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SHORT REPORT

EGFR and KRAS status of primary sarcomatoid carcinomas of the lung: Implications for anti-EGFR treatment of a rare lung malignancy

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Sarcomatoid carcinomas (SC) of the lung are uncommon malignant tumors composed of carcinomatous and sarcomatous cell components and characterized by a more aggressive outcome than other histological subtypes of nonsmall cell lung cancer (NSCLC). Although epidermal growth factor receptor (EGFR)-targeted therapies have emerged as a promising therapeutic approach in patients with advanced typical NSCLC such as adenocarcinoma, the potential clinical activity of these drugs in lung SC is still unknown. To investigate this point, we have analyzed the status of 4 EGFR pathways biomarkers in a series of lung SC. EGFR protein expression, *EGFR* gene copy number, *EGFR* mutational status and *KRAS* mutational status were assessed in a series of 22 consecutive cases of primary lung SC. EGFR protein overexpression was observed in all the cases. High level of polysomy (≥4 copies of the gene in >40% of cells) was detected in 5 cases (23%). No *EGFR* mutation was detected. *KRAS* mutations were found in 8 patients (38%; Gly12Cys in 6 cases and Gly12Val in 2 cases). The consistent EGFR protein overexpression and the high rate of *KRAS* mutation may contribute to the poorer outcome of lung SC in comparison with typical NSCLC. The rare incidence of increased *EGFR* gene copy number, the lack of *EGFR* mutation and the high rate of *KRAS* mutation observed in our series also suggest that most patients with lung SC are not likely to benefit from anti-EGFR therapies.

Key words: lung cancer; lung sarcomatoid carcinoma; EGFR; KRAS

Sarcomatoid carcinomas (SC) of the lung are a group of rare malignancies, accounting for ~1% of all lung tumors and are defined as poorly differentiated nonsmall cell lung carcinomas (NSCLC) containing a component with sarcoma or sarcoma-like (spindle and/or giant cell) features.[1] SC predominantly occurs in heavily smoking males, with an average age at diagnosis of 60 years, and are characterized by an aggressive clinical course.[1] Indeed, recent studies focusing on the clinical outcome of SC have reported significantly worse survival and higher recurrence rates in comparison with patients with other histological subtypes of NSCLC.[2,3]

During the last few years, significant advances in the development of new molecularly targeted agents have been made in the field of NSCLC. These agents aim to inhibit specific pathways and key molecules involved in tumor growth and progression. One example of such targets is the epidermal growth factor receptor (EGFR), a tyrosine kinase receptor that belongs to the ErbB family. EGFR is overexpressed in many human epithelial malignancies, including NSCLC[4,5] where this expression appears to be associated with poor survival.[6] Following stimulation by its natural ligands, this receptor initiates signal transduction cascades that promote cell division, migration and angiogenesis, and inhibit apoptosis. Several molecules have been synthesized that inhibit the EGFR tyrosine kinase domain.[7,8] Gefitinib and erlotinib are small molecules that reversibly target EGFR. These inhibitors produce objective response in about 12–27% of unselected patients with NSCLC.[9–12] Erlotinib has also been shown to improve the survival of patients with advanced NSCLC after a first- or second-line chemotherapy in a multicenter randomized placebo-controlled trial conducted by the National Cancer Institute of Canada.[12] Therefore, this drug has been approved by both the Food and Drug Administration and the European Medicines Agency as monotherapy for the treatment of patients with locally advanced or metastatic non-NSCLC after failure of at least 1 prior chemotherapy regimen.

An important issue concerning EGFR-targeted therapy in NSCLC is the selection of patients who will most likely benefit from EGFR inhibition. There are several lines of evidence indicating that biomarkers such as EGFR protein expression,[13,14] *EGFR* gene copy number,[13,15–20] *EGFR* mutations[13,15,21–28] and *KRAS* mutations[22,28–30] may be used to predict which patients with NSCLC will respond to EGFR tyrosine kinase inhibitors.

Because of its rarity, specific data about the clinical activity of EGFR targeted therapies in lung SC are almost nonexistent. Moreover, there are no data about the EGFR and KRAS status in lung SC. Therefore, whether EGFR inhibitor therapy might be effective in patients with lung SC is still unknown. We report here the first study analyzing EGFR protein expression, *EGFR* gene copy number, *EGFR* mutations and *KRAS* mutations in a series of lung SC.

