1a, b). Although in acute pancreatitis PSC activity is transient, their persistent activity in chronic inflammation and pancreatic ductal adenocarcinoma (PDAC) can impair organ function due to their excessive contraction and abundant extracellular matrix protein deposition especially in the periacinar spaces [5]. It is also becoming clearer that myofibroblast like cells found in the activated stroma of pancreatic cancer significantly impact tumor behavior [6].

**Endothelial cells / neoangiogenesis.** In in vitro- and animal-experiments, pancreatic cancer cells are shown to induce angiogenesis by producing angiogenic substances like vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and IL-8 [7–10]. Nevertheless, in vivo, pancreatic cancer is a hypoxic and hypovascular tumor (Fig. 2a, b) with an 80% reduction of the microvascular density compared to the normal pancreas [5, 11, 12]. This discrepancy between experimental data and the clinical reality results mostly from the inefficiency of our current experimental setups in recapitulating the tumor microenvironment. First, pancreatic cancer cells produce also potent anti-angiogenic substances like angiotatin, endostatin and thrombospondin-1, which in fact renders them dominantly antiangiogenic [5, 13]. Second, when cancer cells interact with pro-angiogenic PSC, the dominant effect of this «system» -resembling pancreatic cancer microenvironment- remains antiangiogenic. This cumulative antiangiogenic effect is due to increased production of endostatin cleaved from collagen 18 (produced by cancer cells) by matrix metalloproteinase-12 (MMP-12) secreted by cancer and stellate cells [5].
Pancreatic stellate cells: pancreatic stellate cells are activated myofibroblasts that typically express alpha-smooth muscle actin (a, immunofluorescence analysis shows the intracellular filaments). Pancreatic cancer cells activate the stellate cells around them (b, immunohistochemistry staining of stellate cells by alpha smooth muscle actin).

**Fig. 1.**

Nerve cells / Pancreatic neuropathy / Intrapancreatic perineural invasion. The pancreas has an abundant nerve supply composed of various myelinated and unmyelinated nerve fibers and aggregates of neural cell bodies known as intrapancreatic ganglia [14]. The autonomic nervous system regulates the secretory functions of the pancreas, constriction and relaxation of the blood vessels and excretory ducts. The pancreas has a sensory nerve supply that is involved in signal transmission to the central nervous system [14]. The nerves are usually involved when pathologic changes occur in an organ. Although the nerve fiber density of the pancreas decreases due to periacinar fibrosis, the number of pathologically enlarged nerves increase [15]. Pain is reported by 75–80% of patients at the initial evaluation. The pain in pancreatic cancer is believed to be a kind of neuropathic pain. Neuropathic pain is caused by injury of peripheral or visceral nerves, rather than stimulation of pain receptors and is a result of nerve compression by tumor, or direct infiltration of nerves by cancer- or inflammatory cells [15–19]. Interestingly, the size of nerve fibers is increased within the areas of pancreatic fibrosis that characterizes chronic pancreatitis, which further supports the theory that stromal changes within the pancreas may precede the development of malignancy [17]. Logically, intrapancreatic nerve invasion should precede extrapancreatic perineural invasion, which causes pain and precludes curative resection. Nonetheless, considering the high frequency of intrapancreatic nerves (Fig. 3a, b), if analyzed in detail, it should be impossible to find any case of PDAC without intrapancreatic perineural invasion, therefore the prognostic value of this finding is highly questionable.

**Fig. 2.**

Pancreatic stellate cell overactivity leads to the fibrotic microenvironment of the cancer. The fine periacinar capillary network of the normal pancreas (a, CD31 staining of the endothelial cells) is lost in pancreatic cancer. Mason trichrom staining of the pancreatic cancer (b) depicts the collagen-rich (blue) microenvironment of the tumor with notable scarcity of the vessels.

