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Blood vessel invasion is a major feature and a factor of poor prognosis in sarcomatoid carcinoma of the lung

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ABSTRACT

Objectives: Pulmonary sarcomatoid carcinomas (SC) are highly disseminated types of non-small-cell lung carcinoma. Their prognosis is poor. New therapeutic targets are needed to improve disease management.
Materials and methods: From 1995 to 2013, clinical and survival data from all consecutive patients with surgically treated SC were collected. Pathological and biomarker analyses were performed: TTF1, P63, c-MET and ALK expression (immunohistochemistry), PAS staining, ALK rearrangement (FISH), and *EGFR*, *KRAS*, *HER2*, *BRAF*, *PIK3CA*, and *MET* genes mutations (PCR).
Results: Seventy-seven patients were included. Median age was 61 years (53–69). Histological subtypes were pleomorphic carcinoma (78%), carcinosarcoma (12%), and giant-cell and/or spindle-cell carcinoma (10%). Blood vessel invasion (BVI) was present in 90% of cases. Morphology and immunohistochemistry were indicative of an adenocarcinoma, squamous, and adenosquamous origin in 41.5%, 17% and 11.5%, respectively, 30% remained not-otherwise-specified. *KRAS*, *PIK3CA*, *EGFR*, and *MET* mutations were found in 31%, 8%, 3%, and 3%, respectively. No tumors had *HER2* or *BRAF* mutations, or ALK rearrangement, whereas 34% had a c-MET positive score. Five-year overall survival (OS) was 29% for the whole population. At multivariate analysis, tumor size <50 mm (HR = 1.96 [1.04–3.73], $p = 0.011$), no lymph-node metastasis (HR = 3.25 [1.68–6.31], $p < 0.0001$), no parietal pleural invasion (HR = 1.16 [1.06–1.28], $p = 0.002$), no BVI (HR = 1.22 [1.06–1.40], $p = 0.005$), and no squamous component (HR = 3.17 [1.48–6.79], $p = 0.01$) were associated with longer OS. Biomarkers did not influence OS.
Conclusion: Dedifferentiation in NSCLC could lead to SC and an epithelial subtype component could influence outcome. BVI was present in almost all SCs and was an independent factor of poor prognosis.

1. Introduction

In 2004, the World Health Organization classification of non-small-cell lung carcinomas (NSCLC) defined pulmonary sarcomatoid carcinomas (SCs) as a small subgroup of poorly differentiated tumors that contain sarcoma or sarcoma-like elements [1]. Diagnosis is usually based on specific morphological aspects. In some cases, immunohistochemical analysis of the expression of multiple keratin and epithelial membrane antigen are necessary to demonstrate the epithelial differentiation in the sarcomatoid component [1]. Retrospective studies show that these tumors are (i) associated with a poor prognosis whatever the stage at diagnosis, i.e., early [2] or metastatic [3,4], and (ii) resistant to conventional chemotherapy, with two-thirds of patients presenting at first evaluation with a progressive disease under a platinum-based regimen [4]. Improving our knowledge of SCs could improve the management of these patients and lead to the development of specific therapeutic trials.

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In this retrospective study, we collected clinical, pathological, and survival data from a cohort of 77 patients with surgically treated primary pulmonary SCs. We also performed periodic-acid-Schiff (PAS) staining, immunohistochemical analysis for TTF1, P63, c-MET and ALK proteins, Fluorescent in situ hybridization (FISH) for ALK rearrangement and genotyping for *EGFR*, *KRAS*, *HER2*, *BRAF*, *PIK3CA*, and *MET* genes.

2. Materials and methods

2.1. Patients

From January 1995 to April 2013, all consecutive patients with pathologically proven primary pulmonary SC based on lung-tumor resections were identified from the archive database at the Department of Pathology (Tumorothèque des Hôpitaux Universitaires de l'Est Parisien). Patients with a tumor biopsy taken by mediastinoscopy or pleuroscopy were not included. All tumors were reviewed by an expert lung pathologist (M.A.) and classified according to the 2004 WHO classification.

Clinical characteristics and surgical staging, according to the seventh edition of the TNM classification, were collected [5]. Disease-free survival (DFS) was defined as the time from surgery to relapse or until the endpoint, defined as the 1st April 2013. Overall survival (OS) was defined as the time from surgery to death or until the endpoint. According to national guidelines, each patient signed a research-approval form.

