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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

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Abstract

Sarcomatoid carcinoma (SC) is a variant of head and neck squamous cell carcinoma characterized by a prominent and sometimes exclusive spindle cell component. Distinction from a sarcoma or reactive stroma can be problematic, particularly in cases in which the conventional component is not obvious. The value of immunohistochemistry is limited because of the loss of cytokeratin expression in a sizable percentage of cases. Staining for p63 can enhance detection of epithelial differentiation, but its usefulness is offset by expression in various soft tissue proliferations. Staining for p40—a squamous-specific isoform of p63—could potentially improve diagnostic accuracy. Immunohistochemistry for pancytokeratin, p63, and p40 was performed on 37 head and neck SCs, 201 soft tissue neoplasms, and 40 reactive stromal proliferations. The SCs were also stained for p16 in the event that some of the tumors were human papillomavirus (HPV) related. HPV in situ hybridization was performed on p16-positive cases. Twenty-three of 37 (62%) SCs were positive for pancytokeratin, 23 of 37 (62%) were positive for p63, and 20 of 37 (54%) were positive for p40. Compared with p63, p40 staining was less likely to be observed in soft tissue tumors (5% vs. 30%) and reactive stromal proliferations (0% vs. 30%). HPV16 was detected in 3 of 10 (30%) SCs of the oropharynx but in none of the nonoropharyngeal SCs. p40 staining does not improve the sensitivity for diagnosing SC, but it does diminish the risk of misdiagnosing a sarcoma or reactive stroma as SC. The presence of a sarcomatoid variant of HPV-related oropharyngeal cancer points to HPV testing as a useful diagnostic tool for atypical spindle cell proliferations of the oropharynx.

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Keywords

head and neck squamous cell carcinoma; spindle cell carcinoma; carcinosarcoma; sarcomatoid carcinoma; human papillomavirus

Sarcomatoid carcinoma (SC) of the upper respiratory tract is a distinct variant of squamous cell carcinoma trademarked by a prominent or even exclusive spindle cell component.^{1–3} Despite the mesenchymal appearance of the spindle cell component, it is of surface epithelial origin.⁴ The diagnosis of SC is uniquely challenging. At one extreme, the spindle cell proliferation may closely resemble a reactive fibroblastic proliferation and be mistaken for a pseudosarcomatous reaction to an associated carcinoma or to radiation.⁵ At the other extreme, the spindle cell proliferation may be mistaken for a true sarcoma.³ In both these instances, distinction of SC from a mesenchymal process rests on the demonstration of epithelial differentiation. Although the presence of an associated conventional squamous cell carcinoma is extremely helpful, up to one third of SCs are entirely spindle.^{4,6–8} Immunohistochemistry is widely used as a tool to identify epithelial differentiation in the spindle cells, but its value is limited by problems related to sensitivity and specificity. Anywhere from 17% to 74% of SCs are cytokeratin negative^{4,6–9}; and cytokeratin is intrinsically or aberrantly expressed in various soft tissue neoplasms^{10–19} and reactive stromal proliferations.^{4,9,20}

P63 is a transcription factor that is consistently expressed in normal squamous epithelium and in squamous cell carcinoma. Retention of p63 staining in some carcinomas that have undergone sarcomatoid change has prompted its use as an epithelial marker for the diagnosis of SC in the head and neck⁹ and other organs.^{21–25} The enhanced sensitivity of p63 staining is offset to some degree by its occasional staining of various soft tissue neoplasms and uncertainty regarding the staining of reactive stromal proliferations.^{9,26,27} Whereas the p63 antibody that is most commonly used in diagnostic practices (ie, p63 4A4) recognizes both the Np63 and TAp63 isoforms of the p63 molecule, p40 is an antibody that recognizes only the squamous-specific isoform Np63.²⁸ In certain diagnostic scenarios in which the recognition of squamous differentiation in a poorly differentiated neoplasm is critical, p40 staining is more reliable than p63 staining.^{29–34} Potentially, p40 immunohistochemistry could help distinguish SC from reactive and neoplastic mesenchymal proliferations of the head and neck.

