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ORIGINAL ARTICLE

Histopathological tumor invasion of the mesenterico-portal vein is characterized by aggressive biology and stromal fibroblast activation

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Abstract

Background: Mesenterico-portal vein resection (PVR) during pancreatoduodenectomy for pancreatic head cancer was established in the 1990s and can be considered a routine procedure in specialized centers today. True histopathologic portal vein invasion is predictive of poor prognosis. The aim of this study was to examine the relationship between mesenterico-portal venous tumor infiltration (PVI) and features of aggressive tumor biology.

Methods: Patients receiving PVR for pancreatic ductal adenocarcinoma of the pancreatic head were identified from a prospectively maintained database. Immunohistochemical staining of tumor tissue was performed for the markers of epithelial–mesenchymal transition (EMT) E-Cadherin, Vimentin and beta-Catenin. Morphology of cancer-associated fibroblasts (CAFs) was assessed as inactive or activated. Statistical calculations were performed with MedCalc software.

Results: In total, 41 patients could be included. Median overall survival was 25 months. PVI was found in 17 patients (41%) and was significantly associated with loss of membranous E-Cadherin in tumor buds ($p = 0.020$), increased Vimentin expression ($p = 0.03$), activated CAF morphology ($p = 0.046$) and margin positive resection ($p = 0.005$).

Conclusion: Our findings suggest that PVI is associated with aggressive tumor biology and disseminated growth less amenable to margin-negative resection.

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Introduction

Since the 1990s, resection of the mesenterico-portal vein (PVR) during pancreatoduodenectomy (PD) has been established as a feasible option in patients with tumor adhesion to the mesenterico-portal vein.[1] Up to now, heterogenous results have been reported regarding perioperative morbidity[2,3] as well as survival benefit[4–6] in patients with PVR for pancreatic cancer. Especially the latter could have significant impact regarding the indication for neoadjuvant therapy[4] on the one hand or alternative palliative surgery[7] on the other hand.

These heterogeneous results might be caused by varying tumor biology, missed in analysis at the clinical level. In recent years, several studies focused on detailed histopathological parameters and disclosed that true cancer cell infiltration of the venous wall (PVI) is not existent in all resection specimens. PVI as well as

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increasing depth of PVI were associated with impaired overall survival.[8–10] To our knowledge, the relationship between tumor biologic aspects and PVI has not yet been investigated.

Epithelial–mesenchymal transition (EMT) is a process of cellular plasticity that has been demonstrated to contribute to cancer cell invasion and metastasis in many experimental models[11] and tumor budding is often regarded as a clinical correlate of EMT.[12] The associated microenvironment plays a substantial role influencing tumor progression and prognosis.[13] In this context, cancer-associated fibroblasts (CAF) have been identified as key players.[14,15] The aim of this study was to assess the role of EMT and CAF in PVI of pancreatic head cancer.

Materials and methods

Patients and operations

Patients with PVR for pancreatic head PDAC operated from 2001 to 2012 at the Clinic for General and Visceral Surgery, University Medical Center Freiburg, Germany, were identified from a prospectively maintained database. All cases with sufficient available tissue left for immunohistochemical re-evaluation were included.

PVR was performed as tangential or segmental resection when adhesion to the mesenterico-portal vein was macroscopically the only presumed barrier to negative resection margins. Complete portal vein occlusion is acceptable for resection according to current national and international guidelines when short and amenable to reconstruction,[16–18] but this condition is considered a general contraindication to pancreatoduodenectomy and PVR at our institution. The following principles of PVR were followed: PVR was performed as segmental or tangential as felt macroscopically appropriate by the surgeon. Vein reconstruction after segmental resection was performed by running suture as end-to-end anastomosis. Tangential resections were reconstructed by running suture. Mobilization of the liver and mesentery by the Cattell–Braasch maneuver[19] was employed for PVR, which in our experience allows for PVR of up to 10 cm with direct reanastomosis. Vascular prostheses or vein grafts were not used. The splenic vein was preserved/included in venous anastomosis whenever possible. Alternatively, the inferior mesenteric vein was preserved to drain splenic blood after occlusion of the splenic vein at the venous confluence of superior mesenteric artery and splenic vein. Standard lymphadenectomy was carried out along the hepatoduodenal ligament, common hepatic artery, portal and superior mesenteric vein and along the right aspect of the superior mesenteric artery.[20]

Standard pathology workup

The mesopancreatic margin, as well as other margins when macroscopically suspicious, were marked intraoperatively by the surgeon. Intraoperative frozen section examination was performed in all cases according to a standard protocol.[21] The portal vein was embedded in relation to the tumor and its resection margins. After frozen section, representative specimens were formalin fixed, paraffin embedded (FFPE), sliced (2 μm thick) and stained with hematoxylin and eosin (H&E) according to routine protocols.[21] Pathology reports included histological WHO type, tumor grade and classification according to UICC (pTNM) and microscopic status of the oral and aboral duodenal, biliary, pancreatic parenchymal, mesopancreatic and mesenterico-portal vein resection margin (R0/1/2). A positive margin was defined as the presence of tumor cells directly at the margin (zero distance rule). Presence (1) or absence (0) of microscopic lymphatic (L), blood vessel (V) and perineural invasion (Pn) were documented, with lymphatic and microvessel invasion defined by presence of intraluminal tumor cells with endothelial adhesion. For this study, all cases and H&E stained tissue slides were re-reviewed by two experienced pathologists (P.B., I.K.).

Resection margin and venous tumor invasion

All tissue samples were histologically examined for tumor cell presence at the resection margins. Each resection margin was reviewed separately. PVI was defined as the presence of tumor cells in the vascular tunica media (smooth muscle) or intima. For this study, H&E stained tissue samples of the resections margin were re-assessed by two experienced pathologists (P.B., I.K.), blinded for outcome variables.

Immunohistochemistry and assessment of EMT features

Immunohistochemical staining for E-Cadherin, beta-Catenin and Vimentin was performed according to routine protocols as previously described.[22] Tumor tissue slides were quantitatively evaluated by two independent pathologists (P.B., I.K.) blinded for outcome variables. Vimentin expression was accordingly defined as positive if more than 10% of tumor cells showed positive cytoplasmic staining. Tumor buds were defined as isolated or up to five cohesive tumor cells without contact to the main tumor mass.[23] E-Cadherin and beta-Catenin expression was graded positive if more than 50% of the tumor cells showed membranous staining and was evaluated in the main tumor mass and tumor buds separately as described previously.[22] Fig. 1 illustrates examples of pathological staining.

CAF grading

To assess CAFs, H&E stained tumor tissue slides were observed at 20-fold magnification. Stromal activity was evaluated according to Ha *et al.*[24] in the main tumor region. “Mature, inactivated” fibroblasts were defined as thin, wavy fibroblasts with small spindle cell morphology while “immature, activated” fibroblasts were defined as large cells with a plump spindle-shaped morphology (Fig. 1). Immature CAF phenotype was assumed, if the proportion of immature fibroblasts was more than 50% in the area of the main tumor volume of all tumor slides.

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