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Original Article

Copy number alterations of *EGFR* and *FGFR1* in pulmonary carcinosarcoma

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Abstract: Carcinosarcoma may develop through sarcomatoid changes in a carcinoma, known as epithelial-mesenchymal transition, or may develop from a single cancer stem cell and subsequently diverge into morphologically dissimilar carcinomatous and sarcomatous components. Thus, we tried to elucidate the histogenesis of pulmonary carcinosarcoma, which has not been thoroughly examined. A key approach to the investigation was use of fluorescence in situ hybridization analysis of *EGFR* and *FGFR1,* because the carcinomatous component of the carcinosarcoma of the available sample was a squamous cell carcinoma, which tends to harbor copy number gain in *EGFR* and *FGFR1*. Consequently, it was postulated that the carcinosarcoma originated from cancer stem cells polysomic for *EGFR*, and only the carcinomatous component received *FGFR1* amplification during divergence into carcinomatous and sarcomatous components.

Keywords: Carcinosarcoma, EGFR, FGFR1, fluorescence in situ hybridization, immunohistochemistry

Introduction

Pulmonary carcinosarcoma has been classified under sarcomatoid carcinoma, which includes four other tumor types, such as pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, and pulmonary blastoma [1]. Pulmonary sarcomatoid carcinoma accounts for 0.1-0.4% of all lung malignancies; carcinosarcoma accounts for only 4% of sarcomatoid carcinoma [1]. Carcinosarcoma may develop through sarcomatoid change in a carcinoma, known as epithelial-mesenchymal transition (EMT) [1, 2]. However, there is another opinion regarding histogenesis, in which carcinosarcoma develops from a single cancer stem cell, and subsequently diverges into morphologically dissimilar carcinomatous and sarcomatous components [3]. It has been previously shown that both components exhibited overlapping and non-overlapping chromosomal alterations; these non-overlapping aberrations were thought to represent events significant in the divergence occurring after initial tumorigenesis [4].

Herein, with use of an available sample, we investigate the tumorigenesis of pulmonary carcinosarcoma having squamous cell carcinoma (SCC) as a carcinomatous component; the sarcomatous component included heterologous elements, such as rhabdomyosarcoma and osteosarcoma. We applied fluorescence in situ hybridization (FISH) analysis of *EGFR* and *FGFR1*, in addition to *TP53* mutational analysis and commonly performed *KRAS and EGFR* mutational analyses. We had particular interest in *EGFR* and *FGFR1* because copy number gain in these is more frequently observed in SCC than in adenocarcinoma [5-9]. Moreover, *KRAS and EGFR* do not often mutate in carcinosarcoma, in contrast to relatively frequent mutations in *TP53* [1]. Thus, the mutational analyses of *KRAS and EGFR* were less interesting for the evaluation of histogenesis. We expected that FISH analysis of *EGFR* and *FGFR1* and mutational analysis of *TP53* would reveal aspects important to the consideration of histogenesis.

Materials and methods

Patient selection

Examining computerized database of our hospital from 2000 to 2014, only one pulmonary carcinosarcoma was identified.

Immunohistochemistry

After fixation with 10% buffered formalin, the sample was trimmed to an appropriate