Human papillomavirus (HPV) is a well-recognized cause of most squamous cell carcinomas arising from the oropharynx.^{35–37} In these HPV-related head and neck cancers, the presence and distribution of HPV can readily be visualized by DNA in situ hybridization and/or p16 immunohistochemistry—a reliable surrogate marker for the presence of HPV.^{38,39} Importantly, the presence of HPV persists, even as a tumor clinically progresses or undergoes phenotypic shifts. For example, HPV is consistently detected in HPV-related cancers that recur, sometimes long after treatment of the primary tumor,^{40,41} and it is detected in HPV-related carcinomas that have undergone transformation to small cell carcinoma.^{42,43} Recently, Watson et al⁴⁴ detected HPV in 2 SCs of the head and neck,

suggesting that HPV testing could also be used as an ancillary diagnostic tool in the evaluation of spindle cell lesions of the head and neck.

METHODS

Cases

The surgical pathology files of The Johns Hopkins Hospital were searched for cases of SC with available tissue blocks or unstained slides. Slides were reviewed to confirm the diagnosis. To ensure inclusion of only true SCs, only biphasic carcinomas having both a malignant spindle cell component and a component of conventional invasive squamous cell carcinoma and/or squamous dysplasia were included.

The nonepithelial lesions included a variety of soft tissue sarcomas and reactive stromal proliferations. Four tissue microarrays (TMAs) containing various malignant soft tissue neoplasms resected at The Johns Hopkins Hospital were previously constructed from formalin-fixed paraffin-embedded tissue blocks. The TMAs included 27 pleomorphic undifferentiated sarcomas, 27 Ewing sarcomas, 26 Kaposi sarcomas, 15 liposarcomas, 14 leiomyosarcomas, 9 angiosarcomas, 8 low-grade fibromyxoid sarcomas, 8 rhabdomyosarcomas (7 embryonal and 1 pleomorphic), 5 chondrosarcomas, 4 malignant peripheral nerve sheath tumors, 3 clear cell carcinomas, 2 acral myxoinflammatory fibroblastic sarcomas, 1 fibrosarcoma, and 1 malignant ossifying fibromyxoid tumor. These TMA cases were supplemented by formalin-fixed and paraffin-embedded tissue blocks from an additional 53 soft tissue neoplasms including 13 inflammatory myofibroblastic tumors, 8 synovial sarcomas, 7 undifferentiated pleomorphic sarcomas, 6 leiomyosarcomas, 3 schwannomas, 2 angiosarcomas, 2 embryonal rhabdomyosarcomas, 2 low-grade fibromyxoid sarcomas, and 1 osteosarcoma. Forty cases of reactive stromal proliferations of the head and neck were also included and ranged from granulation tissue (n=16), radiation fibroblasts (n=12), nodular fasciitis (n=9), and inflammatory sinonasal polyps with atypical stromal cells (n=3).

Immunohistochemistry

Immunohistochemistry was performed on 5- μ m-thick sections prepared from formalin-fixed and paraffin-embedded blocks using the standard autostaining protocols on a Ventana Benchmark XT autostainer (Ventana Medical Systems Inc., Tucson, AZ). The primary antibodies were: p40 (Ab-1; Oncogene Research Products, Cambridge, MA; 1:2000 dilution); p63 (4A4; BioCare Medical, Concord CA; prediluted by manufacturer); pancytokeratin AE1/AE3 (pck-26; Ventana; prediluted by manufacturer); and p16 (INK4a; Ventana; prediluted by manufacturer). AE1/AE3 was utilized because it has usually been shown to be the most sensitive cytokeratin immunostain that is widely available for the diagnosis of head and neck sarcomatoid carcinoma.^{6,7} For p40 and p63, nuclear reactivity was regarded as positive, and for pancytokeratin only cytoplasmic staining was considered positive. Staining in <5% of tumor cells was regarded as “focal,” whereas positivity in $\geq$ 5% was regarded as “nonfocal.” For p16, nuclear and cytoplasmic staining in at least 70% of tumor cells was regarded as positive.³⁹

In Situ Hybridization

To confirm HPV status, HPV16 DNA in situ hybridization using the type 16-specific catalyzed signal amplification method for biotinylated probes (Dako GenPoint, Carpinteria, CA) was performed on each p16-positive SC. A positive result was defined as the detection of punctate signals within tumor nuclei. The SiHa and CaSki cell lines served as positive HPV16 controls.