Material and methods

Patients

Between 1998 and 2007, tumor specimens from 22 consecutive patients with lung SC, who underwent surgery for excision of a primary tumor, were analyzed in the Pathology Department of the University Hospital of Nice, France. All slides from the lung resection specimens were reviewed blindly by 2 pathologists (VH and PH). In all the cases, the diagnosis of lung SC was established according to the World Health Organization classification of tumors.[1]

EGFR *protein expression evaluation*

EGFR protein expression was evaluated by immunohistochemistry (IHC) using methods described elsewhere,[31] with the immunohistochemical system kit EGFR pharmDx (DakoCytomation, Carpinteria, CA) on freshly cut, formalin-fixed, paraffin-embedded

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tissue from the most representative block with adequate viable tumor area (avoiding areas of necrosis). The cell lines, HT-29 and CAMA-1, supplied by the manufacturer as positive and negative controls were pelleted, formalin fixed and paraffin embedded. These sections were analyzed by 2 observers using light microscopy. The observers were unaware of the clinicopathological details. Tumors were considered positive for EGFR when at least 10% of the tumor cells had membranous staining.[17,31] Cytoplasmic staining without associated membrane staining was reported as negative. Staining intensity was defined as negative (0), weak (1+), moderate (2+) or strong (3+).

EGFR gene copy number evaluation

EGFR gene copy number per cell was investigated by FISH on paraffin-embedded sections from the lung resection specimens and metastasectomies. The procedure for deparaffinization and FISH was done as described previously.[31] The 5-μm sections were dewaxed for 3 × 10 min in xylene, washed in 100% ethanol, air dried and incubated in 2 × sodium saline citrate (SSC) at 75°C for 20 min and a proteinase K solution (Roche, Meylan, France) (20 U/ml in 2 × SSC) at 45°C for 50 min. Then, they were washed in 2 × SSC for 5 min at room temperature and stored in 70% ethanol at 4°C before hybridization with the LSI *EGFR* SpectrumOrange/CEP 7 SpectrumGreen probe (Vysis, Abbott Laboratories, Rungis, France) according to the manufacturer's instructions. At least 100 nonoverlapping interphase nuclei per slide were analyzed, independently, by a single observer who was unaware of the patients' clinical characteristics. Patients were classified into 2 strata: (i) FISH-negative, with no or low genomic gain (<4 copies of the gene in >40% of cells) or (ii) FISH-positive with either a high level of polysomy (≥4 copies of the gene in >40% of cells) or with gene amplification. Gene amplification was defined by the presence of tight gene clusters, and a gene/chromosome per cell ratio ≥2 or ≥15 copies of the gene per cell in ≥10% of analyzed cells.[13,17,31]

EGFR mutational status

For mutation detection, DNA was extracted from the areas of fresh formalin fixed, paraffin-embedded tumor sections. *EGFR* mutations were detected using PCR-based direct sequencing of exons 18–21 using primers and annealing conditions as described by Pao *et al*.[32] Aliquots of PCR products were examined by electrophoresis on 2% agarose gel containing ethidium bromide. PCR products were treated with 2 μl ExoSAP-IT (GE Healthcare) at 37°C for 15 min followed by the inactivation at 80°C for 15 min and directly sequenced using Applied Big Dye Terminator v1.1 cycle sequencing method on ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

KRAS mutational status

KRAS mutations screening was first performed using PCR amplification and direct sequencing of exon 1, containing mutation ''hotspots'' at codons 12 and 13, as described elsewhere.[33] All mutations were confirmed by a second independent PCR and sequencing reaction. Mutant-enriched PCR (ME-PCR) was also performed on all samples, as described by Gormally *et al*.[34] This method allows the detection of low levels of mutant DNA against a background of wild-type sequences. Briefly, 2 successive restriction fragment length polymorphism analyses were performed, leading to enrichment in mutant sequence. Primers and conditions have been published elsewhere.[34,35] Extensive measures were taken to prevent the cross contamination of samples. To avoid false positive results generated during successive PCR rounds, all analyses were repeated twice. Results were scored as ''positive'' or ''negative'' with respect to established cutoff sensitivity values as described by Gormally *et al*.[34] Briefly, DNA from SW480 cells (containing *KRAS* mutation at codon 12) was diluted in increasing proportion of wild-type DNA and used as a standard. *KRAS* codon 12 ME-PCR could detect up to 0.1% of mutant DNA in wild-type DNA. However, no systematic attempt was made to obtain a quantitative assessment of the amount of gene mutation present in each sample.