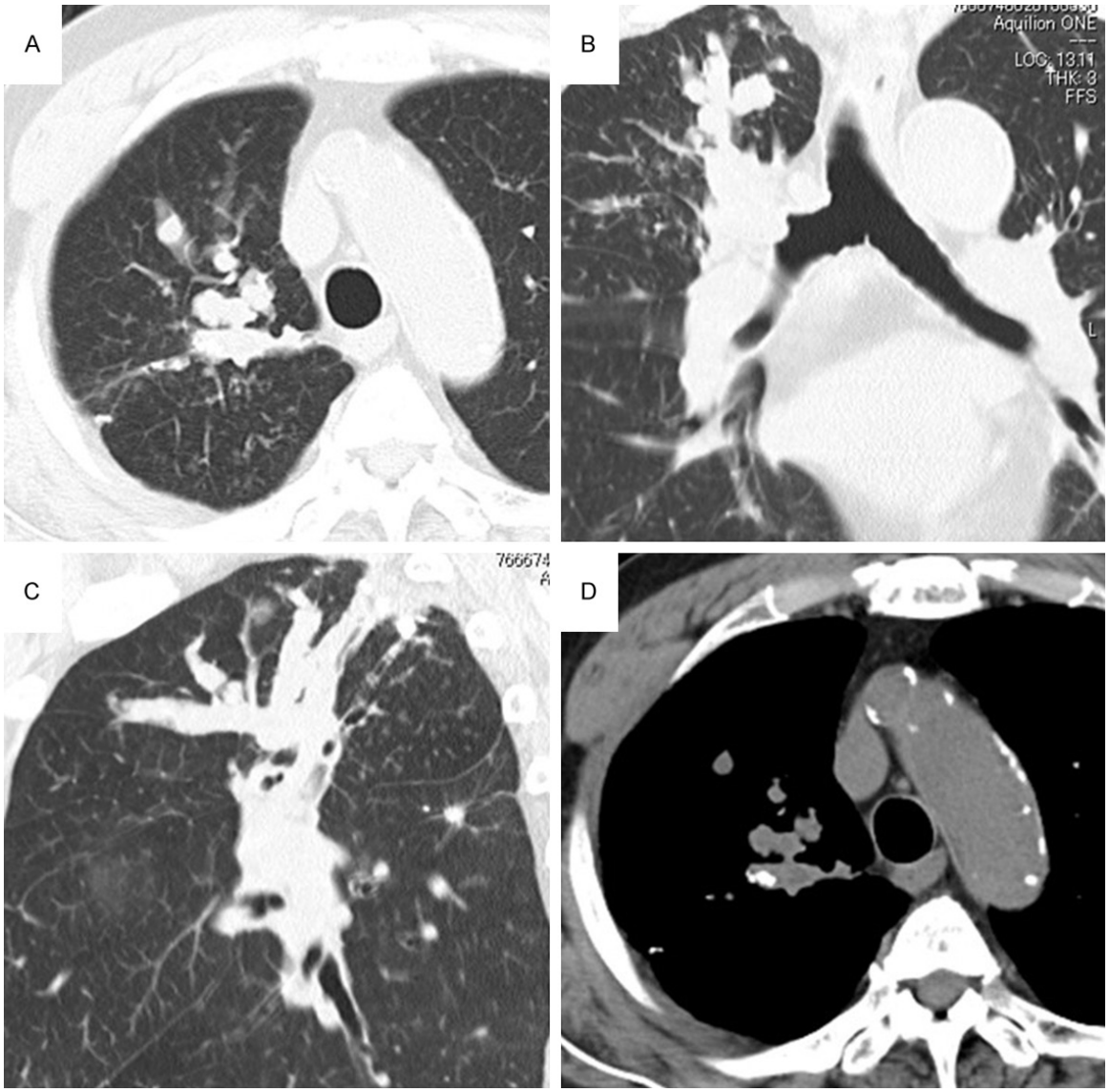

Figure 1. Computed tomography findings. (A) Axial; (B) Coronal; and (C) Sagittal. A branching mass lesion obstructing the right superior bronchial trunk, the peripheral branches of which were also obstructed. The mass appeared to originate at the proximal bronchus and spread distally, following the distribution of the bronchi. (D) Coarse, patchy calcification was observed inside the branching mass, with soft tissue density.

size and embedded in paraffin blocks. Formalin-fixed paraffin-embedded blocks were cut (4-μm thick) for immunohistochemical analysis. An automated slide stainer (BenchMark GX; Ventana Medical Systems, Tucson, AZ, USA) was chosen for immunohisto chemistry.