RESULTS

Of the 47 SCs identified, 37 were biphasic. The results of these 37 biphasic SCs are summarized in Table 1. In all, 26 of 37 (70%) SCs were positive for at least 1 of the 3 epithelial markers (Fig. 1). P40 immunoreactivity was observed in the spindled cell component of 20 (54%) SCs. The staining was focal in 3 cases and nonfocal in 17 cases. P63 staining was observed in 23 (62%) cases (nonfocal staining=17; focal staining=6). Pancytokeratin staining was observed in 23 (62%) cases (nonfocal staining=21; focal staining=2). All cases that were p40 positive were also positive for p63 and pancytokeratin. Three cases were p63 positive but p40 negative. In these p40-negative/p63-positive cases, p63 staining was focal and pancytokeratin was negative. Three SCs were pancytokeratin positive but negative for both p63 and p40.

The results of immunohistochemical analysis of various soft tissue neoplasms and reactive stromal proliferations are summarized in Table 2. In soft tissue neoplasms, p40 staining was observed in only 9 of 201 (4%) cases. P40 positivity was seen in 4 of 27 (15%) Ewing sarcomas, 1 of 7 (14%) epithelioid sarcomas, 1 of 8 (13%) synovial sarcomas, 1 of 11 (9%) angiosarcomas, 1 of 20 (5%) leiomyosarcomas, and 1 of 34 (3%) pleomorphic undifferentiated sarcomas. P40 staining was focal in 7 soft tissue neoplasms, and it was nonfocal in 2 Ewing sarcomas. P40 immunostaining was not observed in any of the 40 reactive stromal proliferations. P63 immunostaining was observed in 60 of 201 (30%) soft tissue neoplasms (Fig. 2). P63 positivity was observed in 1 of 1 (100%) osteosarcoma, 2 of 3 (67%) cellular schwannomas, 2 of 4 (50%) malignant peripheral nerve sheath tumors, 5 of 10 (50%) low-grade fibromyxoid sarcomas, 9 of 20 (45%) leiomyosarcomas, 6 of 15 (40%) liposarcomas, 13 of 34 (38%) pleomorphic undifferentiated sarcomas, 3 of 8 (38%) synovial sarcomas, 10 of 27 (37%) Ewing sarcomas, 3 of 10 (30%) rhabdomyosarcomas, 3 of 11 (27%) angiosarcomas, 1 of 7 (14%) epithelioid sarcomas, 1 of 13 (8%) inflammatory myofibroblastic tumors, and 1 of 26 (4%) Kaposi sarcomas. P63 staining was focal in 26 cases and nonfocal in 34 cases including 9 pleomorphic undifferentiated sarcomas, 7 leiomyosarcomas, 4 Ewing sarcomas, 4 liposarcomas, 2 low-grade fibromyxoid sarcomas, 2 schwannomas, 1 angiosarcoma, 1 malignant peripheral nerve sheath tumor, 1 Kaposi sarcoma, 1 epithelioid sarcoma, 1 osteosarcoma, and 1 rhabdomyosarcoma. Focal p63 staining was observed in 12 of 40 (30%) reactive stromal proliferations including 9 of 16 (56%) cases of granulation tissue and 3 of 12 (25%) cases of fibroblastic proliferations with radiation atypia (Fig. 3).

P16 positivity was observed in 4 of 37 (11%) SCs including 3 of 11 (27%) SCs of the oropharynx and 1 of 26 (4%) SCs of nonoropharyngeal sites. DNA in situ hybridization confirmed the presence of HPV16 in the 3 p16-positive oropharyngeal SCs (Fig. 4), but

HPV was not detected in the p16-positive nonoropharyngeal SC. In the HPV-related SCs, HPV was identified in both the conventional and spindle cell components.

