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MUC4 is expressed in alveolar rhabdomyosarcoma

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Abstract

Aims: MUC4 is a transmembrane glycoprotein normally expressed by several human epithelial surfaces, including those of the colon, vagina, and respiratory tract. Although MUC4 overexpression is seen in various carcinomas, its expression among mesenchymal neoplasms is fairly specific to low-grade fibromyxoid sarcoma and sclerosing epithelioid fibrosarcoma. Having observed unanticipated anti-MUC4 immunoreactivity in rhabdomyosarcoma, we aimed to further characterize its expression.

Methods/Results: Expression of MUC4 was assessed by immunohistochemistry in a total of 97 rhabdomyosarcomas using formalin-fixed paraffin-embedded tissue sections. MUC4 was expressed by 21 of 26 *PAX3/7-FOXO1* fusion-positive cases, wherein immunoreactivity, varying from weak to strong, was present in 20–100% of neoplastic cells. With the exception of one sclerosing rhabdomyosarcoma showing immunoreactivity in 20% of cells, MUC4 was not expressed by embryonal (n=28), sclerosing (n=20), or pleomorphic (n=23) rhabdomyosarcomas. Analyzing published gene expression microarray data from a separate cohort of 33 fusion-positive and 25 fusion-negative rhabdomyosarcomas, we found on average 11.4-fold increased expression in fusion-positive tumors (P=0.0004).

Conclusions: MUC4 is expressed to a variable extent in the majority of *PAX3/7-FOXO1* fusion-positive (alveolar) rhabdomyosarcomas, while expression in other rhabdomyosarcoma subtypes is rare.

Keywords

MUC4; rhabdomyosarcoma; immunohistochemistry; sarcoma; gene expression; soft tissue tumors

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AUTHOR CONTRIBUTIONS

GWC conceived the study. All authors contributed to study design, performed components of the research, and analyzed data. EF and GWC wrote the paper. All authors edited the manuscript.

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INTRODUCTION

Mucins are transmembrane high-molecular weight glycoproteins that are normally expressed by human epithelial surfaces where they function to lubricate the epithelium and protect it from exogenous injury. MUC4 is a mucin, normally expressed in several non-neoplastic epithelia, that is of special interest given its role in human malignancies.[1] Expression of MUC4 is associated with poor prognosis in carcinomas arising from various anatomic sites and there is evidence of a functional role for aberrant MUC4 signaling in epithelial neoplasms.[2] Among mesenchymal tumors, MUC4 expression is relatively specific to low-grade fibromyxoid sarcoma and sclerosing epithelioid fibrosarcoma (SEF), serving as a useful immunohistochemical marker for these entities.[3,4] Recently, additional MUC4-expressing mesenchymal tumors have been identified, including angiomatoid fibrous histiocytoma,[5] sarcoma with *MGA-NUTM1* fusion,[6] meningioma,[7] and intracranial mesenchymal tumor with *FET-CREB* fusion.[8] This expanding repertoire of MUC4-positive mesenchymal neoplasms serves as a potential source of diagnostic difficulty in cases with indistinct morphologic features. Having encountered a case of rhabdomyosarcoma with unexpected MUC4 expression in our consultation practice, we sought to determine the frequency of MUC4-positivity in rhabdomyosarcoma and to explore the association of MUC4 expression with histopathologic subtypes of rhabdomyosarcoma.

MATERIALS AND METHODS

Cases were retrieved from the surgical pathology archives of Stanford Health Care and Brigham and Women's Hospital under an Institutional Review Board-approved protocol (#42887, most recent review 10 September 2020). Representative hematoxylin and eosin-stained slides were reviewed. In total, whole-tissue sections of 97 tumors were evaluated for expression of MUC4: 26 *PAX3/7-FOXO1* fusion-positive (alveolar) rhabdomyosarcomas, 28 embryonal rhabdomyosarcomas, 20 sclerosing rhabdomyosarcomas, and 23 pleomorphic rhabdomyosarcomas. The presence of *FOXO1* gene rearrangement was confirmed in all 26 alveolar rhabdomyosarcoma cases by either 1) interphase fluorescence *in situ* hybridization (FISH) using Vysis break-apart probes (Abbott Laboratories, Abbott Park, IL, USA), or 2) karyotype characterization by metaphase chromosome analysis of disaggregated tumor biopsies in short-term culture using G-banding (GTW method). Among 21 alveolar rhabdomyosarcoma cases for which information regarding anatomic site was available, the anatomic origin was as follows: 14 head/neck, 3 abdomen/pelvis, 2 deep soft tissue of the extremities, 1 paraspinal soft tissue, and 1 chest wall.

