Vascular and ductal elastotic changes in pancreatic cancer

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It is well known that malignant tumors may be the cause of a variety of vascular alterations in the context of paraneoplastic syndromes. Leukocytoclastic vasculitis has been reported as paraneoplastic side effect in various malignancies including carcinomas of the colon, lung, and urinary tract (1) while superficial thrombophlebitis may present the first clinical sign of pancreatic ductal carcinoma (PDAC) (2). Moreover, vascular thrombosis and obstructive changes may occur in the tumor tissue, caused by direct invasion of the vessel wall. Vascular invasion, which may lead to thrombosis and obstruction, is often encountered in pancreatic carcinoma but no other degenerative or inflammatory changes of the vessels have been described at the primary site of the tumor. During routine examination of the PDAC specimens, we came up to vascular changes consistent with elastosis. This unique tumor-associated vasculopathy was topographically linked with the tumor mass, occurred independently from vascular invasion and there was no correlation with paraneoplastic vasculitis either clinically or histologically. Similar changes of the vessels have been described in the past in the context of various carcinomas such as cervical squamous carcinoma, pulmonary bronchioalveolar carcinoma and adenocarcinoma of the large bowel (3). Another example of cancer-associated elastosis concerns ductal elastosis in cases of intraductal and invasive ductal carcinoma of the breast (4–6), salivary gland tumors (7, 8) and prostatic adenocarcinoma (3).

Vascular elastosis in pancreatic carcinomas has not yet been described and both vascular and ductal changes seem to be underrecognized. The aim of this study is to identify and define alterations of arteries, veins and ducts in PDAC and to provide information regarding their diagnostic significance and their relationship with possible sources of origin.
Table 1. Demographics and tumor characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients age, years (mean, range)</td>
<td>67.3 (51–80)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>23 (63.8%)</td>
</tr>
<tr>
<td>Male</td>
<td>13 (36.2%)</td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>II</td>
<td>22 (72.2%)</td>
</tr>
<tr>
<td>III</td>
<td>12 (14.8%)</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0</td>
</tr>
<tr>
<td>T2</td>
<td>0</td>
</tr>
<tr>
<td>T3</td>
<td>36 (100%)</td>
</tr>
<tr>
<td>T4</td>
<td>0</td>
</tr>
<tr>
<td>Nodal status</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>18 (50%)</td>
</tr>
<tr>
<td>N1</td>
<td>18 (50%)</td>
</tr>
<tr>
<td>Metastasis</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>2 (6%)</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

The examined material consists of a series of 36 consecutive cases of pancreatic ductal adenocarcinoma surgically removed during the years 2012–2013. Three of the cases showed additional lesions of chronic pancreatitis in non-tumoral pancreas. For all 36 cases a Whipple procedure was performed and there was no history of previous adjuvant therapy. Patient demographics and tumor characteristics are listed in Table 1.

Surgical specimens were fixed in 10% neutral formalin solution and tissue sections were processed according to the routine protocol.

Paraaffin sections stained with hematoxylin–eosin were re-examined by two experienced pathologists. Three blocks were chosen for further analysis in each case, one enclosing representative tumor tissue, one from the tumor periphery and one from the rest of the pancreas at a distance from the tumor. Selected tissue sections with a mean area of 4 cm² were stained with Verhoeff’s Van Gieson (EVG) stain to reveal the elastic fibers and consecutively were studied with special attention to ductal, vascular and stromal elastosis. In particular, validation was focused on the thickness, density, and distribution pattern of the elastic fibers in the walls of vessels and ducts, in perivascular and periductal tissue as well as in tumor stroma. Additionally, elastosis severity was graded as absent (grade 0), mild (grade 1), moderate (grade 2), and severe (grade 3).

For methodological reasons the examined vessels and ducts were classified into the following groups according to their topographical relationship with the tumor mass.

- **Group A**: vessels and ducts located within the neoplastic tissue.
- **Group B**: vessels and ducts located at the tumor periphery.
- **Group C**: vessels and ducts located in non-tumoral pancreas, at a distance from the tumor.

Vessels and ducts in group A and B were evaluated in comparison with vessels and ducts of analogous diameter in group C which served as control.

Comparative statistical analysis of elastosis grade was performed between the aforementioned topographical groups. In each case, the whole section area was scanned and the number of veins, arteries, and ducts was recorded and categorized according to elastosis grade. Six cases were excluded from the analysis because of the very limited number of vessels and ducts within the tumor, due to extensive necrosis or carcinomatous destruction and extinction.

The small number of cases with well-differentiated carcinoma and the absence of stage I and II in our series did not permit further statistical analysis.