DISCUSSION

Immunohistochemistry is not always reliable in its ability to confirm epithelial origin. In a review of the literature, the frequency of sarcomatoid carcinomas demonstrating cytokeratin positivity by immunohistochemistry ranged from 50% to 75%.² In our evaluation of 37 biphasic SCs, cytokeratin staining of the spindle cell component was entirely negative in 38% of the cases. Clearly, the absence of cytokeratin staining does not exclude the diagnosis of SC. Conversely, the presence of positive cytokeratin staining in an atypical spindle cell proliferation does not necessarily confirm a diagnosis of SC. Focal positive staining should be interpreted with extreme caution given that many sarcomas and even pseudosarcomatous stromal proliferations aberrantly express cytokeratin.^{10,12,16} For example, we have previously noted that 17% of radiation-induced stromal atypias are focally cytokeratin positive.⁴

p63 is a p53 homolog that is consistently expressed in normal squamous epithelium and in squamous cell carcinoma. As p63 staining is sometimes retained in carcinomas that have undergone sarcomatoid change, some have advocated its use as an alternative epithelial marker in head and neck SCs. Lewis et al⁹ reported that p63 staining increased the detection rate of epithelial differentiation by over 30% compared with cytokeratin staining alone. Despite such early promise, we found that p63 staining is only of limited value in discerning epithelial derivation and at times may even undermine the diagnostic process. In our study, addition of p63 staining was not sufficiently sensitive to expand the detection of epithelial staining compared with pancytokeratin staining alone. Both markers were positive in 30% of the cases. There were only 3 cytokeratin-negative cases that were p63 positive, and in these 3 cases p63 staining was focal. This added benefit of including p63 staining to enhance detection of epithelial derivation in a small subset of cases is offset by the repeated finding of p63 staining in various mesenchymal processes. Jo and Fletcher²⁶ first reported p63 staining in a small subset of soft tissue tumors, and our study further expands the breadth of p63 positivity across a broadening spectrum of soft tissue tumor types. Importantly, p63 staining is not limited to neoplastic proliferations. Focal p63 staining was a common finding in granulation tissue and in atypical fibroblastic proliferations after radiation therapy—reactive processes that are notoriously difficult to distinguish from some forms of SC. Owing to suboptimal specificity, the separation of SCs and mesenchymal proliferations solely on the basis of p63 staining is ill advised.

Whereas the p63 antibody detects both main isoforms of the p63 molecule, the p40 antibody detects only the squamous-specific isoform Δ p63.²⁸ Accordingly, p40 expression shows a more restricted distribution that is mostly confined to squamous epithelium and squamous cell carcinomas. We were able to confirm that p40 expression in soft tissue lesions is very limited: p40 staining was observed in only 4% of soft tissue neoplasms and in none of the reactive stromal proliferations. Compared with p63 staining, p40 staining in a suspected SC provides much more compelling evidence of squamous differentiation. Inopportunistically, p40 expression is not invariably retained, as a squamous cell carcinoma undergoes sarcomatoid

transformation. P40 staining was slightly less sensitive than pancytokeratin staining (54% vs. 62%), and there were no instances in which p40 staining confirmed epithelial differentiation in an SC that was pancytokeratin negative. The value of adding p40 to the immunohistochemical evaluation of a suspected SC is primarily in diminishing the risk of mistaking a mesenchymal lesion for an SC.

The rationale for HPV analysis as a tool for diagnosing SC is supported by the observations that: (1) the incidence of HPV-related oropharyngeal squamous cell cancer has been increasing by 11% over the last 2 decades, even as the incidence of HPV-unrelated head and neck cancer has stabilized⁴⁵; (2) HPV persists in a tumor, even when the tumor undergoes morphologic transformation such as the shift from a non-small cell to a small cell phenotype^{42,43}; and (3) HPV has recently been detected in 2 cases of SC.⁴⁴ Using an HPV detection approach that incorporates p16 immunohistochemical staining together with HPV16 DNA in situ hybridization, HPV was identified in both the conventional and spindle cell components of 3 (10%) SCs. This approach was particularly useful in the oropharynx—the site most commonly targeted by high-risk HPV—where HPV was detected in 30% of the SCs. The strict epitheliotropism of HPV infection confirms the epithelial nature of the spindle cell component in a way that cannot be matched by various immunohistochemical epithelial markers that are inevitably expressed in various reactive and neoplastic mesenchymal processes.

