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ORIGINAL PAPER

Consistent LEF-1 and MYB Immunohistochemical Expression in Human Papillomavirus-Related Multiphenotypic Sinonasal Carcinoma: A Potential Diagnostic Pitfall

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Abstract
Human papillomavirus (HPV)-related multiphenotypic sinonasal carcinoma (HMSC) is a distinct, newly-described sinonasal tract neoplasm characterized by a salivary gland tumor-like appearance with myoepithelial and ductal cells, frequent surface squamous dysplasia, and relatively indolent behavior. When considering a diagnosis of HMSC, aggressive high-grade salivary gland carcinomas, particularly those with a basaloid morphology such as basal cell adenocarcinoma and adenoid cystic carcinoma, enter the differential diagnosis. The full morphologic and immunophenotypic profile of HMSC continues to be unraveled. In this series of ten cases, we demonstrate that this tumor has consistent, strong immunohistochemical expression of LEF-1 yet lacks nuclear expression of β-catenin, and also has consistent yet variable expression of MYB protein. While LEF-1 expression may be a useful diagnostic adjunct, it can also be a pitfall, as other salivary tumors such as basal cell adenocarcinoma have been previously shown to express LEF-1. Additionally, MYB protein expression is not a discriminatory marker when trying to separate HMSC from adenoid cystic carcinoma.

Keywords Human papillomavirus · Multiphenotypic sinonasal carcinoma · Adenoid cystic carcinoma · Carcinoma with adenoid cystic-like features · LEF-1 · MYB

Introduction

Human papillomavirus-related multiphenotypic sinonasal carcinoma (HMSC) is a distinct, newly recognized sinonasal tract neoplasm that demonstrates a prominent basaloid morphology [1, 2]. It is characterized by a salivary gland tumor-like appearance accompanied by frequent surface dysplasia, an association with high-risk types of HPV (especially type 33), and relatively indolent behavior. HMSC was originally known as "HPV-related carcinoma with adenoid cystic carcinoma-like features" to highlight its close morphologic similarity to adenoid cystic carcinoma [2]. However, since its initial description, the morphologic spectrum of this tumor has expanded, with other basaloid tumors including basal cell adenocarcinoma and epithelial-myoepithelial carcinoma now also entering the differential diagnosis of HMSC [1–3].

Recently, data has suggested that β-catenin and lymphoid enhancer binding factor 1 (LEF-1) immunoexpression are useful diagnostic adjuncts for the diagnosis of salivary gland basal cell adenoma and basal cell adenocarcinoma [4–9]. Indeed, mutations in *CTNNB1*, the gene that encodes β-catenin on chromosome 3p21 as well as other genes involved in the Wnt/β-catenin pathway such as *APC, AXIN1* and *AXIN2* have been identified in a subset of basal cell adenomas and adenocarcinomas [5–7]. The protooncogenic effects of alterations of Wnt signaling pathway both canonical (β-catenin dependent) and non-canonical

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(β-catenin independent) have been well-documented [10, 11]. β-catenin is potent transcriptional activator of Wnt targeted gene expression, but by itself it cannot bind DNA, and relies on its association with DNA-binding transcription factors such as the LEF/TCF family [10]. LEF-1 is a member of the LEF/TCF family, and while it is mediator of the Wnt/β-catenin signaling pathway, it can also modulate gene transcription independently [12, 13].

Of the salivary gland tumors, HMSC most frequently mimics adenoid cystic carcinoma. Fusions involving *MYB* or *MYBL1* are very common in adenoid cystic carcinoma, and consequently the majority of adenoid cystic carcinomas demonstrates overexpression of the MYB protein [14–16]. While MYB protein expression is recognized to be neither entirely sensitive nor specific, it is commonly used in salivary gland pathology as supporting evidence of adenoid cystic carcinoma [17, 18].