Fluorescence in situ hybridization

Formalin-fixed paraffin-embedded sections were trimmed (4-μm thick) for fluorescence in situ hybridization (FISH) analysis. FISH was performed using two probe sets targeting the EGFR gene and chromosome 7 (Vysis EGFR/CEP 7 FISH Probe Kit; Abbott Molecular, Chicago, IL, USA) and FGFR1 gene (FGFR1 Break Apart FISH Probe; Empire Genomics, Buffalo, NY) and chromosome 8 (CEP 8 SpectrumOrange Direct Labeled Fluorescent DNA Probe Kit; Abbott Molecular). The centromere probe of chromosome 7 is recognized by a green signal; the probe matching the EGFR gene is recognized by an orange signal.

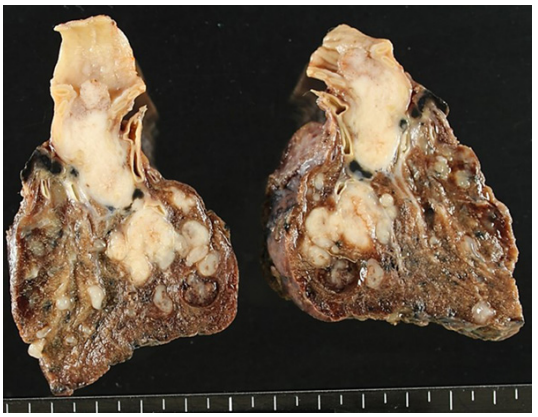

Figure 2. Macroscopic findings. Multiple discrete nodules exhibiting elastic induration and whitish color were present on the cut surfaces.

Mutational analysis

Mutational analysis was performed with a polymerase chain reaction (PCR) at a commercial laboratory (LSI medience, Tokyo, Japan).

Results

Clinical findings

A 70-year-old male current tobacco smoker with a 360-pack-year history presented to our hospital because of bloody sputum. Chest computed tomography (CT) revealed a branching mass lesion obstructing the right superior bronchial trunk, peripheral branches of which were also obstructed. The mass appeared to originate at the proximal bronchus and spread distally, following the distribution of the bronchi (**Figure 1A-C**). Coarse, patchy calcification was observed inside the branching mass with soft tissue density (**Figure 1D**). There were no elevated tumor markers for lung carcinoma; transbronchial biopsy of the mass strongly suggested SCC of the lung. The patient underwent right upper lobectomy and lymph node dissection.

Pathological findings

Gross examination of the tumor revealed multiple discrete nodules exhibiting elastic induration and whitish color on the cut surfaces (**Figure 2**). Reconstruction of their relationship suggested that all the nodules in a cut surface were continuous with other nodules in adjacent cut surfaces, and that the nodules represented primarily intrabronchial growth of the tumor.

Histologically, the proximal part of the tumor was primarily composed of a carcinomatous component; however, the stroma in contact with carcinomatous nests consisted of a sarcomatous component. The tumor mainly grew in the bronchus, but invasion restricted to the bronchial wall was observed in some areas (**Figure 3A**). The tumor showed intrabronchial growth extending to the peripheral lung; it was confined within the bronchus at the periphery, and invasion into the bronchial wall was not observed. The sarcomatous component predominated over the carcinomatous component at the periphery (**Figure 3B**). Both carcinomatous and sarcomatous components were intermingled closely, but a transition between the two components was not identified. The carcinomatous component was composed of solid nests of tumor cells with a high nucleus-to-cytoplasm ratio, without keratinization or gland formation; the nuclei were hyperchromatic with a few nucleoli. The sarcomatous component consisted of spindle cells and short spindle cells with enlarged nuclei and a few nucleoli (**Figure 3C**). Foci of tumor cells showing rhabdomyoblastic morphology were prominent (**Figure 3D**). Patches of tumor cells directly formed osteoid (**Figure 3E**). Nodules found in the cut surfaces of the specimen were essentially intrabronchial lesions, and no metastatic nodules were identified. Lymph node metastasis was also not observed. The surgical margin was free of tumor cells.