In their review of SCs of the upper aerodigestive tract, Batsakis and Suarez² sagaciously recommended the term “pseudosarcomatous proliferative lesion” as an interim diagnosis for the sizeable number of atypical spindle cell proliferations in which histologic and immunohistochemical evidence is insufficient to establish a definite diagnosis of SC. Over the ensuing decade, the availability of additional immunohistochemical markers such as p63 and p40 has done little to substantiate epithelial differentiation in these morphologically complex tumors. In contrast, HPV detection assays may be useful in “closing the diagnostic loop” on these difficult lesions, at least for a subset of tumors arising in the oropharynx.

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REFERENCES

1. Nappi O, Wick MR. Sarcomatoid neoplasms of the respiratory tract. *Semin Diagn Pathol*. 1993; 10:137–147. [PubMed: 8367623]
2. Batsakis JG, Suarez P. Sarcomatoid carcinomas of the upper aerodigestive tracts. *Adv Anat Pathol*. 2000; 7:282–293. [PubMed: 10976907]
3. Cardesa, A.; Zidar, N. Spindle cell carcinoma. In: Barnes, L.; Eveson, JW.; Reichart, P., et al., editors. *World Health Organization Classification of Tumours Pathology and Genetics of Head and Neck Tumors*. Lyon, France: IARC Press; 2005. p. 127–128.
4. Ansari-Lari MA, Hoque MO, Califano J, et al. Immunohistochemical p53 expression patterns in sarcomatoid carcinomas of the upper respiratory tract. *Am J Surg Pathol*. 2002; 26:1024–1031. [PubMed: 12170089]
5. Lane N. Pseudosarcoma (polypoid sarcoma-like masses) associated with squamous-cell carcinoma of the mouth, fauces, and larynx. *Cancer*. 1957; 10:19–41. [PubMed: 13413797]