In this study, we examine the immunohistochemical expression profile of LEF-1, β-catenin, and MYB protein in HMSC in order to explore the utility of these markers to distinguish HMSC from basal cell adenocarcinoma and adenoid cystic carcinoma.

Materials and Methods

Ten HMSCs were retrieved from our case files; all cases have been documented in prior publications [1–3, 19]. Immunohistochemistry for LEF-1, β-catenin and MYB was performed at a large reference laboratory (ProPath, Dallas, Texas) using a manual staining protocol with horseradish peroxidase-conjugated polymer followed with diaminobenzidine. 4 μm formalin-fixed paraffin embedded tissue sections were mounted on adhesive slides, along with multitumor control tissues. After drying for 1 min in a microwave followed by 20 min in a 60 °C oven, the slides were deparaffinized in xylene and rehydrated in graded alcohols to tap water. Endogenous peroxidase activity was quenched for 10 min at room temperature, using 0.3% H2O2 with 0.1% Sodium Azide added.

Epitope retrieval for β-catenin was achieved by placing the slide in 1 mM EDTA, pH 8.5 for 48 min. The MYB and LEF-1 slides were placed in a 0.25 M Tris solution, pH 9.0, for 48 min in a pressure cooker. After rinsing the slides in phosphate buffered saline (PBS) buffer, primary antibody incubation (Mouse monoclonal anti-β-catenin, clone 14, diluted 1:100, BD Biosciences-San Jose, CA; Mouse monoclonal anti-MYB, clone 5E11, diluted 1:10, Santa Cruz Biotechnologies-Dallas, TX; Rabbit monoclonal anti-LEF-1, clone EPR2029Y, diluted 1:150, Abcam-Cambridge, U.K.) was performed for 50 min at room temperature, using gentle orbital rotation at 40 rpm.

Following another rinse in PBS, incubation with anti-mouse or anti-rabbit horseradish peroxidase-conjugated polymer (PowerVision Poly-HRP anti-Mouse IgG or PowerVision Poly-HRP anti-Rabbit IgG, Leica-Buffalo Grove, IL) was performed for 45 min at 25 °C, using gentle orbital rotation at 40 rpm. The slides were then immersed for 8 min in 25 °C diaminobenzidine (DAB) (Invitrogen-Carlsbad, CA) to develop the brown colored staining. After rinsing in tap water, the slides were placed in 0.5% copper sulfate for 1 min at RT to enhance the appearance of chromogen. Finally, the slides were rinsed in water, counterstained in Thiazin (Wescor-Logan, UT) followed by Hematoxylin (Leica), dehydrated in graded alcohols and xylene, and coverslipped. Every stain performed included control tissue including a variety of benign tumors and normal tissue types to ensure optimized staining and titration with no background staining. The overall intensity (weak, moderate, or strong) and percentage of tumor cells with nuclear staining was recorded. An H-score was calculated by multiplying the intensity score (0 = none, 1 = weak, 2 = moderate, 3 = strong) with proportion score.

A break-apart fluorescence in situ hybridization (FISH) assay for *MYB* (Empire Genomics, Buffalo, NY) was performed in six of the cases according to the manufacturer's protocol using the HYBrite platform (Abbott Molecular, Des Plaines, IL), as previously described [2].

Results

The results are summarized in Table 1. The ten cases represented 6 females and 4 males ranging from 37 to 75 years (mean 56.6 years). Six tumors involved the nasal cavity only, three involved the nasal cavity and maxillary sinus, and 1 case involved only the maxillary sinus. Four of 10 tumors exhibited an associated component of surface squamous dysplasia. As previously demonstrated, all 10 cases harbored high-risk HPV [1–3, 19], and no *MYB* rearrangements were detected in the 6 cases previously tested by fluorescence in-situ hybridization [2].