Vimentin and α-SMA were used as markers for carcinoma-associated fibroblasts (9–13) and for the depiction of normal fibroblasts (vimentin+, α-SMA-) and myofibroblasts (vimentin+, α-SMA+). Immunohistochemistry was performed in 15 cases on 4-μm sections from the groups A and C, using the alpha-smooth muscle actin monoclonal mouse anti-human antibody (1:20; clone 1A4, DAKO, Glostrup, Denmark) and the vimentin monoclonal mouse anti-human antibody (1:50; clone V9, DAKO, Glostrup, Denmark). The two-step peroxidase-conjugated polymer technique (DAKO Envision kit, DAKO, Carpinteria, CA, USA) was applied.

Evaluation included both cellular density and distribution pattern.

The chi-square test was used for the statistical analysis.

RESULTS

In H&E sections, vascular changes were visible in 8 of the 36 cases. Small and medium sized arteries were affected, characterized by increased wall thickness, stenosis and occasionally obliteration of their lumen. Tortuous elastic fibers were discernible often associated with mild and rarely moderate mononuclear inflammatory infiltration (Fig. 1A) while in one case elastic fiber deposition was accompanied by giant cell reaction reminiscent of giant cell arteritis (Fig. 1B).

Verhoeff’s Van Gieson stain revealed elastotic changes in both vessels and pancreatic ducts in all 36 cases. Vascular elastosis of mild degree was characterized by circumferential or focal elastic fiber deposition in the adventitia of arteries and veins (Fig. 1C,D). Elastosis of moderate degree showed additionally to adventitia changes, multiplication, and tortuosity of elastic laminae in arteries, and accumulation of evenly distributed elastic fibers throughout the wall in veins (Fig. 1E,F). In severe elastosis, a trans-mural and perivascular dense network of entangled elastic fibers was seen causing wall thickening and partial or total lumen obliteration of arteries and veins (Fig. 1G,H).

Within the neoplastic tissue (group A) elastosis ranged from mild to severe affecting both arteries and veins in variable degrees even in the same case. The majority of the cases showed mild and moderate vascular alterations while severe elastotic lesions...
were observed in one-third of the cases. Some of the obliterated vessels with elastosis exhibited carcinomatous invasion.

The same range of severity was also demonstrated in pancreatic ducts. Almost all ducts including those showing PanIn 1-3 showed mild to severe ductal elastosis. Mild elastosis was characterized by focal or circumferential periductal elastic fiber accumulation (Fig. 2A). In moderate and severe ductal elastosis, progressive increase and condensation of elastic fibers was observed throughout the thickened wall (Fig. 2B–D). Besides few foci of elastic fiber deposition in the stroma, elastosis was not depicted as a stroma component either in a diffuse manner or around carcinomatous structures.

At the tumor periphery (group B) both vascular and ductal alterations were observed which most often ranged from mild to moderate. Severe

**Fig. 1.** (A) Thickened artery with tortuous elastic fibers and mononuclear inflammatory infiltration. H&E (×100). (B) Arterial wall with elastic fiber deposition accompanied by inflammatory infiltration and giant cell reaction. H&E (×200). (C) and (D) Vascular elastosis of mild degree: focal and circumferential elastic fiber deposition in the adventitia of arteries (C, D) and vein (C). EVG (C×200, D×100). (E) and (F) Vascular elastosis of moderate degree: arteries showing multiplication and tortuosity of elastic laminae (arrow). Severe elastosis in the paired vein with dense accumulation of elastic fibers throughout the wall (arrowhead). EVG (E,F×200). (G) and (H) Severe vascular elastosis: intra-mural dense and tangled network of elastic fibers with partial lumen obliteration of arteries and veins. EVG (G×40, H×200).
changes were less frequently encountered compared to group A (p < 0.01). Data are shown in Table 2. In the non-tumoral pancreatic tissue (group C) no vascular or ductal elastosis was identified (Fig. 3A, B) with rare exception of a small number of arteries which showed mild elastosis of the internal elastic lamina, probably age related.

In the tumor stroma, α-SMA-positive and vimentin-positive cells considered to represent carcinoma-associated fibroblasts were observed in large numbers. In addition, α-SMA-positive cells were notably arranged around all carcinomatous structures (Fig. 2E,F) exhibiting a complete or incomplete perineoplastic ring. In contrast, the presence of α-SMA-positive cells around elastotic and non-elastotic vessels was usually scant while periductally their density ranged from absent/minimal to moderate, irrespectively of location (Figs 2E and 3C).

Vimentin-expressing cells surrounded ducts and vessels forming a thin zone irrespective of elastosis degree while no spatial relationship was found with carcinomatous structures.

In cases of chronic pancreatitis, ductal elastosis was mild to moderate and never severe, while α-SMA-positive cells were circumferentially arranged in a large number of native ducts.