Immunohistochemical detection of MUC4 was performed with a monoclonal antibody raised in mouse (Clone 8G7) purchased from Cell Marque (Rocklin, CA, USA). Anti-MUC4 antibody was used at a dilution of 1:200 (by volume) following heat-induced antigen retrieval using Leica Epitope Retrieval Solution 2 on the Leica BOND-III platform. Anti-MUC4 immunoreactivity was assessed as a percentage of neoplastic cells exhibiting cytoplasmic or membranous chromogen using a single, whole representative histologic section of tumor. Cases were considered positive for MUC4 expression if more than 1% of neoplastic cells exhibited anti-MUC4 immunoreactivity; the intensity of staining was graded as weak, moderate, or strong. Positive and negative controls were used throughout.

Publicly available microarray data were accessed via Gene Expression Omnibus (GEO) between January 1, 2020, and October 15, 2020. Microarray data (GSE66533)[9] were analyzed using the Partek Genomics Suite (version 6.6) with inclusion of all specimens after sample quality and principal component analyses.

RESULTS

MUC4 expression was observed in 21 of 26 (81%) alveolar rhabdomyosarcomas by immunohistochemistry; the staining was consistently cytoplasmic and granular-appearing. Immunoreactivity among neoplastic cells in positive cases varied from patchy to diffuse (20%−100%, median 50%; Figure 1). The proportion of MUC4-positive cells was equal to or greater than 50% in 14 of 26 (54%) cases. The intensity of immunoreactivity was also variable, even within a given tumor, with most positive cases exhibiting a spectrum of weak, moderate, and strong staining (Figure 1). In tumors with patchy expression, MUC4-positive areas were not morphologically distinctive on adjacent H&E-stained sections. The *FOXO1* fusion partner (*PAX3* or *PAX7*) was characterized in 13 of the alveolar rhabdomyosarcoma cases (9 by conventional cytogenetics, 4 by next generation sequencing). All 13 cases exhibited t(2;13), consistent with a *PAX3-FOXO1* rearrangement; the MUC4 expression in these cases spanned from negative (0%) to strong/diffuse (100%).

Embryonal, sclerosing, and pleomorphic rhabdomyosarcoma cases were negative for MUC4 expression by immunohistochemistry with the exception of one sclerosing rhabdomyosarcoma exhibiting granular, cytoplasmic immunoreactivity in 20% of neoplastic cells (Table 1). Although ancillary studies for *FOXO1* rearrangement were not performed in this case of sclerosing rhabdomyosarcoma, the tumor showed relatively limited expression of myogenin and diffuse expression of MyoD1 by immunohistochemistry, providing additional support for the classification. Among negative cases, one sclerosing rhabdomyosarcoma, one pleomorphic rhabdomyosarcoma, and two embryonal rhabdomyosarcomas showed immunoreactivity in rare cells (<1%). Within the embryonal rhabdomyosarcoma cohort, 5 cases, each with absence of *FOXO1* rearrangement by FISH and lack of MUC4 expression by immunohistochemistry, exhibited a dense or primitive appearance mimicking alveolar rhabdomyosarcoma.

Publicly available gene expression microarray data from an independent cohort of 33 *PAX3/7-FOXO1* fusion-positive and 25 fusion-negative rhabdomyosarcomas were also analyzed to interrogate *MUC4* expression.[9] This analysis revealed overall approximately 11-fold increased expression of *MUC4* in fusion-positive tumors relative to fusion-negative ones (P=0.0004; Figure 1). Paralleling the immunohistochemistry results, *MUC4* expression among *PAX3/7-FOXO1* fusion-positive cases was variable; 23/33 fusion-positive cases (70%) showed a level of expression that was more than 2-fold that of the average of all fusion-negative cases (maximum 54-fold increased expression). Conversely, only 2/25 *PAX3/7-FOXO1* fusion-negative cases (8%) had *MUC4* expression above this threshold (2.3-fold and 2.6-fold). Among fusion-positive cases, expression of *MUC4* did not differ with regard to involvement of *PAX7* (n=7) versus *PAX3* (n=26) in the pathogenic translocation (1.1-fold difference; P=0.79).

DISCUSSION

Our finding of MUC4 expression in alveolar rhabdomyosarcoma warrants consideration of the potential for diagnostic confusion with SEF. Although the epithelioid cytomorphology and stromal hyalinization of SEF generally distinguish these entities, SEF can manifest with a “small round cell” appearance.[10] Still, alveolar rhabdomyosarcoma and SEF are readily separated by immunohistochemistry, as SEF does not express the myogenic markers desmin, myogenin, or MyoD1. Moreover, although both entities exhibit cytoplasmic MUC4 staining with a somewhat granular appearance, we observed that MUC4 expression in alveolar rhabdomyosarcoma is often heterogeneous, whereas in SEF, expression tends to be strong and diffuse. Even in cases with substantial histologic overlap, the finding of recurrent *EWSR1/FUS-CREB3L2* rearrangement in SEF and *PAX3/7-FOXO1* rearrangement in alveolar rhabdomyosarcoma would enable appropriate classification.