Inflammatory cells. A relationship between inflammation and cancer was hypothesized by Rudolph Virchow back in the 1850s [20]. Epidemiological, clinical and experimental evidence has then contributed to support and further elucidate the functional role of inflammatory cells in tumor progression. Accordingly, malignant tumors are considered as «wounds that do not heal» [21], where microenvironment-derived growth promoting factors sustain the survival and proliferation of initiated cells. All components of the innate (e.g., macrophages, dendritic cells, mast cells, granulocytes) and adaptive immunity (B- and T-lymphocytes) are present in the tumor microenvironment, although in different proportions and with different — and sometimes probably opposite functions [22]. Cells of the innate immunity are recruited to tumor sites by growth factors and chemokines that are often produced by the cancer cell themselves, including colonystimulating factor-1 (CSF-1), CC chemokines (CCL2, CCL3, CCL4, CCL5, CCL8), transforming growth
factor β-1 (TGFβ-1), vascular endothelial growth factor. In return, inflammatory cells produce mediators that contribute to cancer growth, invasion and metastasis [23]. Tumor-associated macrophages exhibit a distinct phenotype that share many characteristics with M2-polarized macrophages and influence every step of tumor progression [24]. Inflammatory cells are part of the stromal reaction that characterizes pancreatic cancer, a fact that highlights the close relation between a chronic inflammatory condition and this tumor [25]. Indeed, similarities between the stroma composition in chronic pancreatitis and pancreatic cancer further emphasize the pathogenetic link between them. Macrophages, mast cells, neutrophils, dendritic cells, B- and T-lymphocytes have all been described in the stroma of pancreatic cancer [26, 27]. Interestingly, the number of mast cells and macrophages showed a positive correlation with the microvessel count and mast cell/macrophase-rich tumors with high microvessel density displayed a tendency for a worse prognosis [26]. In addition to their role in promoting an angiogenic phenotype, mast cells are also probably involved in the establishment of the stromal reaction in primary and metastatic pancreatic cancer. An invasive front of neutrophil granulocytes has been reported at the invasive edge of pancreatic ductal adenocarcinoma, where they produce TGFβ-1, which further affects the stromal reaction that accompanies pancreatic cancer [28]. Taken together, recent evidence supports an active protumorgenic role of inflammatory cells in the development and progression of human pancreatic cancer.

**Extracellular matrix.** The extracellular matrix (ECM) exerts both pro- and anti-tumorigenic effects in pancreatic cancer. Although a fibrotic matrix was initially regarded as a host barrier against tumor invasion, it has become evident that it can modulate and even initiate tumorigenesis [4, 6, 29, 30]. *In vitro*, extracellular matrix proteins influence growth, differentiation, survival and motility of cancer cells both by providing a physical scaffold and by acting as a reservoir for soluble mitogens. Cancer cells are believed to exploit this tumor-supportive microenvironment [4, 29]. The replacement of the normal parenchyma by excessive desmoplastic tissue rich in collagen, fibronectin and periostin ostensibly plays a role in the aggressive behavior of PDAC. The initial stellate cell activity apparently starts at the periacinar spaces on the invading front of the activated stroma [5]. Therefore, on the invasive front of the activated stroma, continuous activation of PSCs and the subsequent fibrosis of the periacinar spaces may physically cause tissue hypoxia and parenchymal loss by hindering the blood circulation, oxygen diffusion and damaging fine innervation. Normally, the collagen type IV-rich basement membrane, which separates epithelial cells from the interstitial matrix, exerts inhibitory effects on both cancer cells and stellate cells. In various tumors, including PDAC, breaching of the basement membrane is a critical step in tumor progression. This step brings malignant cells into direct contact with ECM proteins such as fibrillar collagens, supporting their growth and contributing to their chemo-resistance [4, 29].

![Fig. 3. Replacement of the fine periacinar nerve fibers of the normal pancreas with pathologic nerves in pancreatic cancer. Similar to the capillary network, periacinar spaces of the normal pancreas harbor fine nerve fibers (a, GAP-43 staining without counterstaining). In the fibrotic parts of the pancreas the number of the pathologically enlarged nerves increase (b, GAP-43 staining without counterstaining).](image)

**CONCLUSION**

Activated stroma of the pancreatic cancer comprising activated stellate cells, inflammatory cells impacts on carcinogenesis significantly. Therefore, targeting the activated stroma in order to uncouple epithelial-stromal interactions may interrupt multiple aberrant autocrine and paracrine pathways that promote pancreatic cancer cell growth, invasion, metastasis, and angiogenesis.

**REFERENCES**