TABLE I – PATIENTS CHARACTERISTICS (*N* = 22)

		No. of patients	%
Age at diagnosis (years)			
Median	59		
Range	49–84		
Sex			
Male		15	68.0
Female		7	32.0
TNM* stage at diagnosis			
IB		6	27.0
IIA		3	13.5
IIB		3	13.5
IIIA		6	27.0
IIIB		2	9.0
IV		2	9.0

*UICC International Union Against Cancer. TNM classification of malignant tumours. 6th ed. New York: Wiley-Liss; 2002.

Results

Patients

The clinical and histological characteristics of the 22 lung SC are described in Table I. All the patients had a history of smoking.

EGFR *protein expression*

Using IHC, we found EGFR overexpression in all the cases (Fig. 1). The average percentage of the stained cells was 66% (range 35–90). Staining was weak in 3 cases (14%), moderate in 13 cases (59%) and strong in 6 cases (27%).

EGFR gene copy number

By using FISH analysis, high level of polysomy (≥4 copies of the gene in >40% of cells) was detected in 5 cases (23%) (Fig. 1). *EGFR* amplification (>10 copies/cell) was detected in none of the cases. Therefore, 5 cases (23%) were considered as FISH positive and 17 (77%) as FISH negative.

EGFR mutation analysis

Results for the mutation analysis for *EGFR* exons 18, 19, 20 and 21 were obtained in 19 cases. No *EGFR* mutation was detected. No result could be obtained in 3 cases.

KRAS mutation analysis

Mutation analysis for *KRAS* exon 2 was available in 21 cases. *KRAS* mutations were found in 8 patients (38%). In 2 cases, the mutation was detected only by ME-PCR. The mutations were Gly12Cys in 6 cases and Gly12Val in 2 cases. We have failed to obtain a result in 1 case. *KRAS* mutations and increase in *EGFR* gene copy number (high level of polysomy) were mutually exclusive.

Discussion

SC of the lung is a rare aggressive disease characterized by a high rate of distant recurrence after initial surgery for localized disease.[2,3] There is presently no available data indicating the optimal management of patients with advanced disease. In particular, the role of chemotherapy remains unclear and these patients are often treated according to the results of trials having enrolled patients with lung squamous cell carcinoma or adenocarcinoma. The inhibitors of EGFR, such as erlotinib, are widely used for the treatment of advanced patients with NSCLC in the 2nd or 3rd line setting. However, whether these drugs have some activity in lung SC is still unknown. By assessing the status of EGFR and *KRAS* in lung SC, our study provides important information about the potential role of anti-EGFR targeted therapies for patients with advanced SC.

In our series, EGFR was overexpressed in 100% of the cases. This proportion is higher than that reported in series of typical NSCLC using similar scoring system (57–67%).[17,30] Since EGFR overexpression has been associated with poor prognosis in typical NSCLC,[6] this result may represent a biological explanation for the