All 10 HMSCs had diffuse and strong nuclear expression of LEF-1, with H-scores ranging from 210 to 300 (mean, 258) (Fig. 1). All tumors entirely lacked nuclear expression of β-catenin. All 10 tumors had some degree of MYB protein expression. The MYB immunostaining ranged from 20 to 90% in extent, and was variably weak (4 of 10), moderate (4 of 10), and strong (2 of 10) in intensity; H-score ranged from 20 to 270 (mean 108) (Fig. 2). The immunoexpression of both LEF-1 and MYB was predominantly seen in the myoepithelial-like cells of the HMSCs. In the 4 cases with surface squamous dysplasia, the dysplastic foci were negative for both LEF-1 and MYB, and lacked nuclear expression of β-catenin.

Table 1 Demographics for all 10 HMSC cases and immunohistochemical staining results for LEF-1, β-catenin and MYB protein

Case	Age years	Sex	Tumor site	LEF-1 extent, intensity (H-score)	β-catenin extent, intensity (H-score)	MYB extent, intensity (H-score)
1	69	Female	Nasal cavity	100%, strong (300)	Negative (0)	30%, moderate (60)
2	57	Male	Nasal cavity	90%, strong (270)	Negative (0)	20%, weak (20)
3	45	Male	Nasal cavity and maxillary sinus	100%, strong (300)	Negative (0)	90%, strong (270)
4	65	Female	Nasal cavity and maxillary sinus	80%, strong (240)	Negative (0)	30%, weak (30)
5	53	Female	Nasal cavity	80%, strong (240)	Negative (0)	60%, weak (60)
6	37	Female	Nasal cavity and maxillary sinus	90%, strong (270)	Negative (0)	50%, moderate (100)
7	46	Female	Nasal cavity	80%, strong (240)	Negative (0)	90%, moderate (180)
8	53	Male	Nasal cavity	70%, strong (210)	Negative (0)	30%, weak (30)
9	66	Male	Maxillary sinus	80%, strong (240)	Negative (0)	30%, moderate (60)
10	75	Female	Nasal cavity	90%, strong (270)	Negative (0)	90%, strong (270)