2.2. Histological analyses

For each tumor sample, the following pathological characteristics were collected: histological subtype, presence of an epithelial component, and its subtype (i.e., adenocarcinoma, squamous, or large-cell carcinoma), lymphatic and vascular emboli, visceral and parietal pleural invasion, blood vessel invasion, and necrosis. Vascular or lymphatic emboli were defined by the presence of tumoral cells inside blood or lymphatic vessels distant from the primary tumor. Blood vessel invasion was defined by contiguous tumoral invasion of the blood vessels. The percentage of tumoral necrosis was evaluated. PAS staining was performed. The threshold for positivity was set at 5% of positive cells, as previously described [6].

2.3. Biomarker analyses

Formalin-fixed paraffin-embedded samples were used for immunohistochemistry and molecular analyses. For immunohistochemistry, 3-μm sections were used. TTF1 (clone 8G7G3/1, 1/100, 32 min, Dako®, Les Ulis, France) and P63 (clone 4A4, 1/100, 40 min, Clinisciences®, Nanterre, France) immunohistochemistry were performed on a benchmark system (Ventana Medical System®, USA), as previously described [6]. The threshold for positivity was set at 5% of positive cells with an intensity of ≥1.

The ALK protein was evaluated using a monoclonal mouse antibody (clone 5A4, Abcam®, UK) on a benchmark system [7]. ALK was considered positive when at least 10% of tumor cells with an intensity ≥2 were stained [8]. The H2228 cell line was used as an external positive control. c-MET expression was evaluated using a monoclonal rabbit antibody (clone SP44) (Genentech®, USA) on a benchmark system [9]. Scoring was based on intensity and percentage of positive cells [10]. The internal control was bronchial epithelium staining of moderate intensity. Four categories were defined: score 3+ (≥50% of tumor cells with strong intensity), 2+ (≥50% of tumor cells with moderate intensity), 1+ (≥50% of tumor cells with weak intensity), 0 (no staining or <50% of tumor cells with weak intensity). c-MET positivity was defined as a score ≥2+ [10].

Genotyping analysis of *EGFR*, *KRAS*, *HER2*, *BRAF*, *PIK3CA*, and *MET* genes was performed (methods detailed in Supplementary Table 1). The presence of tumor cells was evaluated on hematoxylin-and-eosin-stained sections. For genotyping, DNA was extracted from 3 × 10-μm sections with a QIAmp DNA mini kit® (Qiagen, Hilden, Germany).

ALK FISH was performed using Vysis LSI ALK (Dual Color, Break Apart Rearrangement Abbott Molecular, Des Plaines, IL, USA). ALK gene rearrangement was defined as positive when ≥15% of scored tumor cells had split ALK 5′ and 3′ probe signals or had 3′ isolated signals, as described previously [11]. If there were ≤50 tumor nuclei within the scribed area that could be enumerated per sample, the sample was considered uninformative.

Table 1
Characteristics of patients with pulmonary sarcomatoid carcinoma ($n = 77$).

Characteristics	n (%) or median (Q1–Q3)
Age (years)	61 (53–69)
Gender	
Male	59 (77)
Female	18 (23)
Tobacco status	
Former/current smoker	74 (96)
Never-smoker	3 (4)
Localization	
Peripheral	57 (74)
Central	14 (18)
Both central and peripheral	6 (8)
Type of surgery	
Lobectomy	48 (62)
Pneumonectomy	28 (36)
Wedge	1 (2)
Neo-adjuvant chemotherapy	20 (26)
Tumor size (mm)	50 (35–80)
pT extension	
T1	7 (9)
T2	33 (43)
T3	32 (42)
T4	5 (6)
pN extension	
N0	40 (52)
N1	25 (33)
N2	12 (15)
Pathological stage (7th TNM)	
I	16 (21)
II	33 (43)
III	23 (30)
IV	5 (6)
Histological subtypes	
Pleomorphic carcinomas	60 (78)
Carcinosarcomas	9 (12)
Giant-cell carcinomas and or spindle-cell carcinomas	8 (10)
Necrosis (%)	40 (10–51)
Blood vessel invasion	69 (90)
Vascular emboli	8 (10)
Lymphatic emboli	9 (12)
Pleural extension	
Visceral	41 (53)
Parietal	18 (23)
Endobronchial extension	20 (26)

2.4. Statistical analyses

Categorical variables were compared by Chi-squared or Fisher's exact test. Non-normal continuous variables were expressed as medians (Interquartile). The Mann–Whitney test was used on non-normal continuous variables. For survival analysis (DFS, OS), Cox's model was used for univariate and multivariate analyses. Independent variables with a $p < 0.150$ in univariate analyses were