We unexpectedly found that MUC4 expression in rhabdomyosarcoma was associated with the presence of the pathogenic *PAX3/7-FOXO1* fusion that characterizes the alveolar subtype. Given that identification of the *PAX3/7-FOXO1* fusion is important for appropriate prognostication and treatment of rhabdomyosarcoma, especially in pediatric patients, there is interest in diagnostic approaches that enable classification of fusion-positive tumors. In addition to molecular-cytogenetic techniques that test directly for the translocation itself, immunohistochemistry can be used to assess for distinctive gene expression changes mediated by the fusion oncoprotein. Such fusion-specific immunohistochemical markers of *PAX3/7-FOXO1* include AP2β, NOS1, HMGA2, and OLIG2.[11–13] In addition to being more accessible than alternative molecular methods in certain laboratories, these immunohistochemical surrogates may be more readily applied to scant biopsy specimens. Our observation that MUC4 expression is largely limited to the fusion-positive alveolar subtype among rhabdomyosarcomas implies that MUC4 may be used as a biomarker of the fusion. The estimated specificity is 98.6%; however, with a negative predictive value of 93% in this study, absence of MUC4 staining alone does not exclude a pathogenic *PAX3/7-FOXO1* fusion.

Our finding of MUC4 expression adds to the literature describing features of epithelial differentiation sometimes seen in rhabdomyosarcoma. For instance, about half of alveolar rhabdomyosarcomas are immunoreactive with antibodies recognizing broad-spectrum keratins.[14] Rhabdomyosarcomas also occasionally express synaptophysin, chromogranin A, and INSM1.[14,15] The observation of MUC4 expression thus underscores the importance of considering alveolar rhabdomyosarcoma in the differential diagnosis of poorly differentiated malignancies with immunohistochemical evidence of epithelial differentiation, particularly in head and neck sites where alveolar rhabdomyosarcoma often arises.

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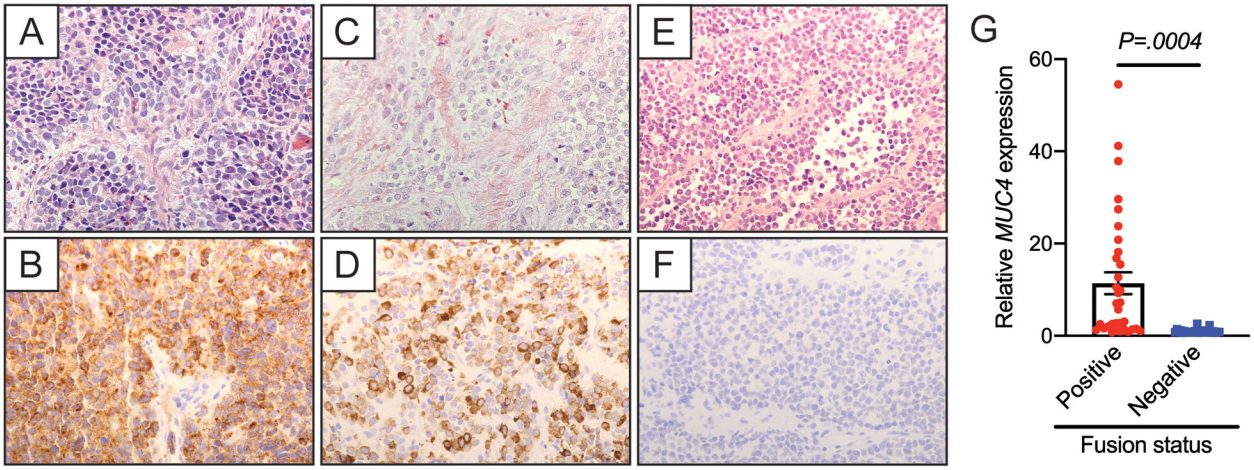

Figure 1.

Representative photomicrographs of H&E stain (A, C, E) and MUC4 immunohistochemistry (B, D, F) in three fusion-positive alveolar rhabdomyosarcoma cases demonstrating MUC4 expression that is diffuse and strong (A, B), heterogeneous (C, D), or absent (E, F). (G) Relative expression of *MUC4* in *PAX3/7-FOXO1* fusion-positive and -negative rhabdomyosarcomas from analysis of publicly available gene expression microarray data (GSE66533). Expression values are normalized to the mean of fusion-negative cases. Each dot represents expression in a distinct tumor. Error bars represent standard error of the mean.

Table 1.

Summary of immunohistochemical staining for MUC4 in rhabdomyosarcomas.

Tumor type	Total cases	MUC4 positive (%)
PAX3/7-FOXO1 fusion-positive (alveolar) rhabdomyosarcoma	26	21 (81)
Sclerosing rhabdomyosarcoma	20	1 (5)
Pleomorphic rhabdomyosarcoma	23	0 (0)
Embryonal rhabdomyosarcoma	28	0 (0)

MUC4 positive indicates >1% immunoreactivity