Immunohistochemistry (IHC) was performed using an autoimmune stainer (BenchMark GX; Roche Ventana Medical Systems Inc., Tokyo, Japan). CK5/6 (D5/16 B4, 1:100; Dako, Glostrup, Denmark) (**Figure 4A**) and p40 (polyclonal, 1:200; Nichirei Biosciences, Tokyo, Japan) (**Figure 4B**) were positive only in the carcinomatous component; and the staining was diffuse. Thus, the carcinomatous component was considered to be poorly differentiated SCC. Corresponding to an area with rhabdomyoblastic morphology, some tumor cells were positive for desmin (D33, 1:100; Dako) (**Figure 4C**), myogenin (F5D, 1:100; Dako) (**Figure 4D**), and myoglobin (polyclonal, prediluted; Ventana, Tucson, AZ, USA) (**Figure 4E**). Some tumor cells surrounding osteoid were positive for D2-40 (D2-40, 1:100; Dako) (**Figure 4F**). Thus, the sarcomatous component was considered to contain two heterologous elements, rhabdomyo-

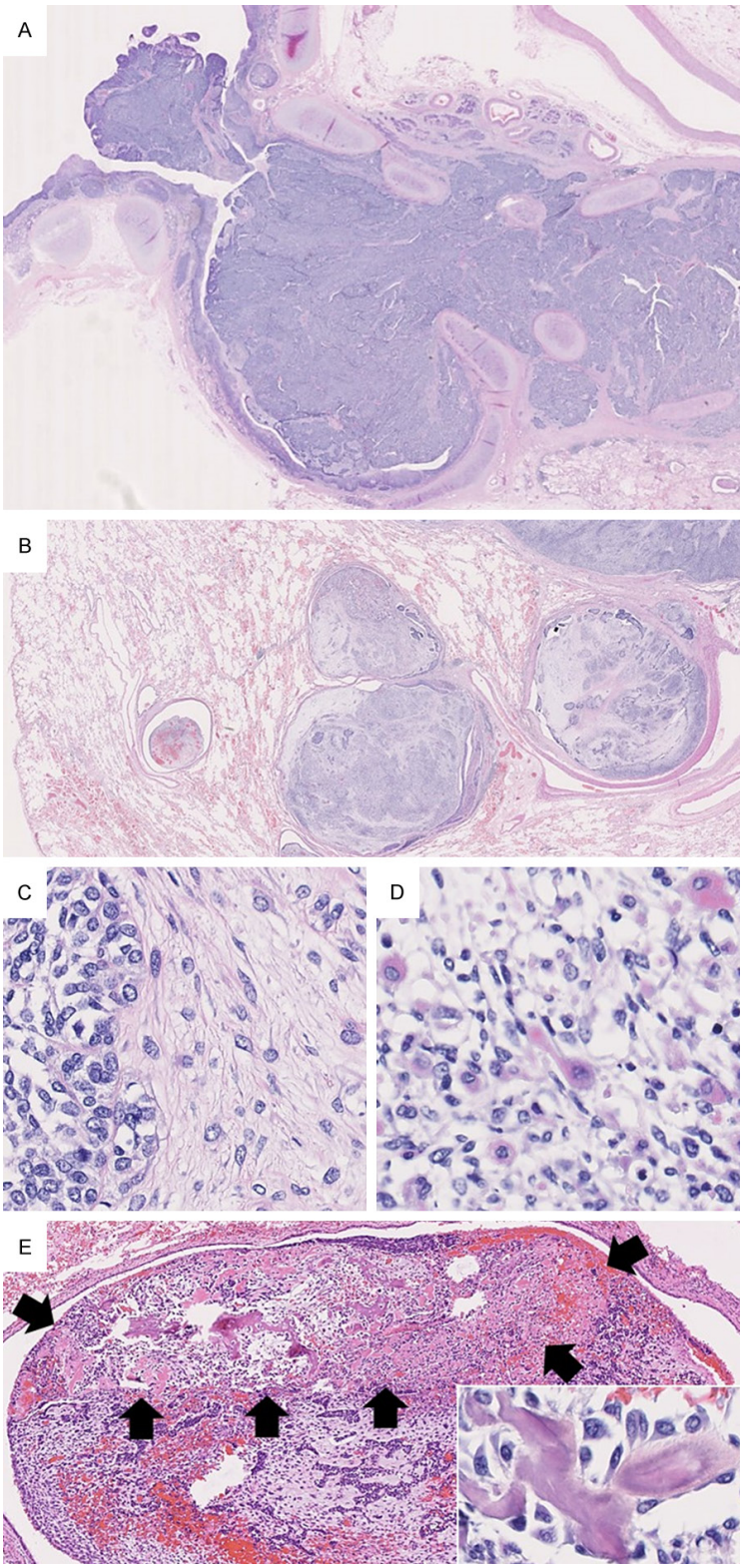

Figure 3. Histological findings. A. The proximal part of the tumor was primarily composed of a carcinomatous component. The stroma in contact with carcinomatous nests consisted of a sarcomatous component. The tumor mainly grew in the bronchus, but invasion restricted to the bronchial wall was observed in some areas. B. The tumor showed intrabronchial growth extending to the peripheral lung; at the periphery, the tumor was confined within the bronchus, and invasion into the bronchial wall was not observed. The sarcomatous component predominated over the carcinomatous component at the periphery. C. The