Epidermal Growth Factor Receptor Expression in Spindle Cell Carcinomas of the Head and Neck

R. F. Watson · R. D. Chernock · K. H. Zhang ·
L. S. Michel · D. R. Adkins · S. K. El-Mofty ·
J. S. Lewis Jr.

Received: 2 November 2014 / Accepted: 30 December 2014 / Published online: 7 January 2015
© Springer Science+Business Media New York 2015

Abstract Spindle cell carcinoma (SpCC) is an uncommon head and neck squamous cell carcinoma (SCC) variant consisting of spindled and/or pleomorphic cells with epithelial differentiation. Epidermal growth factor receptor (EGFR) is expressed by >90 % of conventional SCC, and high level expression is associated with a poorer prognosis. Anti-EGFR therapies are commonly used to treat head and neck SCC. However, no studies have evaluated EGFR expression in SpCC. Cases of SpCC were retrieved from department files. The diagnosis required either a biphasic lesion with a squamous neoplastic component, or a purely spindle cell or pleomorphic tumor with immunohistochemical positivity for epithelial markers. EGFR immunohistochemistry was performed and was quantified in quartiles. Medical records were reviewed for clinical follow up information. EGFR was expressed in 21/30 (70 %) cases, including in the squamous component in 18/19 (95 %) and the spindle cell component in only 12/30 (40 %). Where the spindle cell component was positive,

the intensity and distribution were lower than for the squamous component. Recurrent tumors were predominantly (80–90 %) of the spindle cell component, and had low (or absent) EGFR expression. Kaplan–Meier survival analysis showed no statistically significant differences in overall or disease free survival between the EGFR expressing and non-expressing groups ($p = 0.414$ and 0.19 , respectively). SpCCs of the head and neck have a poor prognosis, and markedly reduced EGFR expression. EGFR-specific therapies may not be ideal for SpCC patients, which may lack EGFR expression, but further studies are needed.

Keywords Spindle cell carcinoma · Sarcomatoid carcinoma · Epidermal growth factor receptor · Immunohistochemistry · Cetuximab

Introduction

Sarcomatoid carcinoma is an unusual, histologically diverse tumor type. It is common in the head and neck as a variant of squamous cell carcinoma (SCC), and are specifically referred to as spindle cell carcinoma (SpCC) when arising there [1–5]. The tumors frequently present as exophytic, polypoid masses, particularly when arising in the larynx and hypopharynx, and are classically associated with a biphasic histologic appearance combining conventional SCC and spindle cell (or sarcomatoid) components [6]. A minority of cases display only the spindle cell component for which immunohistochemistry for epithelial markers such as cytokeratin and p63 are critical in differentiating them from true sarcomas and other tumors like melanoma [3, 5, 7, 8]. Molecular and ultrastructural studies have clearly shown that the spindle cell/sarcomatoid

Electronic supplementary material The online version of this article (doi:10.1007/s12105-014-0604-y) contains supplementary material, which is available to authorized users.

R. F. Watson · R. D. Chernock · S. K. El-Mofty ·
J. S. Lewis Jr. (✉)
Pathology and Immunology, Washington University
School of Medicine, 660 S. Euclid Ave.,
Campus Box 8118, St Louis, MO, USA
e-mail: jlewis@path.wustl.edu

R. D. Chernock · S. K. El-Mofty · J. S. Lewis Jr.
Otolaryngology Head and Neck Surgery, Washington University
School of Medicine, St Louis, MO, USA

K. H. Zhang · L. S. Michel · D. R. Adkins
Division of Oncology, Department of Internal Medicine,
Washington University School of Medicine, St Louis, MO, USA

component of SpCCs is derived from the same clone as the epithelial component and thus represents divergent differentiation [3, 9, 10, 11, 12]. Although these tumors have both unique macroscopic and microscopic appearances, the demographics are very similar to conventional SCCs including the major risk factors smoking and alcohol use [1–3, 5, 13]. SpCCs do, however, occur much more frequently in sites of previous radiation therapy (as many as 15–20 % of cases) and only rarely harbor high risk human papillomavirus (HPV), regardless of the head and neck subsite involved [1–5, 7, 8, 14]. Based on a limited number of studies, early, polypoid SpCC appear to do well clinically, while higher stage tumors tend to do poorly compared to similarly staged conventional SCC [5, 13, 14]. The prognosis for SpCC patients, overall, though, appears to be similar to conventional SCCs.

