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Osteoclast-like giant cell tumor in mucinous cystadenocarcinoma of the pancreas: an immunohistochemical and molecular analysis

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Abstract

Osteoclast-like giant cell tumors (OLGT) are rare neoplasms of the pancreas and mostly associated with ductal adenocarcinomas. In this report, we present the rare case of OLGT associated with mucinous cystadenocarcinoma (MCC). We investigated the expression profile of both tumors by methods of molecular biology and immunohistochemistry. The panel of markers included osteopontin, her2/neu, mismatch repair genes, *K-ras*, *p53*, E-cadherin, VEGF-C, and podoplanin. Osteopontin was expressed by the osteoclast-like giant cells but not by the mononuclear tumor cells of the OLGT. We detected an amplification and overexpression of *her2/neu* in the MCC but not in the OLGT. Although we observed an immunohistochemical expression of hMSH2 and hMLH1 in the OLGT, we were not able to confirm this result by western blot analysis. We also did not find any microsatellite instability (D2S123, BAT26). While mutation of *K-ras* codon 12 was found in both tumor components, there was wild-type DNA of *p53*. E-cadherin was expressed in MCC but not in OLGT. VEGF-C was only positive in osteoclast-like giant cells and some of the mononuclear cells of OLGT. The vessel-rich stroma of OLGT did not present any podoplanin-positive lymphatic vessel. The observation of our case and others in the published literature may indicate separating OLGT with undifferentiated carcinoma from OLGT with MCC for the better clinical outcome of the latter.

Keywords: Osteoclast-like giant cell; Tumor; Pancreas; Mucinous cystic neoplasm

1. Introduction

Giant cell tumors of the pancreas are rare neoplasms, which appear in two variations. One variation is the undifferentiated (anaplastic) carcinoma (UC) with pleomorphic/sarcomatoid growth pattern and multinucleated tumor giant cells [1]. At the time of diagnosis, UC were often found with widespread metastasis, thus associated with a poor prognosis. Different to this type, Rosai described in 1968 a carcinoma of the pancreas resembling a giant cell tumor of the bone presenting osteoclast-like giant cells [2]. The osteoclast-like giant cell tumor (OLGT) has been found together with UC or ductal adenocarcinoma (DC) or without any other tumor component. A much better prognosis was described for the combination of OLGT with DC than for OLGT and UC [1,3]. Even less frequent is the combination of OLGT with mucinous cystic neoplasms (MCN) [4–7]. Recently, a patient's survival over 10 years was described for OLGT with mucinous cystadenocarcinoma (MCC) [8]. Few OLGTs were found without any other tumor component that may explain the former confusion on whether OLGTs were epithelial or mesenchymal in origin. For a long time, the origin of OLGT has been an issue of debate. Initially an acinar origin was suggested [2,9], others favored the mesenchymal nature of OLGT [10–14]. The immunohistochemical phenotype with positive staining of vimentin in the absence of keratins and carcinoembryonic antigen (CEA)

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corroborated this latter opinion. Meanwhile, evidence gained that mononuclear cells are neoplastic and epithelial, while the osteoclast-like giant cells are histiocytes [15–17]. Osteoclast-like giant cells are regarded as bone-marrow-derived monocytes that are secondarily recruited into the tumor [18,19]. The aim of this case report was to investigate new relevant markers that were not studied before. We included markers of the mismatch repair genes (MLH1, MSH2) whether these are involved in this particular tumorigenesis.

Because OLGTs are rich in vessels the vascular endothelial growth factor-C (VEGF-C) and podoplanin (marker for lymphatic vessels) were included. There is growing evidence that the overexpression of the *Her2/neu* protein that is of importance in breast cancer, may also affect other malignancies. The detection of *Her2/neu* overexpression in pancreatic adenocarcinomas and its relevance for antibody treatment, prompted us to include this marker too. Osteopontin (OPN) can function to regulate tumor growth and progression. Thus, OPN was considered as a potential marker for tumor progression and prognosis [20].