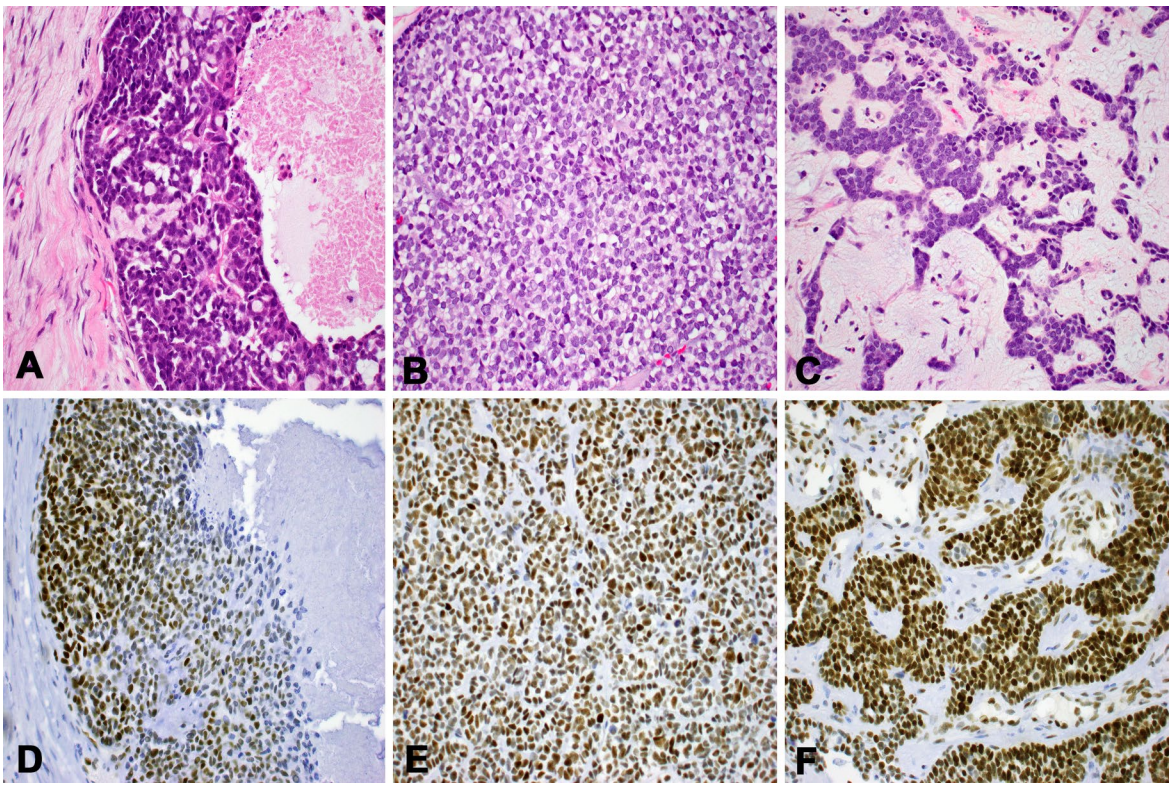

Fig. 1 Corresponding hematoxylin and eosin images for three HMSC cases (**a**–**c**). LEF-1 expression in each case was strong, ranging from 80 to 100% (**d**–**f**)

Discussion

HMSC is distinct sinonasal tract carcinoma that has a salivary gland tumor-like appearance accompanied by frequent surface squamous dysplasia; both components have a basaloid tumor cell morphology [1–3]. The tumor cells demonstrate a spectrum of ductal, myoepithelial and squamous differentiation, hence, the use of term "multiphenotypic" to describe this peculiar neoplasm [1]. In general, the majority of HMSC have a prominent solid growth pattern with minor cribriform and/or tubular patterns. High-grade features such as necrosis, brisk mitotic activity and cellular anaplasia are common, but despite this high-grade morphology, HMSC pursues a relatively indolent clinical course [1–3]. Because of this indolent behavior, it is important to distinguish HMSC from other, more aggressive carcinomas.