carcinomatous component was composed of solid nests of tumor cells showing a high nucleus-to-cytoplasm ratio; the nuclei were hyperchromatic with a few nucleoli. The sarcomatous component consisted of spindle cells and short spindle cells with enlarged nuclei and a few nucleoli. D. Tumor cells showing rhabdomyoblastic morphology. E. Osteoid formation was observed (arrows). Inset: Osteoid was directly produced by tumor cells.

sarcoma and osteosarcoma, and the tumor was diagnosed as carcinosarcoma. The tumor was negative (null pattern) for p53 (DO-7, 1:100; Dako), suggesting *TP53* mutation. If *TP53* is wild type, tumor cells are expected to show variable positivity. Tumor cells in the carcinomatous component were moderately positive for EGFR (EGFR pharmDx™; Dako) with membranous immunostaining (**Figure 4G**); those in the sarcomatous component were also moderately positive, but membranous immunostaining was not easily observable, compared with the carcinomatous component (**Figure 4H**). Strong positivity for FGFR1 (ab10646, 1:100; Abcam, Cambridge, MA, USA) was observed in the carcinomatous component (**Figure 4I**); however, FGFR1 was faintly positive in the sarcomatous component (**Figure 4J**).

By FISH analysis, similar balanced polysomy of *EGFR* and chromosome 7 where the *EGFR* gene is located was observed in both carcinomatous (**Figure 5A**) and sarcomatous components (**Figure 5B**). In detail, the balanced polysomy was observed in 80% and 70% of the tumor cells of carcinomatous and sarcomatous components, respec-