While SCC is the most common malignancy in the head and neck, there is increasing recognition of the morphologic and molecular heterogeneity within it. Large numbers of studies have shown that high risk HPV, especially in the oropharynx, acts as a causative agent in SCC by suppression of the p53 and retinoblastoma proteins by the viral oncoproteins E6 and E7 [15, 16]. These HPV-related oropharyngeal SCCs also have improved prognosis when compared to their HPV negative counterparts, with HPV status clearly shown to be an independent risk factor [16, 17, 18]. In addition to HPV-related carcinogenic pathways, other drivers of development and growth for SCCs are the epidermal growth factor receptor (EGFR), transforming growth factor β (TGF- β), and phosphatidylinositol-3-kinase (PI3K)/AKT pathways [19]. EGFR belongs to the ERBB family of receptor tyrosine kinases signaling through the Ras-MAPK, PI3K-AKT-PTEN, and phospholipase C pathways [20]. Numerous studies have shown that not only is EGFR overexpressed in the majority of head and neck SCCs, but its overexpression is also associated with worse prognosis [21–25]. Based on these findings, cetuximab became the first targeted monoclonal antibody therapy against EGFR to be approved for use in head and neck SCCs. In combination with radiotherapy or cisplatin-based chemotherapy, cetuximab improves loco-regional control and overall survival [26, 27]. Several other EGFR-related targeted agents are in development [28].

There is abundant data on EGFR expression in conventional SCC which suggests that overexpression is prognostically adverse, although this is not a consistent finding [29]. Expression of EGFR and downstream molecules also has been suggested to predict treatment response to EGFR-targeted therapies, particularly in cell lines, but no consistent relationship between EGFR expression or mutation and treatment response for patients in routine clinical practice has been identified [30]. There is no published data on EGFR expression in head and neck

SpCCs. In this study, we evaluated a series of well-characterized head and neck SpCC for EGFR expression by immunohistochemistry and correlated results with clinical and pathologic features and patient outcomes.

Materials and Methods

Study Population

This study was approved by the Human Research Protection Office of Washington University. The pathology files of Barnes-Jewish Hospital were searched from 1988 to 2009 for cases of head and neck tumors diagnosed as “spindle cell carcinoma”, “sarcomatoid carcinoma,” or “SCC with a spindle cell component.” All biopsy and/or surgical resection specimen slides were retrieved and reviewed by two study pathologists (RFW and JSL). Representative slides from each case were subsequently independently reviewed, and the diagnosis confirmed by two other study pathologists with expertise in head and neck pathology (RDC and SEM). Cases were included only if they met the current 2005 World Health Organization definition [6] under which tumors either consist of a combination of conventional SCC and SpCC components or consist only of a spindle cell or pleomorphic, sarcomatoid tumor along a head and neck mucosal site for which there was clear demonstration of epithelial differentiation by immunohistochemistry. The latter tumors had to show positivity for at least one epithelial marker (pan-cytokeratin AE1/AE3, p63, or epithelial membrane antigen). All immunohistochemistry for epithelial differentiation was performed as part of the original workup of these tumors (i.e. no additional immunohistochemistry was performed as part of this study). In addition, pathology specimens from a recurrence or metastasis to any site which were obtained by biopsy or resection were collected and reviewed by two study pathologists (RFW and JSL) to confirm the diagnosis and determine the morphologic pattern of the recurrent/metastatic tumor.

All clinical information including basic demographics, treatment data, clinical follow up, and survival information was obtained by review of the patients’ paper and/or electronic medical records.

Immunohistochemistry

Immunohistochemistry was performed on 4 μ m sections from formalin-fixed, paraffin-embedded tissue blocks using the VECTASTAIN[®] Elite ABC Kit following manufacturer’s instructions using a monoclonal antibody to EGFR (Cell Signaling; clone D38B1; monoclonal; 1:100 dilution). Included in the VECTASTAIN[®] Elite ABC Kit was

a secondary antibody cocktail to detect binding of the primary antibody, and this complex was subsequently visualized using hydrogen peroxide substrate and a 3,3'-diaminodenzidine tetrahydrochloride (DAB) chromogen. Antigen retrieval was performed by boiling for 15 min in citrate buffer. Blocking was performed using normal donkey serum. EGFR cytoplasmic positivity in the basal cell layer of normal squamous epithelium on a separate positive control and also in the surrounding non-neoplastic squamous epithelium of the tumor sections was used as an internal positive control.

All primary tumors were immunostained as were the tumor specimens from recurrences and metastases, when they were biopsied or surgically resected. The distribution of staining was graded in quartiles as follows: 0 = negative, 1+ = 1 to 25 % of cells positive, 2+ = 26 to 50 % of cells positive, 3+ = 51 to 75 % of cells positive, and 4+ = 76 to 100 % of cells positive. Intensity of staining was graded subjectively in tertiles as follows: 0 = negative, 1 = weak, 2 = moderate, and 3 = intense. The antibody used does not recognize nuclear EGFR so no nuclear staining was observed. For study purposes, cases were considered EGFR positive if at least 1 % of tumor cells showed any intensity of staining. All EGFR immunostains were reviewed by two of the study pathologists (RFW and JSL), and discrepancies were resolved by consensus review.