2. Patient's history

An inconspicuous cyst (1 cm in diameter) in the cauda pancreatis of a 44-year-old woman was checked sonographically four times during 7 years in different hospitals. Two years after the last follow-up, in April 2000, ultrasound and computed tomography (CT) revealed enlargement of the cyst up to 12 cm with multiple cystic spaces. In addition, the patient presented anemia and occult blood containing stool was observed through occult blood testing (Haemoccult® test). The diagnosis of a biopsy was that of a mucinous cystic tumor which led to surgical intervention. A pancreatectomy with splenectomy was performed. The antrum ventriculi and the proximal parts of the jejunum were also removed.

3. Histology and immunohistochemistry

Fresh tissue of the operation specimen was divided in two parts: one for molecular analysis, one for frozen sections. Parts for molecular analysis were immediately shock-frozen in liquid nitrogen. Remaining material of the frozen sections and the entire tumor were fixed in 4% formalin and submitted to histological routine procedure. Serial sections (3-μm thick) were used for hematoxylin and eosin (HE) staining as well as for immunohistochemistry. For immunohistochemical study formalin-fixed, paraffin-embedded tissues were stained by the (strept)avidin-biotinylated peroxidase complex (ABC), and by the alkaline phosphatase-anti alkaline phosphatase (APAAP) methods. Primary antibodies raised against Cam 5.2 (low molecular weight cytokeratin), cytokeratins 7, 8, 18, 19, CD34, CD45 (leukocyte common antigen; LCA), Her2/neu, Ki-67, Lu-5, CD68 (KP-1, PGM1), Mdm-2 (Ab-1), MLH1, MSH2 (Ab-2), p53, Podoplanin, OPN, VEGF-C, and vimentin were used. A list of primary antibodies, their sources, and the dilution ratio are given in Table 1.

4. Tumor tissue preparation and FISH

For fluorescence in situ hybridization (FISH), paraffin embedded tissue sections of the tumor and of tumor free pancreatic parenchyma as a control material was evaluated for amplification of *Her2/neu.* Before denaturation, tissue

Table 1
Characteristics of antibodies

Antibody	Clone	Source		Dilution
Cam 5.2	P3/NS1/1-Ag-4	BD Transduction	San Diego, USA	1:100
Cytokeratin 7	OV-TL 12/30	DAKO	Glostrup, DK	1:200
Cytokeratin 8	35pH11	DAKO	Glostrup, DK	1:25
Cytokeratin 18	DC 10	BioGenex	San Ramon, USA	1:100
Cytokeratin 19	BA17	DAKO	Glostrup, DK	1:200
CD34	QBEnd/10	Novacastra Lab.	Newcastle, UK	1:50
CD45 (LCA)	PD7/26, 2B11	DAKO	Glostrup, DK	1:10
CD68	KP-1, PGM-1	DAKO	Glostrup, DK	1:100
E-cadherin	HECD-1	R&D Systems	Abingdon, UK	1:1000
Her2/neu	Polyclonal	DAKO	Glostrup, DK	HercepTest™
Ki-67	MIB-1	Immunotech	Marseille, F	1:50
Lu-5	Ascites	Biomedical AG	Augst, CH	1:250
Mdm-2 (Ab-1)	IF2	Oncogene Sci	Cambridge, USA	1:50
MLH1	G168-728	BD PharMingen	San Diego, USA	1:50
MSH2 (Ab-2)	FE-11	Oncogene Sci	Cambridge, USA	1:100
Osteopontin	10A16	IBL	Gunma, Jp	1:30
p53	DO-1	Immunotech	Marseille, F	1:5
Podoplanin	Polyclonal	Breiteneder et al.	Ref. [30]	1:200
VEGF-C	Polyclonal	Zymed Lab. Inc.	San Francisco, USA	1:20
Vimentin	V9	DAKO	Glostrup, DK	1:20

VEGF-C: vascular endothelial growth factor-C.