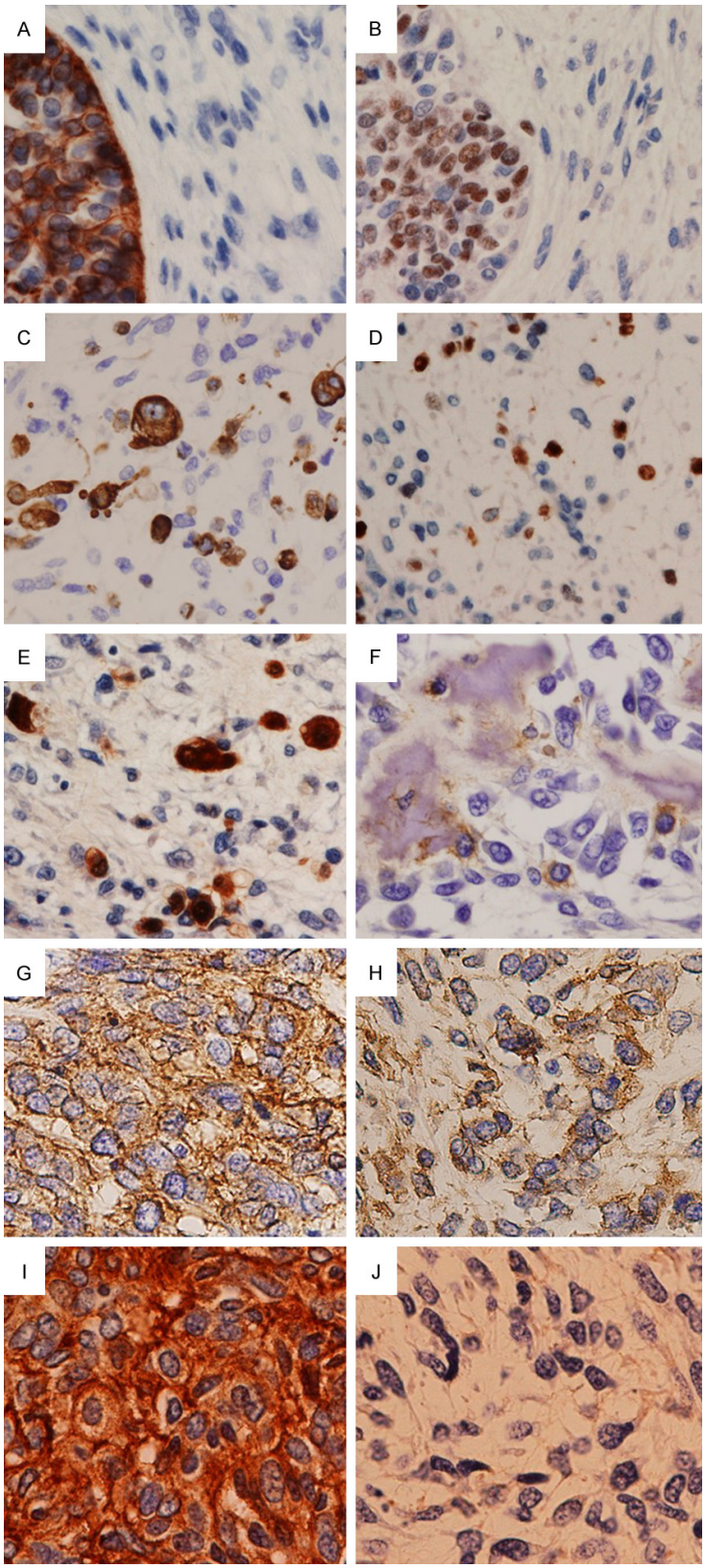

Figure 4. Immunohistochemical findings. A. Positivity for CK5/6 only in the carcinomatous component. B. Positivity for p40 only in the carcinomatous component. C. Positivity for desmin in tumor cells showing rhabdomyoblastic morphology. D. Positivity for myogenin in tumor cells showing rhabdomyoblastic morphology. E. Positivity for myoglobin in tumor cells showing rhabdomyoblastic morphology. F. Positivity for D2-40 in some tumor cells surrounding osteoid. G. Moderate EGFR positivity with membranous staining in the carcinomatous component. H. Moderate EGFR positivity without easily appreciable membranous staining in the sarcomatous component. I. Strong FGFR1 positivity in the carcinomatous component. J. Faint FGFR1 staining in the sarcomatous component.

tively; tumor cells not showing the polysomy harbored balanced trisomy or disomy of *EGFR* and chromosome 7. Regarding *FGFR1* gene, the FISH probe targeting it was recognized as yellow signal because the probe was a break-apart type probe. Amplification of the *FGFR1* gene in the carcinomatous component was represented by a much higher number of yellow than green signals corresponding to the centromere probe of chromosome 8; yellow signals were characteristically observed as large clusters in approximately 80% of the tumor cells (**Figure 5C**). Amplification of the *FGFR1* gene was not identified in the sarcomatous component, with nearly the same number of yellow and green signals (**Figure 5D**).