Statistical Analysis

Statistical analyses were performed using SPSS statistics software version 20.0 (SPSS, Chicago, IL, USA) and GraphPad Prism (GraphPad Prism version 5.00 for Windows, San Diego, CA, USA). Student's *t* test was used in the comparison of continuous variables while Fisher's exact was used for categorical variable. Survival was estimated using the Kaplan–Meier method comparing the EGFR expressing and non-expressing tumor groups. Overall survival was defined as time from initial diagnosis to date of death, and disease-free survival was defined as time from initial diagnosis to date of first tumor recurrence or death.

Results

The basic demographic and pathologic information for the 31 identified patients is summarized in Table 1. Overall, the demographic features were comparable to those reported in conventional SCC and other SpCC series, being predominantly Caucasian men in their 50s, 60s, or 70s with a history of smoking and/or alcohol use (over 95 % of patients). Nine (36 %) of the 25 of the patients with information available had a

Table 1 Demographic features and pathologic staging of the entire patient cohort

Demographics (n = 31)	
Age range (years)	39–82 (mean = 64.4)
Gender	
Male	25 (80.6 %)
Female	6 (19.4 %)
Male:female ratio	4:1
Race	
Caucasian	25 (80.6 %)
African–American	5 (16.1 %)
Other	1 (3.3 %)
Risk factors ^a	
Tobacco use (n = 24)	23 (95.8 %)
Alcohol use (n = 22)	21 (95.5 %)
History of radiation (n = 25)	6 (24.0 %)
History of prior SCC (n = 25)	9 (36.0 %)
Location	
Oral cavity	12 (38.7 %)
Oropharynx	5 (16.1 %)
Larynx	11 (35.5 %)
Hypopharynx	3 (9.7 %)
Clinical stage at diagnosis	
I	7 (22.6 %)
II	6 (19.4 %)
III	1 (3.2 %)
IV	12 (38.7 %)
Not staged	5 (16.1 %)
Lymph node metastasis	6 (19.4 %)
Distant metastasis	0

SCC squamous cell carcinoma

^a Adequate clinical documentation of risk factors was available only in 25 patients

previous history of conventional SCC of the head and neck, and 6 (24 %) had a history of prior radiation to the head and neck. The most common primary site was the oral cavity (38.7 %) followed closely by the larynx (35.5 %). Histologically, 19 (59.4 %) of the tumors were biphasic with both spindle cell and conventional SCC components, or at least with a component of squamous dysplasia (Fig. 1a, b). The spindle cell components ranged in appearance from cases with bland, monomorphic cells in fascicles to those with highly pleomorphic morphology similar to that of undifferentiated pleomorphic sarcoma. Only one case had focal areas that resembled a defined sarcoma, in that case, an osteosarcoma. All eight monophasic tumors (spindle cell component only) were positive for at least one epithelial marker by immunohistochemistry. Six of the 8 were positive for pan-cytokeratin (AE1/AE3) or high molecular weight cytokeratin (34βE12), 3 of 8 for p63, and 1 of 8 for epithelial

Fig. 1 Histologic features of SpCC and EGFR immunohistochemistry. **a** A classically biphasic SpCC with both conventional SCC and spindle cell components (H&E; $\times 100$ magnification). **b** The same SpCC showing a keratinizing-type squamous cell carcinoma (*left side*) and a pleomorphic, spindle cell component (*right side*) with variably-sized and shaped, hyperchromatic nuclei with prominent

fibrous stroma and prominent mitoses (H&E; $\times 400$). **c** The tumor shows diffuse cytoplasmic positivity for EGFR in the conventional SCC component ($\times 400$). **d** The tumor shows almost absent EGFR staining with both low distribution and intensity ($\times 400$). *SpCC* spindle cell carcinoma, *SCC* squamous cell carcinoma, *EGFR* epidermal growth factor receptor

membrane antigen. Complete staging information was available in 26 patients. The majority had high stage (either III or IV) disease. Lymph node metastases were present at presentation in 6 (19.4 %) patients. None of the patients had distant metastases at presentation.