6. 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; 26:153–170. [PubMed: 11812937]
7. 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; 10:741–753. [PubMed: 2430474]
8. Lewis JE, Olsen KD, Sebo TJ. Spindle cell carcinoma of the larynx: review of 26 cases including DNA content and immunohistochemistry. *Hum Pathol.* 1997; 28:664–673. [PubMed: 9191000]
9. Lewis JS, Ritter JH, El-Mofty S. Alternative epithelial markers in sarcomatoid carcinomas of the head and neck, lung, and bladderp63, MOC-31, and TTF-1. *Mod Pathol.* 2005; 18:1471–1481. [PubMed: 15976812]
10. Brown DC, Theaker JM, Banks PM, et al. Cytokeratin expression in smooth muscle and smooth muscle tumours. *Histopathology.* 1987; 11:477–486. [PubMed: 2440790]
11. Gray MH, Rosenberg AE, Dickersin GR, et al. Cytokeratin expression in epithelioid vascular neoplasms. *Hum Pathol.* 1990; 21:212–217. [PubMed: 1689691]
12. Fletcher CD, Beham A, Bekir S, et al. Epithelioid angiosarcoma of deep soft tissue: a distinctive tumor readily mistaken for an epithelial neoplasm. *Am J Surg Pathol.* 1991; 15:915–924. [PubMed: 1718176]
13. Corson JM, Weiss LM, Banks-Schlegel SP, et al. Keratin proteins and carcinoembryonic antigen in synovial sarcomas: an immunohistochemical study of 24 cases. *Hum Pathol.* 1984; 15:615–621. [PubMed: 6204918]
14. Muro-Cacho CA. The role of immunohistochemistry in the differential diagnosis of soft-tissue tumors. *Cancer Control.* 1998; 5:53–63. [PubMed: 10761017]
15. Gu M, Antonescu CR, Guiter G, et al. Cytokeratin immunoreactivity in Ewing's sarcoma: prevalence in 50 cases confirmed by molecular diagnostic studies. *Am J Surg Pathol.* 2000; 24:410–416. [PubMed: 10716155]
16. Miettinen M, Rapola J. Immunohistochemical spectrum of rhabdomyosarcoma and rhabdomyosarcoma-like tumors. Expression of cytokeratin and the 68-kD neurofilament protein. *Am J Surg Pathol.* 1989; 13:120–132. [PubMed: 2464953]
17. Miettinen M. Immunoreactivity for cytokeratin and epithelial membrane antigen in leiomyosarcoma. *Arch Pathol Lab Med.* 1988; 112:637–640. [PubMed: 2454091]
18. Coindre JM. Immunohistochemistry in the diagnosis of soft tissue tumours. *Histopathology.* 2003; 43:1–16. [PubMed: 12823707]
19. Hibshoosh H, Lattes R. Immunohistochemical and molecular genetic approaches to soft tissue tumor diagnosis: a primer. *Semin Oncol.* 1997; 24:515–525. [PubMed: 9344317]
20. Nakayama M, Wenig BM, Heffner DK. Atypical stromal cells in inflammatory nasal polyps: immunohistochemical and ultrastructural analysis in defining histogenesis. *Laryngoscope.* 1995; 105:127–134. [PubMed: 8544590]
21. Hall JM, Saenger JS, Fadare O. Diagnostic utility of P63 and CD10 in distinguishing cutaneous spindle cell/sarcomatoid squamous cell carcinomas and atypical fibroxanthomas. *Int J Clin Exp Pathol.* 2008; 1:524–530. [PubMed: 18787630]
22. Tse GM, Tan PH, Chaiwun B, et al. p63 is useful in the diagnosis of mammary metaplastic carcinomas. *Pathology.* 2006; 38:16–20. [PubMed: 16484002]
23. Reis-Filho JS, Schmitt FC. P63 expression in sarcomatoid/metaplastic carcinomas of the breast. *Histopathology.* 2003; 42:94–95. [PubMed: 12493033]
24. Westfall DE, Folpe AL, Paner GP, et al. Utility of a comprehensive immunohistochemical panel in the differential diagnosis of spindle cell lesions of the urinary bladder. *Am J Surg Pathol.* 2009; 33:99–105. [PubMed: 18941404]
25. Velazquez EF, Melamed J, Barreto JE, et al. Sarcomatoid carcinoma of the penis: a clinicopathologic study of 15 cases. *Am J Surg Pathol.* 2005; 29:1152–1158. [PubMed: 16096403]
26. Jo VY, Fletcher CD. p63 immunohistochemical staining is limited in soft tissue tumors. *Am J Clin Pathol.* 2011; 136:762–766. [PubMed: 22031315]