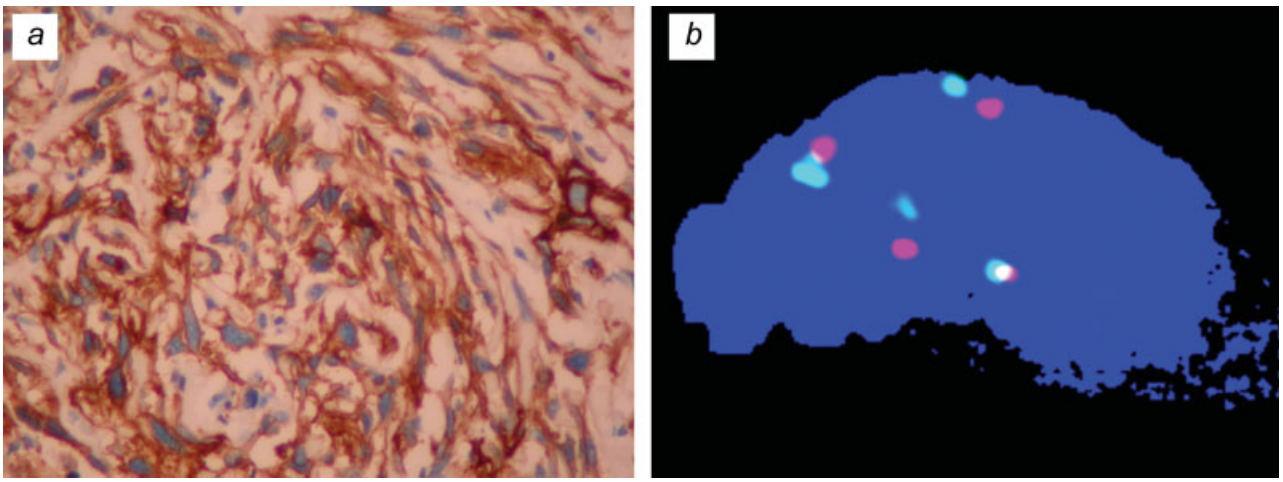

Figure 1 – EGFR protein expression (*a*) and gene copy number (*b*) in a primary lung sarcomatoid carcinoma. (*a*) EGFR immunohistochemistry (×200): strong membranous epidermal growth factor receptor (EGFR)-positive staining (score >10% of the tumor cell) in the primary tumor. (*b*) Dual-color FISH assays using *EGFR* (red) and chromosome 7 centromere (green) probes showing polysomy 7 (tetrasomy 7).

poorer outcome of lung SC in comparison with typical NSCLC. However, whether this pattern of EGFR expression represents a rationale for the use of EGFR-targeted therapies in lung SC remains questionable. Indeed, although some authors reported a relationship between EGFR protein expression assessed by IHC and clinical benefit from EGFR tyrosine kinase inhibitors in NSCLC,[13,14] such a correlation was not confirmed by others.[11,36,37] Therefore, the predictive value of EGFR IHC in NSCLC care remains to be validated.

In our series, the proportion of patients with primary tumors that were *EGFR* positive as assessed by FISH (23%) was lower than that observed in other studies investigating typical NSCLC (45–59%) and using the same scoring system.[17,20,31] We and others have previously shown that the correlation between increased *EGFR* gene copy number per cell and EGFR protein expression is not absolute in lung squamous cell carcinoma and adenocarcinoma.[5,31] Our additional results concerning lung SC underscores the potential crucial role, in this group of tumors, of mechanisms controlling EGFR expression unrelated to the number of genes per cell, such as post-transcriptional regulation. Moreover, none of the cases included in our series harbored *EGFR* mutations. This result is consistent with previous data indicating that *EGFR* mutations are mainly observed in lung adenocarcinomas from patients with no or limited exposure to cigarette smoking.[32] Several studies have shown that increased *EGFR* gene copy number by FISH and/or *EGFR* gene mutations are reliable markers for the prediction of clinical benefit from anti-EGFR drugs such as gefitinib, erlotinib, or cetuximab in typical NSCLC.[13,15–28] The rare occurrence of these molecular events in lung SC suggests that these tumors are not ideal candidates for anti-EGFR therapy. This is consistent with the high rate of *KRAS* mutations observed in our series (38%). Indeed, such mutations, which are found in about 20% of typical NSCLC (mainly adenocarcinomas), are predictive of resistance to erlotinib or gefitinib.[29,30] Although some data are controversial,[38] *KRAS* mutations have also been associated with poor prognosis in resected typical NSCLC.[39–41] The high rate of *KRAS* mutations in lung SC may therefore contribute to the aggressive outcome of these tumors.

In summary, our study has demonstrated that lung SC are characterized by a consistent EGFR protein overexpression, a low rate of increased *EGFR* gene copy number, a lack of *EGFR* mutation and a high rate of *KRAS* mutations. The frequent EGFR overexpression and *KRAS* mutations may contribute to the poorer outcome of this rare lung malignancy than in typical NSCLC such as squamous cell carcinoma or adenocarcinoma. The rare incidence of increased *EGFR* gene copy number, the lack of *EGFR* mutation and the high rate of *KRAS* mutation observed in our series also suggest that most patients with lung SC are not likely to benefit from anti-EGFR therapies.

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