EGFR immunohistochemistry was performed in all 31 cases, and the results are summarized in Table 2, and results for each case are shown in supplementary Table 1. One case was excluded due to the lack of an adequate internal positive control. Considering the tumors as a whole, the majority (21/30; 70 %) were positive for EGFR expression, with 12 out of the 21 cases showing positivity in at least 50 % of the tumor cells and 17 of the 21 (81 %) positive cases showing at least moderate (2+) staining intensity. The spindle cell component was only positive in 12 of the 30 (40 %) cases, however. There were 11 tumors consisting only of a spindle cell component, and only 3 (27 %) of these were positive for EGFR. For the 19 histologically biphasic tumors, EGFR expression was evaluated separately in the conventional SCC and spindle cell components. Eighteen of these 19 (95 %) biphasic cases were EGFR positive in at least one component. The SCC

component was positive in all 18 of them, but the spindle cell component was positive in only 9 of the 18 (50 %). In addition to the spindle cell components being less often positive for EGFR than the SCC components, the distribution and staining intensity in the spindle cell components were also lower (1.75 vs. 2.78 for distribution and 1.92 vs. 2.22 for intensity, respectively; Fig. 1c, d).

In total, 9 out of the 31 patients had a recurrence or metastasis for which a biopsy or resection specimen was available in our pathology files and with adequate material for characterization. Histologic review of these showed that 7 of 9 (78 %) were purely spindle cell/sarcomatoid. Of the two cases that were not purely sarcomatoid, one was mixed, with the majority (70 %) being spindle cell, and the other showed only the conventional SCC component. Immunostaining for EGFR was performed and showed the SCC component of the two cases that had one to be strongly and diffusely EGFR positive. The spindle cell component was positive in all 8 of the cases that had one, but distribution and intensity were low in 5 of the 8. Specifically, most had less than 50 % of the cells positive with an intensity of 1+ or 2+ only. Interestingly, 6 of the 8

Table 2 EGFR immunohistochemistry results

	Primary tumors (n = 30)	Recurrent tumors ^a (n = 9)
<i>Overall</i>		
Positive	21 (70 %)	9 (100 %)
Distribution		
1+	3 (14.3 %)	4 (44.4 %)
2+	5 (23.8 %)	1 (11.1 %)
3+	8 (38.1 %)	2 (22.2 %)
4+	5 (23.8 %)	2 (22.2 %)
Intensity		
1+	4 (19.1 %)	2 (22.2 %)
2+	7 (33.3 %)	4 (44.4 %)
3+	10 (47.6 %)	3 (33.3 %)
Negative	9 (30 %)	0
<i>By component</i>		
EGFR epidermal growth factor receptor		
Spindle cell (n = 30)	12 (40 %)	8 (100 %)
Distribution (mean)	1.75	2
Intensity (mean)	1.92	1.75
Conventional (n = 19)	18 (94.7 %)	2 (100 %)
Distribution (mean)	2.78	3.5
Intensity (mean)	2.22	3

^a Results are for patients whose tumor recurred and was biopsied or surgically resected so had tissue available for review and immunohistochemical staining

SpCCs for which the EGFR status could be evaluated were completely negative on the spindle cell component of the primary tumor but were then positive for EGFR on the recurrence.

Clinical demographics, treatment, and outcomes for all cases by EGFR status are presented in Table 3. Aside from the EGFR negative patients being slightly older than the EGFR positive patients (61.9 vs. 68.9 years, $p = 0.036$), no statistically significant differences were seen by sex, race, location of primary tumor, or stage at diagnosis between the EGFR positive and negative cases. Surgery or surgery in combination with radiation therapy was the most common primary treatment modality (78 %). The mean and median follow up times were 48.2 and 18.8 months. Overall, of the 25 patients who underwent curative intent therapy, 16 (64 %) patients suffered disease recurrence of which 13 (52 %) were local, 8 (32 %) regional, and 6 (24 %) distant. Although limited by sample size, there were minimal differences in outcomes by EGFR status, with similar rates of patients in both EGFR positive and negative groups showing regional failure (29.4 vs. 37.5 %, EGFR positive vs. negative) and distant metastases (27.8 vs. 25.0 %, EGFR positive vs. negative). There was a slightly higher rate of local recurrence in patients with EGFR positive tumors versus those that were negative (58.8 vs. 37.5 %), but small sample size precluded statistical characterization of this difference. Disease status at the end of the follow period was also similar between groups. Considering just the patients with tumors that were EGFR negative versus all other patients, 8 of 15 (53 %) patients with an EGFR negative SpCC tumor or tumor component suffered disease recurrence versus 10 of 12 (83 %) patients with an EGFR positive SpCC tumor or tumor component. This suggests that although the spindle cell component is more often EGFR completely negative, that this does not predict disease recurrence/progression.