27. Kallen ME, Nunes Rosado FG, Gonzalez AL, et al. Occasional staining for p63 in malignant vascular tumors: a potential diagnostic pitfall. *Pathol Oncol Res.* 2011; 18:97–100. [PubMed: 21667345]
28. Crum CP, McKeon FD. P63 in epithelial survival, germ cell surveillance, and neoplasia. *Annu Rev Pathol.* 2010; 5:349–371. [PubMed: 20078223]
29. Bishop JA, Teruya-Feldstein J, Westra WH, et al. p40 (DeltaNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. *Mod Pathol.* 2012; 25:405–415. [PubMed: 22056955]
30. Lin Z, Liu M, Li Z, et al. DeltaNp63 protein expression in uterine cervical and endometrial cancers. *J Cancer Res Clin Oncol.* 2006; 132:811–816. [PubMed: 16804722]
31. Hibi K, Trink B, Patturajan M, et al. AIS is an oncogene amplified in squamous cell carcinoma. *Proc Natl Acad Sci USA.* 2000; 97:5462–5467. [PubMed: 10805802]
32. Pelosi G, Sonzogni A, Papotti M, et al. Different prevalence of transactivating (TA) p63 and non-TAp63 isoforms in pulmonary adenocarcinomas: a useful diagnostic tool. *Mod Pathol.* 2010; 23:411A–412A.
33. Righi L, Graziano P, Fornari A, et al. Immunohistochemical subtyping of nonsmall cell lung cancer not otherwise specified in fine-needle aspiration cytology: a retrospective study of 103 cases with surgical correlation. *Cancer.* 2011; 117:3416–3423. [PubMed: 21246522]
34. Del Vescovo V, Cantaloni C, Cucino A, et al. miR-205 Expression levels in nonsmall cell lung cancer do not always distinguish adenocarcinomas from squamous cell carcinomas. *Am J Surg Pathol.* 2011; 35:268–275. [PubMed: 21263248]
35. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med.* 2010; 363:24–35. [PubMed: 20530316]
36. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst.* 2000; 92:709–720. [PubMed: 10793107]
37. Adelstein DJ, Ridge JA, Gillison ML, et al. Head and neck squamous cell cancer and the human papillomavirus summary of a National Cancer Institute State of the Science Meeting, November 9–10, 2008, Washington, D.C. *Head Neck.* 2009; 31:1393–1422. [PubMed: 19787782]
38. Lewis JS Jr. p16 Immunohistochemistry as a standalone test for risk stratification in oropharyngeal squamous cell carcinoma. *Head Neck Pathol.* 2012; 6(suppl 1):S75–S82. [PubMed: 22782226]
39. Singhi AD, Westra WH. Comparison of human papillomavirus in situ hybridization and p16 immunohistochemistry in the detection of human papillomavirus-associated head and neck cancer based on a prospective clinical experience. *Cancer.* 2010; 116:2166–2173. [PubMed: 20186832]
40. Ruzevick J, Olivi A, Westra WH. Metastatic squamous cell carcinoma to the brain: an unrecognized pattern of distant spread in patients with HPV-related head and neck cancer. *J Neurooncol.* 2013; 112:449–454. [PubMed: 23408186]
41. Bishop JA, Ogawa T, Chang X, et al. HPV analysis in distinguishing second primary tumors from lung metastases in patients with head and neck squamous cell carcinoma. *Am J Surg Pathol.* 2012; 36:142–148. [PubMed: 22173119]
42. Bishop JA, Westra WH. Human papillomavirus-related small cell carcinoma of the oropharynx. *Am J Surg Pathol.* 2011; 35:1679–1684. [PubMed: 21997688]
43. Kraft S, Faquin WC, Krane JF. HPV-associated neuroendocrine carcinoma of the oropharynx: a rare new entity with potentially aggressive clinical behavior. *Am J Surg Pathol.* 2012; 36:321–330. [PubMed: 22301491]
44. 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; 7:250–257. [PubMed: 23536041]
45. Ernster JA, Sciotto CG, O'Brien MM, et al. Rising incidence of oropharyngeal cancer and the role of oncogenic human papilloma virus. *Laryngoscope.* 2007; 117:2115–2128. [PubMed: 17891052]

FIGURE 1. Sarcomatoid carcinoma. All of the sarcomatoid carcinomas included in the study were biphasic, consisting of an epithelial component (bottom left) in addition to a spindle cell component (A). This sarcomatoid carcinoma was positive in both areas for pancytokeratin (B), p63 (C), and p40 (D).

FIGURE 2. p40 and p63 staining in soft tissue tumors. These cases of leiomyosarcoma (A–C) and low-grade fibromyxoid sarcoma (D–F) were negative for p40 (B and E) but diffusely positive for p63 (D and F).

FIGURE 3.
P63 staining of reactive stromal proliferations. In this example of atypical fibroblasts after radiation therapy (A), p63 was focally positive (B).

FIGURE 4.

HPV-related sarcomatoid carcinoma. This example of sarcomatoid carcinoma (A) exhibited pancytokeratin staining in the conventional epithelial component but not in the spindle cell component (B). P16 staining (C) and HPV16 in situ hybridization signals (D) were present in both components.

