GRM1 immunohistochemistry distinguishes chondromyxoid fibroma from its histologic mimics

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GRM1 Immunohistochemistry Distinguishes Chondromyxoid Fibroma From its Histologic Mimics

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Abstract: Chondromyxoid fibroma (CMF) is a rare benign bone neoplasm that manifests histologically as a lobular proliferation of stellate to spindle-shaped cells in a myxoid background, exhibiting morphologic overlap with other cartilaginous and myxoid tumors of bone. CMF is characterized by recurrent genetic rearrangements that place the glutamate receptor gene *GRM1* under the regulatory control of a constitutively active promoter, leading to increased gene expression. Here, we explore the diagnostic utility of GRM1 immunohistochemistry as a surrogate marker for *GRM1* rearrangement using a commercially available monoclonal antibody in a study of 230 tumors, including 30 CMF cases represented by 35 specimens. GRM1 was positive by immunohistochemistry in 97% of CMF specimens (34/35), exhibiting moderate to strong staining in more than 50% of neoplastic cells; staining was diffuse (>95% of cells) in 25 specimens (71%). Among the 9 CMF specimens with documented exposure to acid decalcification, 4 (44%) exhibited diffuse immunoreactivity (>95%) for GRM1, whereas all 15 CMF specimens (100%) with lack of exposure to decalcification reagents were diffusely immunoreactive ($P = 0.003$). High *GRM1* expression at the RNA level was previously observed by quantitative reverse transcription polymerase chain reaction in 9 CMF cases that were also positive by immunohistochemistry; low *GRM1* expression was observed by quantitative reverse transcription polymerase chain reaction in the single case of CMF that was negative by immunohistochemistry. GRM1 immunohistochemistry was negative (<5%) in histologic mimics of CMF, including conventional chondrosarcoma, enchondroma, chondroblastoma, clear cell chondrosarcoma, giant cell tumor of the bone, fibrous dysplasia, chondroblastic osteosarcoma, myoepithelial tumor, primary aneurysmal bone cyst, brown tumor, phosphaturic mesenchymal tumor, CMF-like osteosarcoma, and extraskeletal myxoid chondrosarcoma. These results indicate that GRM1 immunohistochemistry may have utility in distinguishing CMF from its histologic mimics.

Key Words: GRM1, chondromyxoid fibroma, promoter swapping, immunohistochemistry, bone tumors

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Chondromyxoid fibroma (CMF) is a rare benign bone tumor that most commonly arises in the metaphysis of long bones and typically affects adolescents or young adults, although it can be seen in a wide range of ages and in a variety of anatomic sites.[1] Histologically, CMF consists of lobules of stellate to spindle-shaped cells in a predominantly myxoid background. The lobules generally are more cellular at their periphery, imparting a characteristic zonal architecture. Whereas myofibroblastic spindle-shaped cells tend to occupy the peripheral zone, stellate and chondroid-appearing cells are found in the center.[2] Hyaline cartilage is identified in a minority of cases.[3] Enlarged, hyperchromatic, and pleomorphic nuclei, likely degenerative in nature, are sometimes present, mimicking malignancy. In keeping with the morphologic features of both cartilaginous and myofibroblastic differentiation, by immunohistochemistry the lesional cells express S100 protein and SOX9,[4,5] along with smooth muscle actin.[2] ERG expression has also been observed in CMF.[6]

CMF exhibits recurrent rearrangements involving chromosome arm 6q,[7] resulting in translocation or chromoplexy-mediated fusion of the glutamate receptor gene *GRM1*, a G protein-coupled receptor primarily expressed in neurons of the central nervous system.[8,9] This recurrent genetic abnormality places the entire protein-coding sequence of *GRM1* downstream of any one of several strongly active gene promoters, such as *COL12A1*, *BCLAF1*, or *MEF2A*. The result of this promoter swapping is an up to 1400-fold increase in *GRM1* expression in CMF. While *GRM1* rearrangement and overexpression have

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been observed in 90% of CMF cases, *GRM1* expression was found to be negligible in 174 non-CMF mesenchymal tumors, suggesting that aberrantly increased *GRM1* expression is a distinctive feature of CMF among mesenchymal neoplasms.[8]

Immunohistochemical markers are increasingly being used as efficient and cost-effective surrogates for recurrent, diagnostically relevant genetic events in bone and soft tissue tumors. For instance, immunohistochemistry can be used to detect aberrant expression of components of fusion oncoproteins, such as CAMTA1 in epithelioid hemangioendothelioma and STAT6 in solitary fibrous tumors.[10–14] In addition, immunohistochemistry enables identification of protein overexpression secondary to either gene amplification, as observed with *MDM2* in low-grade central, parosteal, and dedifferentiated osteosarcoma,[15–17] or dysregulation of transcript and protein degradation, as occurs with *FOS* in osteoid osteoma and osteoblastoma.[18–20] Here, we explore the diagnostic utility of GRM1 immunohistochemistry as a surrogate for GRM1 overexpression resulting from recurrent promoter-swapping rearrangements in CMF.