Kaplan–Meier survival analysis was performed comparing both groups, and this showed no significant differences in overall ($p = 0.414$; Fig. 2a) or disease specific ($p = 0.19$; Fig. 2b) survival, respectively. In further analysis, overall and disease specific survival between patients with EGFR positive spindle cell components were compared to patients with EGFR negative ones (ignoring the EGFR status of the conventional SCC component, if present), and again, no significant differences were seen ($p = 0.18$ and 0.54 , respectively; Fig. 2c, d).

We also examined the medical records for whether or not patients received EGFR inhibitor therapy. Five patients did receive cetuximab during the course of their cancer therapy and one received gefitinib. The EGFR-targeted therapy was used in two patients for primary treatment of their disease in conjunction with radiotherapy while the remaining four patients received treatment for either recurrent or metastatic disease after traditional radiation ($\pm$ chemotherapy) was utilized. Interestingly, all of the patients who received EGFR-targeted therapy had tumors which were positive for EGFR in at least one component of their tumors, although this information was not known to the clinicians at the time of treatment. However, among these six patients, three had tumors with a spindle cell

Table 3 Clinical characteristics, treatment, and outcomes by EGFR status

Clinical characteristics	EGFR positive (n = 21)	EGFR negative (n = 9)	p value
Age (mean)	61.9	68.9	0.036*
Male sex	19 (90.5 %)	6 (66.7 %)	0.14
Caucasian race	17 (81 %)	7 (77.8 %)	1.00
Location			
Larynx/hypopharynx	12 (57.1 %)	2 (22.2 %)	0.12
Oral cavity	6 (28.6 %)	5 (55.6 %)	0.23
Oropharynx	3 (14.3 %)	2 (22.2 %)	0.62
TNM stage			
I	4 (19 %)	2 (22.2 %)	1.00
II	5 (23.8 %)	1 (11.1 %)	0.64
III	0	1 (11.1 %)	0.30
IV	10 (47.6 %)	2 (22.2 %)	0.25
Not staged	2 (9.5 %)	3 (33.3 %)	0.14
Treatment (n = 23) ^a			
Surgery only	7	7	
Surgery with radiation	5	0	
Radiation only	2	0	
Neoadjuvant chemotherapy	0	2	
Information not available	7	0	
Anti-EGFR therapy (n = 6)			
EGFR positive (either component)	6 (100 %)	0	
EGFR positive (spindle component)	3 (50 %)	0	
Outcomes (n = 28) ^a			
Disease progression			
Local failure	10/17 (58.8 %)	3/8 (37.5 %)	0.41
Regional failure	5/17 (29.4 %)	3/8 (37.5 %)	1.00
Distant metastasis	5/18 (27.8 %)	2/8 (25 %)	1.00
Overall survival			
Alive, disease free	5/19 (26.3 %)	2/9 (22.2 %)	1.00
Deceased with disease	11/19 (57.9 %)	5/9 (55.6 %)	1.00
Deceased, disease free	3/19 (15.8 %)	2/9 (22.2 %)	1.00

EGFR epidermal growth factor receptor

^a Adequate clinical follow up information was available in 28 patients. The remaining two patients were lost to follow up immediately after primary diagnosis. Specific treatment information was available on 23 patients

* *p* values less than 0.05 were considered statistically significant

component that was completely EGFR negative. Additional clinical follow up for these six patients, including clinical responses based on the Response Criteria in Solid Tumors (RECIST) criteria, is presented in Table 4. Briefly, they presented with a wide age range and sites of primary tumor. Despite EGFR-targeted therapy, four out of the six patients either had progression or only a partial response to therapy with time to progression ranging from 84 to 155 days. All four patients died with disease within 2 years of diagnosis. Of the remaining two patients, one died shortly after treatment initiation due to complications unrelated to malignancy (patient was “censored”). However, one patient treated with EGFR-targeted therapy was alive and disease free at last follow up of almost 5 years. He had a p16 and HPV negative, strongly EGFR positive (both components), stage IVA (T1/N2b/M0) biphasic SpCC arising in the oropharynx that was treated with

primary surgery with post-operative radiation and cetuximab. Time to progression for the three patients who received anti-EGFR therapies for which the tumors had a spindle cell component that was completely EGFR negative was, on average, 84 days, versus those three patients whose spindle cell component was EGFR positive, was 131 days.