Immunohistochemical Staining and HPV Analysis of the Spindle Cell Component of 37 Biphasic Sarcomatoid Carcinomas of the Head and Neck

TABLE 1

Case	Site of Primary Tumor	IHC for Epithelial Differentiation			HPV Testing	
		AE1/AE3	P63	P40	P16 IHC	HPV16 ISH
1	Hypopharynx	-	-	-	-	ND
2	Hypopharynx	++	+	+	-	ND
3	Hypopharynx	-	-	-	-	ND
4	Hypopharynx	-	-	-	-	ND
5	Hypopharynx	++	++	++	-	ND
6	Larynx	-	-	-	-	ND
7	Larynx	++	++	++	-	ND
8	Larynx	++	++	++	-	ND
9	Larynx	-	-	-	-	ND
10	Larynx	-	+	-	-	ND
11	Larynx	++	+	+	-	ND
12	Larynx	++	++	++	-	ND
13	Larynx	-	-	-	-	ND
14	Larynx	-	-	-	-	ND
15	Larynx	-	+	-	-	ND
16	Larynx	++	++	++	-	ND
17	Larynx	++	++	++	-	ND
18	Larynx	-	-	-	++	-
19	Nasopharynx	++	++	++	-	ND
20	Oral cavity	-	-	-	-	ND
21	Oral cavity	++	++	++	-	ND
22	Oral cavity	++	++	++	-	ND
23	Oral cavity	++	++	++	-	ND
24	Oral cavity	+	++	++	-	ND
25	Oropharynx	++	++	++	-	ND
26	Oropharynx	++	++	++	-	ND
27	Oropharynx	++	++	++	-	ND

Case	Site of Primary Tumor	IHC for Epithelial Differentiation			HPV Testing		
		AE1/AE3	P63	P40	P16 IHC	HPV16 ISH	
28	Oropharynx	+	++	++	-	ND	
29	Oropharynx	++	-	-	-	ND	
30	Oropharynx	-	-	-	-	ND	
31	Oropharynx	++	++	++	-	ND	
32	Oropharynx	-	+	-	++	+	
33	Oropharynx	++	++	++	++	+	
34	Oropharynx	-	-	-	++	+	
35	Sinonasal tract	++	+	+	-	ND	
36	Sinonasal tract	++	-	-	-	ND	
37	Sinonasal tract	++	-	-	-	ND	

+ indicates focal staining; ++, nonfocal staining; HPV, human papillomavirus; IHC, immunohistochemistry; ISH, in situ hybridization; ND, not determined.

TABLE 2

P63 and p40 Immunohistochemical Staining in Soft Tissue Tumors and Reactive Stromal Proliferations

Type of Spindle Cell Proliferation	p63 (n [%])	p40 (n [%])
Soft tissue neoplasms		
Acral myxoinflammatory fibroblastic sarcoma	0/2 (0)	0/2 (0)
Angiosarcoma	3/11 (27)	1/11 (9)
Cellular schwannoma	2/3 (67)	0/3 (0)
Chondrosarcoma	0/5 (0)	0/5 (0)
Clear cell sarcoma	0/3 (0)	0/3 (0)
Epithelioid sarcoma	1/7 (14)	1/7 (14)
Ewing sarcoma/primitive neuroectodermal tumor	10/27 (37)	4/27 (15)
Fibrosarcoma	0/1 (0)	0/1 (0)
Inflammatory myofibroblastic tumor	1/13 (8)	0/13 (0)
Kaposi sarcoma	1/26 (4)	0/26 (0)
Low-grade fibromyxoid sarcoma	5/10 (50)	0/10 (0)
Liposarcoma	6/15 (40)	0/15 (0)
Leiomyosarcoma	9/20 (45)	1/20 (5)
Malignant ossifying fibromyxoid tumor	0/1 (0)	0/1 (0)
Malignant peripheral nerve sheath tumor	2/4 (50)	0/4 (0)
Osteosarcoma	1/1 (100)	0/1 (0)
Pleomorphic undifferentiated sarcoma	13/34 (38)	1/34 (3)
Rhabdomyosarcoma	3/10 (30)	0/10 (0)
Synovial sarcoma	3/8 (38)	1/8 (13)
Total	60/201 (30)	9/201 (4)
Reactive stromal proliferations		
Granulation tissue	9/16 (56)	0/16 (0)
Inflammatory sinonasal polyp	0/3 (0)	0/3 (0)
Nodular fasciitis	0/9 (0)	0/9 (0)
Radiation fibroblasts	3/12 (25)	0/12 (0)
Total	12/40 (30)	0/40 (0)