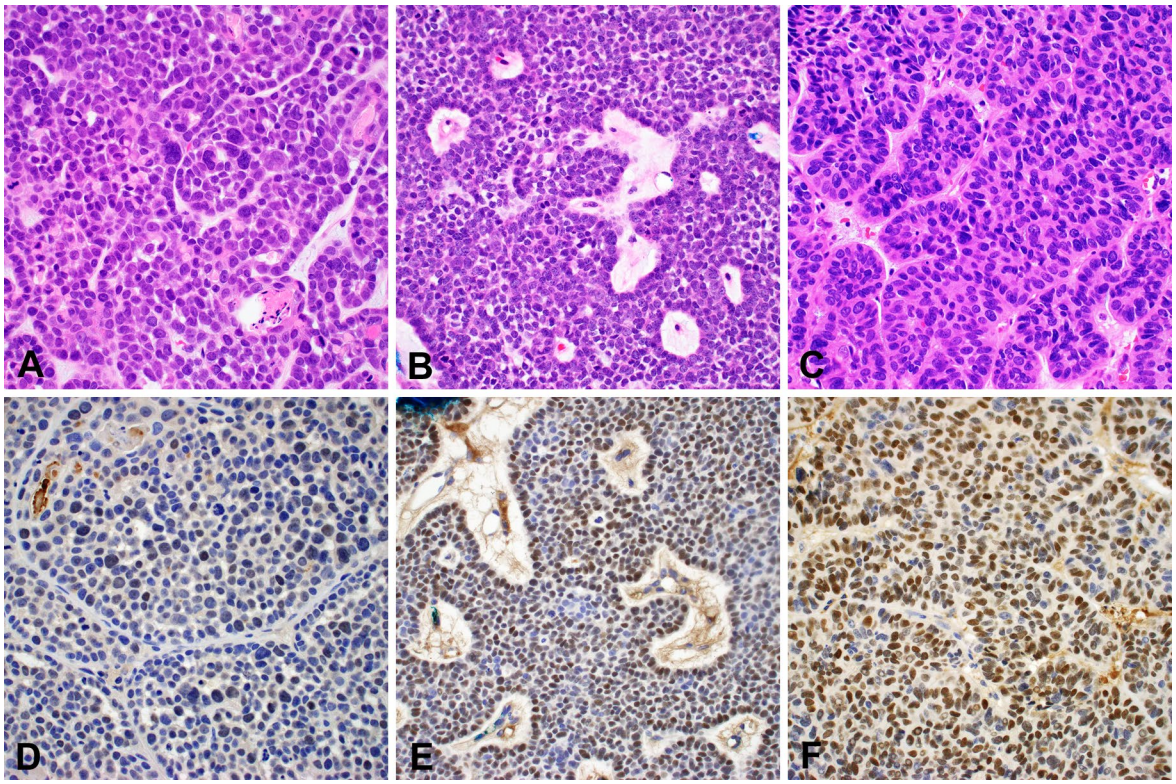

Fig. 2 Corresponding hematoxylin and eosin images for three HMSC cases (**a**–**c**). MYB expression was variable from case to case, examples of weak (**g**), moderate (**h**), and strong (**i**) expression are demonstrated

Recently, there has been literature that suggests that LEF-1 and β-catenin can be useful diagnostic markers in salivary tumors, with preferential staining in basal cell adenomas/adenocarcinomas [4–9]. Since basal cell adenocarcinoma may closely resemble HMSC, we sought to examine the expression of LEF-1 and β-catenin in HMSC. All 10 of our HMSCs demonstrated strong and diffuse nuclear expression of LEF-1, representing a significant pitfall in the distinction of HMSC and basal cell adenocarcinoma. Interestingly, the expression of LEF-1 was in all cases independent of nuclear expression of β-catenin.

Recurrent fusions involving *MYB* or *MYBL1* have been documented in adenoid cystic carcinoma and consequently a subset of adenoid cystic carcinoma demonstrate overexpression of the MYB protein [14–16]. Andreasen and colleagues found that MYB protein expression was limited to two of six cases of HMSC, with staining in only few abluminal cells whereas their four solid adenoid cystic carcinomas were diffusely positive [3]. However, in our study we demonstrated that HMSC has a variable spectrum of staining with MYB protein, with more than half of our cases having moderate to strong intensity, despite consistently lacking *MYB* rearrangements. If MYB protein positivity is defined by nuclear staining in at least 5% of tumor cells, as has been used in prior studies [3, 18], then all our cases would be considered positive for MYB, albeit with variable intensity. Even if more stringent criteria for a positive MYB were applied—for example, > 50% of tumor cells with strong intensity as used by West et al. [17]—2 of 10 cases would still be regarded as positive. Thus, regardless of scoring criteria, MYB protein has limited utility in separating adenoid cystic carcinoma from HMSC.

Unlike true salivary gland neoplasms, HMSC has a consistent association with high-risk human papillomavirus subtypes, particularly type 33 [1–3]. While strong and diffuse expression of the surrogate high-risk HPV marker p16 is very sensitive for HMSC, it is not specific, as there are other non-HPV related mechanisms that can lead to upregulation of p16 [20]. Indeed, many sinonasal carcinomas, salivary-type and surface-type, are often p16-positive in the absence of HPV [21–23]. Since there is such an immunophenotypic overlap between HMSC and other basaloid neoplasms that enter the differential diagnosis, demonstration of high-risk human papillomavirus subtypes either by in-situ hybridization or PCR, remains the best way to establish the diagnosis of HMSC.

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Compliance with Ethical Standards

Conflict of interest Authors have no conflicts of interest or disclosures as it pertains to this manuscript.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standard.

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