Discussion

Spindle cell (sarcomatoid) carcinoma is a histologically diverse and uncommon variant of head and neck SCC with focal to extensive mesenchymal differentiation [14, 31]. They are histologically very aggressive appearing, yet prior studies on the outcome of SpCC suggest that rates of recurrence and metastases are comparable to conventional

Fig. 2 Kaplan–Meier survival analysis of spindle cell carcinoma by EGFR status and component EGFR status. **a** Overall survival by EGFR status. **b** Disease-free survival by EGFR status. **c** Overall survival by the EGFR status of just the spindle cell component. **d** Disease-free survival by the EGFR status of just the spindle cell component. *EGFR* epidermal growth factor receptor

Table 4 Outcomes in EGFR positive cases treated with tyrosine kinase inhibitors

	Age	Sex	Race	Site	Stage	Best response (RECIST)	Outcome
EGFR positive—both components							
Patient 14 ^a	62	M	C	Oropharynx	IV	Complete	Alive w/o disease, f/u 79.2 mo
Patient 17	59	M	AA	Oral cavity	IV	Partial	Deceased w/ disease, f/u 10 mo
Patient 6	56	M	C	Larynx	IV	Progression	Deceased w/ disease, f/u 14 mo
EGFR positive—conventional component only							
Patient 21	82	M	C	Oral cavity	N/A	Partial	Deceased 6 months after diagnosis, likely with disease
Patient 15	39	M	C	Larynx	I	Progression	Deceased w/ disease, f/u 18.8 mo
Patient 18	76	F	C	Larynx	II	Censored ^b	Deceased w/ disease, f/u 22.2 mo

EGFR epidermal growth factor receptor, *mo* months, *w/* with, *w/o* without, *f/u* follow up, *C* caucasian, *AA* African–American, *RECIST* response evaluation criteria in solid tumors

^a Patient numbers correlate to those in supplemental Table 1 where the raw EGFR expression data are presented

^b Patient died of non-cancer pulmonary disease shortly after starting tyrosine kinase inhibitor therapy

SCC, at least across all stages of disease [1, 3, 5]. The current study finds overall and disease free survival to be quite poor for SpCC patients, with only 25 % of patients alive at last follow up.

EGFR is an extensively studied indicator of poor prognosis in head and neck SCCs and has been found to be expressed by tumor cells in the vast majority of cases including being markedly overexpressed in many of them [21–25]. Very few (<5 %) conventional SCC of the head and neck show completely absent EGFR expression [32, 33]. EGFR overexpression by immunohistochemistry or by FISH showing amplification has been associated with poorer

prognosis in conventional SCC, but neither of these findings has been associated with treatment response to cetuximab [33, 34]. Activation of EGFR leads to signaling through three separate intracellular pathways including the Ras/MAPK, PI3K/AKT, and Jak2/STAT3 pathways contributing to increased proliferation, invasion and metastasis, resistance to apoptosis, and stimulation of angiogenesis [34, 35]. These findings have led to the development and use of monoclonal antibody-directed and small molecule inhibitor therapy against EGFR in combination with conventional treatment modalities. Anti-EGFR directed therapies have been shown to improve both overall survival and

loco-regional control in many clinical trials for conventional SCC [26, 27].

To date, despite the importance of EGFR in tumor prognosis and treatment, there has not been a study to address the immunohistochemical expression of EGFR in SpCC. This is surprising as tumors with sarcomatoid morphology lose their epithelial characteristics so should be very likely to lose EGFR expression, especially since this has been suggested in tumors with epithelial to mesenchymal transition. In this study, we found that 30 % of SpCCs completely lacked EGFR expression. This rate is significantly more than what would be expected in conventional SCC [33]. No significant differences were found in the treatment of these patients, although six patients did receive targeted anti-EGFR therapy. Although limited by the relatively small number of patients, survival analysis showed no difference in overall survival by EGFR status ($p = 0.414$). Although there was trend toward possible poorer outcomes in disease-free survival in EGFR positive patients, this was not statistically significant ($p = 0.19$). Separate analysis of survival by just the EGFR expression of the spindle cell component alone also did not demonstrate any statistically significant differences in overall or disease-free survival, either. These findings suggest that, in contrast to conventional SCC, where levels of EGFR are prognostic, no such relationship exists for this biomarker in SpCC, although this clearly requires confirmation. Examination of the recurrences and metastases showed that they are predominantly of the spindle cell/sarcomatoid component, suggesting that it is this component that is the more aggressive. Analysis of the EGFR expression in these recurrences showed that all of these recurrences showed at least some amount of EGFR expression, although slightly less robust than the primary tumor. This shows that the recurrent tumors are not necessarily those that are completely EGFR negative, again suggesting that it is not the EGFR status that is driving the behavior and prognosis, in and of itself. The situation may simply be complicated by the fact that EGFR overexpression is generally an adverse feature, but the aggressive spindle cell component of these tumors, which we found to be the most frequent pattern in the recurrent tumors, generally has lower levels (or completely absent expression) of EGFR, such that, for prognosis, the two may “cancel each other out”.