Mutational analysis of *EGFR* [exon 18, 19 (deletions), 20, and 21] and *KRAS* (codons 12 and 13) was performed, and the result was negative in both carcinomatous and sarcomatous components. Mutational analysis of exons 5, 6, 7, and 8 of *TP53* in carcinomatous and sarcomatous components was conducted; the results of direct sequencing revealed no mutation found in these exons.

Discussion

Pulmonary carcinosarcoma containing an osteosarcoma compo-

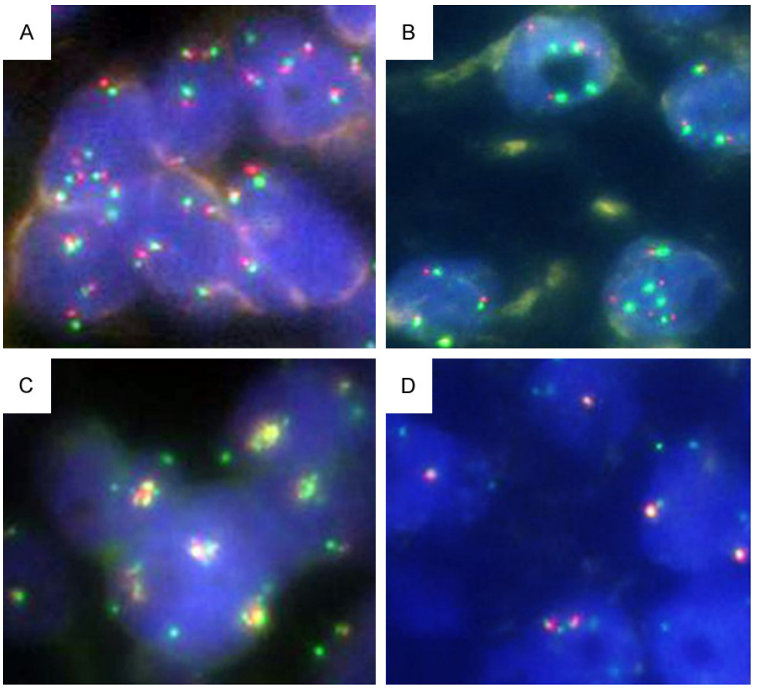

Figure 5. Fluorescence in situ hybridization findings. The centromere probe of chromosome 7 was recognized by a green signal; the probe matching the *EGFR* gene was recognized by an orange signal. Similar balanced polysomy of *EGFR* and chromosome 7 in which the *EGFR* gene is located was observed in both carcinomatous (A) and sarcomatous components (B). Amplification of the *FGFR1* gene in the carcinomatous component was recognized by a much higher number of yellow signals, corresponding to *FGFR1* probe, than green signals, representing the centromere probe of chromosome 8; yellow signals were characteristically observed as large clusters (C). Amplification of the *FGFR1* gene was not identified in the sarcomatous component, which showed nearly the same number of yellow and green signals (D).

nent is rare. It is reported that the most frequent epithelial component is SCC, followed by adenocarcinoma and adenosquamous carcinoma, while the most frequent sarcomatous component is rhabdomyosarcoma, followed by chondrosarcoma, osteosarcoma, or combinations of these elements [1]. In this case, IHC using D2-40 aided in definite confirmation of osteosarcoma [10]. By comparison of CT and pathological findings, calcification observed on CT was found to correspond to osteosarcoma. An intrabronchial growth pattern, which is conspicuously noted on CT, was an unusual feature particularly observed in this case. Only a minority of carcinosarcomas may adopt an intrabronchial growth pattern [1], and this might be attributable to the proportions of carcinomatous and sarcomatous components, as observed in our case. When the sarcomatous component overwhelms the carcinomatous component, this growth pattern can occur. Mesenchymal tumors could take the form of an intraluminal (e.g., intrabronchial and intravascular) growth pattern, as was observed in intravenous leiomyomatosis of the uterus [11].