Fig. 2. (A) Mild ductal elastosis: circumferential periductal elastic fiber deposition (arrow). EVG (A×200) (B–D) Moderate (B) and severe (C, D) ductal elastosis: accumulation and condensation of elastic fibers throughout the thickened wall and in the periductal tissue. EVG (B×100, C×100, D×200). No elastic fibers around carcinomatous structures (asterisks). (E) and (F) α-SMA+ cells within the stroma and surrounding neoplastic duct-like structures (asterisks). Native ducts without α-SMA cuffing (arrows). α-SMA (E×40, F×200).
DISCUSSION

Elastosis of pancreatic vessels and ducts was a common finding in the examined PDAC cases. Although constantly encountered, it was not always recognizable in H&E sections, thus requiring the use of special stains. Vascular and ductal elastotic alterations varied in severity showing variable degrees within the same tumor with escalating differences between the main carcinoma mass and the periphery. Most prominent elastotic reaction was usually observed within the neoplastic tissue while the deposition of elastic fibers significantly diminished at the tumor periphery and adjacent area. Both small- and medium-sized arteries and veins as well as pancreatic ducts were affected. Early elastotic changes became visible in the adventitia of the vessel wall and in the periductal tissue. The lesions progressed to tortuosity and multiplication of the internal elastic lamina of the arteries and to a homogenous increase in the elastic fibers in the wall of veins and ducts. The end result was a condensed intramural network of elastic fibers together with wall thickening and occasionally lumen obliteration. Elastosis was occasionally accompanied by mild and rarely moderate chronic inflammation of the vessel wall while in one case giant cell reaction reminiscent of giant cell arteritis was seen. It has to be stressed that no elastic fibers were found either around carcinomatous structures or diffusely within the tumor stroma.

The presence of elastotic changes has been documented some decades ago in both benign and malignant conditions of the breast (4–6), in colonic adenocarcinoma, in salivary gland tumors (7, 8), in neuroendocrine tumors of the small bowel (4, 14), and also in prostatic, gastric, cervical squamous, and bronchioalveolar pulmonary carcinoma (3). Elastosis was restricted to ducts in ductal and lobular carcinomas of the breast and in carcinomas of
the prostate and the salivary glands (3, 7) while in bronchioalveolar carcinoma it was described only in veins (3). It has to be noted that the appearance of elastosis in PDAC has not been mentioned before while the concomitant involvement of both vessels and ducts observed in our series, has been reported only in breast carcinoma (6). It is known that elastic fibers are one of the components of both pancreatic ducts and vessels wall (15) while cells involved in elastin production and formation of elastic fibers are native fibroblasts, endothelial cells, and smooth muscle cells (16). Fibroblasts are localized around ducts and in the adventitia of vessels, where they reside in a quiescent state. Their activation, expressed by α-SMA positivity, is induced by pancreatic injury and secretion of paracrine factors (17, 18).

The ascertainment that deposition of elastic fibers occurs both in vessels and ducts irrespective of carcinomatous infiltration of their wall, restrains the possibility of an after-invasion effect and provides indications of putative carcinoma-related factors and underlying paracrine pathways taking place in the tumor milieu.

In our study the discrepancy between elastic fiber deposition and density of α-SMA-positive and vimentin-positive cells can be ascribed to the existence of different fibroblasts subtypes in neoplastic and non-neoplastic tissue. Carcinoma-associated fibroblasts despite their abundance in the carcinomatous stroma and their perineoplastic “cuffing”, do not seem to be involved in the production of elastic fibers. This finding is in accordance with the absence of any reference regarding their association with elastic fiber deposition. However, the absence of elastic fibers in the carcinomatous area does not rule out a rapid dissolution of small elastin amounts by matrix metalloproteinases (MMPs), since MMPs have been shown to break down elastic fibers in vitro (19, 20). Regarding vascular and ductal elastosis, the identified inverse association between prominent elastosis and scant α-SMA-positive adventitial cells as well as the inconstant correlation with α-SMA-positive periductal cells point toward down-regulation or absence of elastic fiber degradation.

The significance of tumor-related vascular and ductal elastosis still remains unanswered while no association with tumor aggressiveness and prognosis has been documented. An attempt to relate the degree of elastosis in breast carcinomas with the response to therapy and estrogen receptors level did not yield convincing results (21, 22). In our study, elastosis of vessels and ducts was present in every case of pancreatic carcinoma irrespective of tumor differentiation, while there was no evidence of any protective effect against vessel invasion.

The importance of this study does not rely only on the description of elastosis in PDAC but also on its potential diagnostic significance. The depiction of ductal elastotic alterations is a marker of benignity and may be proven to present a useful tool in distinguishing native ducts from well-differentiated neoplastic duct-like structures especially in biopsies and at the resection margins. Limitations of our study constitute the relatively small number of cases and its retrospective nature. The findings should be confirmed in larger study groups encompassing adequate number of cases of all different stages and grades.

In conclusion, vascular and ductal elastosis is a tumor-associated secondary phenomenon in PDAC. It seems to be the product of fibrogenic cells associated with ducts and vessels cells and inherent to benignity.

**CONFLICT OF INTEREST**

We declare that we have no conflict of interest.

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