So even if the behavior and prognosis of SpCC is not dependent on EGFR expression, does the expression correlate with response to anti-EGFR therapies, particularly among those patients whose tumors completely lack EGFR expression? We separately assessed patients who received anti-EGFR therapy. There were three patients who had EGFR expression in both components of their cancers, and three that did not have expression in the spindle cell component. Although these are small numbers, most of

these patients did poorly, similar to the remaining patients in the study. The only patient who remained alive and disease-free at last follow up had a tumor with strong EGFR expression in both tumor components. Thus, the EGFR completely negative patients did not appear to respond as well to anti-EGFR therapies in this study. Although very small numbers, this study does suggest that SpCC do not always lose all EGFR expression in the spindle cell component and thus, may still respond to anti-EGFR targeted therapies. However, our findings also raise the possibility that complete lack of EGFR expression may signify lack of response to anti-EGFR targeted therapies in SpCC patients. Further study is warranted.

In summary, SpCCs appear to be aggressive tumors frequently occurring after head and neck radiation. The majority of SpCCs express EGFR in at least one of the components, but there is a higher fraction of EGFR negative tumors than for conventional head and neck SCC. EGFR expression, when present, is generally low in the spindle cell components. EGFR expression status does not appear to be associated with patient outcomes suggesting that prognosis in these patients is largely dependent on the clinical stage and/or other factors. Analysis of the patients treated with anti-EGFR therapies demonstrated poor responses in the three patients with EGFR completely negative spindle cell components. Therefore, further evaluation of treatment response to anti-EGFR therapies in a larger number of patients with SpCC is warranted.

Acknowledgments We would like to acknowledge Autumn Watson of the Anatomic and Molecular Core Laboratory for her help with sectioning of tumor cases and also Sora Lim of the Michel laboratory for her assistance with the immunohistochemistry.

References

1. Ellis GL, Corio RL. Spindle cell carcinoma of the oral cavity: a clinicopathologic assessment of fifty-nine cases. *Oral Surg Oral Med Oral Pathol.* 1980;50(6):523–33.
2. Leventon GS, Evans HL. Sarcomatoid squamous cell carcinoma of the mucous membranes of the head and neck: a clinicopathologic study of 20 cases. *Cancer.* 1981;48(4):994–1003.
3. Lewis JE, Olsen KD, Sebo TJ. Spindle cell carcinoma of the larynx: review of 26 cases including DNA content and immunohistochemistry. *Hum Pathol.* 1997;28(6):664–73.
4. Olsen KD, Lewis JE, Suman VJ. Spindle cell carcinoma of the larynx and hypopharynx. *Otolaryngol Head Neck Surg.* 1997;116(1):47–52.
5. Thompson LD, Wieneke JA, Miettinen M, et al. Spindle cell (sarcomatoid) carcinomas of the larynx: a clinicopathologic study of 187 cases. *Am J Surg Pathol.* 2002;26(2):153–70.
6. Cardesa A, Zidar N. Spindle cell carcinoma. In: Barnes EL, et al., editors. *World Health Organization pathology and genetics—head and neck tumours.* Lyon: IARC Press; 2005. p. 127–8.
7. Lewis JS, Ritter JH, El-Mofty S. Alternative epithelial markers in sarcomatoid carcinomas of the head and neck, lung, and bladder—p63, MOC-31, and TTF-1. *Mod Pathol.* 2005;18(11):1471–81.