In considering the molecular features of carcinosarcoma in this case, we emphasized the characteristics of SCC, because the carcinomatous component was SCC, and the sarcomatous component may also share the same molecular features as the carcinomatous component. In a study be Hirsch et al., EGFR was more frequently overexpressed in SCC than in adenocarcinoma [5]. In their study, the FISH patterns of *EGFR* and chromosome 7 in SCC were balanced disomy (37%), balanced trisomy (34%), balanced polysomy (18%), and gene amplification (11%); balanced polysomy and gene amplification were more frequently observed in SCC than in adenocarcinoma [5]. Gene copy number of *EGFR* was correlated with EGFR expression [5]. In a study by Italiano et al., EGFR overexpression was observed in all cases of sarcomatoid carcinoma [12]. Using FISH analysis, they found balanced polysomy of *EGFR* and chromosome 7 in 23% of the cases; *EGFR* amplification was not detected [12]. As expected from these other studies, increased expression of EGFR and balanced polysomy of *EGFR* and chromosome 7 were observed in both carcinomatous and sarcomatous components in our case.

To the best of our knowledge, the status of *FGFR1* has been well examined in SCC, [6-9] but not in sarcomatoid carcinoma. In the meta-analysis of the relationship between SCC and *FGFR1*, amplification is detected in approximately 19%; gender, stage, and tumor differentiation do not seem to affect *FGFR1* amplification in SCC [9]. In contrast, Schildhaus et al. examined 97 adenocarcinomas and did not

observe an amplification of *FGFR1* [6]. Whether *FGFR1* amplification influences survival remains controversial [9], although Kohler et al. showed that survival analysis of SCC according to different cut-off values of *FGFR1* gene copy numbers indicated a tendency toward worse prognosis in cases with increased gene copy numbers [8]. In FISH analysis, *FGFR1* amplification was sometimes seen as a cluster of signals [6, 7]. In our case, large clusters of *FGFR1* signals were observed in the carcinomatous component (SCC) in FISH, which corresponded to high-level amplification according to the criteria proposed by Schildhaus et al. [6]; meanwhile, *FGFR1* amplification was not identified in the sarcomatous component. In addition, strong FGFR1 expression was observed in the carcinomatous component (SCC), but its expression was faint in the sarcomatous component; this could be surmised from the finding by Kohler et al. that high expression of FGFR1 was associated with increased *FGFR1* gene copy numbers in SCC [8].

Combining the results of FISH analysis for *EGFR* and *FGFR1* in our case, it is assumed that cancer stem cells were polysomic for *EGFR,* and that only the carcinomatous component (SCC) received *FGFR1* amplification during divergence into carcinomatous and sarcomatous components. These results support the hypothesis that carcinosarcoma is a monoclonal neoplasm arising from a single stem cell that develops into different cell lineages [3]. However, this did not support the EMT-based hypothesis [1, 2], because *FGFR1* amplification was expected to be present in the sarcomatous component if carcinomatous component (SCC) transitioned to the sarcomatous component.

In conclusion, pulmonary carcinosarcoma in this case exhibited a unique, predominant growth pattern regarded as intrabronchial growth. Histogenesis was investigated using FISH analysis of *EGFR* and *FGFR1,* and it was postulated that cancer stem cells diverged into carcinomatous and sarcomatous components. Mutational analysis of *TP53* did not contribute to the investigation of histogenesis because no mutation was found targeting exons 5-8.

Disclosure of conflict of interest

None.

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