8. Bishop JA, Montgomery EA, Westra WH. Use of p40 and p63 immunohistochemistry and human papillomavirus testing as ancillary tools for the recognition of head and neck sarcomatoid carcinoma and its distinction from benign and malignant mesenchymal processes. *Am J Surg Pathol*. 2014;38(2):257–64.
9. Choi HR, Sturgis EM, Rosenthal DI, et al. Sarcomatoid carcinoma of the head and neck: molecular evidence for evolution and progression from conventional squamous cell carcinomas. *Am J Surg Pathol*. 2003;27(9):1216–20.
10. Takata T, Ito H, Ogawa I, et al. Spindle cell squamous carcinoma of the oral region. An immunohistochemical and ultrastructural study on the histogenesis and differential diagnosis with a clinicopathological analysis of six cases. *Virchows Arch A Pathol Anat Histopathol*. 1991;419(3):177–82.
11. Thompson L, Chang B, Barsky SH. Monoclonal origins of malignant mixed tumors (carcinosarcomas): evidence for a divergent histogenesis. *Am J Surg Pathol*. 1996;20(3):277–85.
12. Zarbo RJ, Crissman JD, Venkat H, et al. Spindle-cell carcinoma of the upper aerodigestive tract mucosa: an immunohistologic and ultrastructural study of 18 biphasic tumors and comparison with seven monophasic spindle-cell tumors. *Am J Surg Pathol*. 1986;10(11):741–53.
13. Viswanathan S, Rahman K, Pallavi S, et al. Sarcomatoid (spindle cell) carcinoma of the head and neck mucosal region: a clinicopathologic review of 103 cases from a tertiary referral cancer centre. *Head Neck Pathol*. 2010;4(4):265–75.
14. Watson RF, Chernock RD, Wang X, et al. Spindle cell carcinomas of the head and neck rarely harbor transcriptionally-active human papillomavirus. *Head Neck Pathol*. 2013;7(3):250–7.
15. Rampias T, Sasaki C, Weinberger P, et al. E6 and e7 gene silencing and transformed phenotype of human papillomavirus 16-positive oropharyngeal cancer cells. *J Natl Cancer Inst*. 2009;101(6):412–23.
16. Ragin CC, Taioli E. Survival of squamous cell carcinoma of the head and neck in relation to human papillomavirus infection: review and meta-analysis. *Int J Cancer*. 2007;121(8):1813–20.
17. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med*. 2010;363(1):24–35.
18. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst*. 2008;100(4):261–9.
19. Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. *Nat Rev Cancer*. 2011;11(1):9–22.
20. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat Rev Cancer*. 2005;5(5):341–54.
21. Grandis JR, Tweardy DJ. Elevated levels of transforming growth factor alpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. *Cancer Res*. 1993;53(15):3579–84.
22. Chung CH, Ely K, McGavran L, et al. Increased epidermal growth factor receptor gene copy number is associated with poor prognosis in head and neck squamous cell carcinomas. *J Clin Oncol*. 2006;24(25):4170–6.
23. Ang KK, Berkey BA, Tu X, et al. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. *Cancer Res*. 2002;62(24):7350–6.
24. Grandis JR, Melhem MF, Gooding WE, et al. Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. *J Natl Cancer Inst*. 1998;90(11):824–32.
25. Maurizi M, Almadori G, Ferrandina G, et al. Prognostic significance of epidermal growth factor receptor in laryngeal squamous cell carcinoma. *Br J Cancer*. 1996;74(8):1253–7.
26. Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med*. 2006;354(6):567–78.
27. Vermorken JB, Mesia R, Rivera F, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. *N Engl J Med*. 2008;359(11):1116–27.
28. Schmitz S, Ang KK, Vermorken J, et al. Targeted therapies for squamous cell carcinoma of the head and neck: current knowledge and future directions. *Cancer Treat Rev*. 2014;40(3):390–404.
29. Keren S, Shoude Z, Lu Z, et al. Role of EGFR as a prognostic factor for survival in head and neck cancer: a meta-analysis. *Tumour Biol*. 2014;35(3):2285–95.
30. Cohen RB. Current challenges and clinical investigations of epidermal growth factor receptor (EGFR)- and ErbB family-targeted agents in the treatment of head and neck squamous cell carcinoma (HNSCC). *Cancer Treat Rev*. 2014;40(4):567–77.
31. Lewis JS Jr. Spindle cell lesions—neoplastic or non-neoplastic? Spindle cell carcinoma and other atypical spindle cell lesions of the head and neck. *Head Neck Pathol*. 2008;2(2):103–10.
32. Burtneess B, Goldwasser MA, Flood W, et al. Phase III randomized trial of cisplatin plus placebo compared with cisplatin plus cetuximab in metastatic/recurrent head and neck cancer: an Eastern Cooperative Oncology Group study. *J Clin Oncol*. 2005;23(34):8646–54.
33. Licitra L, Storkel S, Kerr KM, et al. Predictive value of epidermal growth factor receptor expression for first-line chemotherapy plus cetuximab in patients with head and neck and colorectal cancer: analysis of data from the EXTREME and CRYSTAL studies. *Eur J Cancer*. 2013;49(6):1161–8.
34. Licitra L, Mesia R, Rivera F, et al. Evaluation of EGFR gene copy number as a predictive biomarker for the efficacy of cetuximab in combination with chemotherapy in the first-line treatment of recurrent and/or metastatic squamous cell carcinoma of the head and neck: EXTREME study. *Ann Oncol*. 2011;22:1077–87.
35. Mak MP, William WN Jr. Targeting the epidermal growth factor receptor for head and neck cancer chemoprevention. *Oral Oncol*. 2014;50(10):918–23.