MATERIALS AND METHODS

Cases were retrieved from the surgical pathology archives of Stanford Medical Center, Dartmouth-Hitchcock Medical Center, and Leiden University Medical Center under Institutional Review Board-approved protocols. Representative hematoxylin and eosin–stained slides were reviewed to confirm the diagnostic classification. A combination of whole tissue sections and tissue microarrays (TMA) were used to evaluate 230 cases altogether: CFM (30 total, 4 TMA; 5 cases had 2 specimens, representing primary tumor, and recurrence), primary aneurysmal bone cyst (35 total, 25 TMA), giant cell tumor of the bone (27 total, 17 TMA), chondroblastoma (24 total, 11 TMA), conventional chondrosarcoma with myxoid stroma (20 total, 0 TMA), fibrous dysplasia (15 total, 0 TMA), chondroblastic osteosarcoma (15 total, 0 TMA), extraskeletal myxoid chondrosarcoma (15 total, 3 TMA), myoepithelial tumor (13 total, 0 TMA), enchondroma (10 total, 0 TMA), chordoma (10 total, 0 TMA), clear cell chondrosarcoma (5 total, 0 TMA), phosphaturic mesenchymal tumor (5 total, 0 TMA), brown tumor of hyperparathyroidism (4 total, 4 TMA), and CFM-like osteosarcoma (2 total, 0 TMA). The TMAs were constructed using a tissue arrayer (Beecher Instruments, Silver Spring, MD) as previously described.[21] Tissues were evaluated as single cores, ranging from 0.6 to 2.0 mm in diameter, taken from representative areas of each formalin-fixed paraffin-embedded block. Cores were not considered if the targeted tissue was not included on the array, as assessed morphologically for each core.

Immunohistochemistry for GRM1 was performed on 4-μm-thick formalin-fixed paraffin-embedded tissue sections following pressure cooker antigen retrieval (0.01 M citrate buffer, pH 6.0) using a rabbit monoclonal antibody directed against an epitope within amino acids 280 to 420 of human GRM1 (1:500; clone JM11-61; Thermo Fisher Scientific Inc., Waltham, MA). Immunodetection was completed using the VECTASTAIN ABC kit (Vector Laboratories, Inc., Burlingame, CA) and DAB chromogen (Abcam, Cambridge, UK), according to the manufacturers' specifications. Appropriate positive control (cerebellum; Supplemental Fig. 1, Supplemental Digital Content 1, http://links.lww.com/PAS/B357) and negative control were employed throughout, including independent controls for each iteration of immunohistochemistry. The extent of immunoreactivity was graded according to the percentage of positive tumor cells and the intensity of staining was graded as weak, moderate, or strong.

Quantitative reverse transcription polymerase chain reaction (RT-qPCR) for *GRM1* was previously performed on freshly frozen tissue in 10 cases.[8] In brief, the TaqMan Gene Expression assay (Hs00168250_m1, Thermo Fisher Scientific Inc.) was used with *TBP* (4333769-F), *ACTB* (4333762-T), and *HPRT1* (Hs02800695_m1) housekeeping genes as endogenous RNA controls. Control tissues for comparison of *GRM1* expression levels included three cases each of extraskeletal myxoid chondrosarcoma, central conventional chondrosarcoma, chondroma, and osteochondroma, in addition to 2 chondroblastic osteosarcomas and 2 synovial chondromatoses. RT-qPCR reactions were performed in triplicate using the 7500 RT-PCR system (Thermo Fisher Scientific Inc.). Relative gene expression levels were calculated using the comparative C_t ($\Delta\Delta C_t$) method. Assessment of GRM1 immunohistochemistry was performed by A.M.S.T. and G.W.C. while blinded to the results of RT-qPCR. The Fisher exact test was used to assess the association between GRM1 immunostaining intensity and tissue decalcification.

TABLE 1. Summary of Immunohistochemical Staining for GRM1

Tumor Type	Total Cases	GRM1 Positive (%)*	GRM1 Negative (%)
Chondromyxoid fibroma	30	29 (97)	1 (3)
Primary aneurysmal bone cyst	35	0	35 (100)
Giant cell tumor of the bone	27	0	27 (100)
Chondroblastoma	24	0	24 (100)
Conventional chondrosarcoma	20	0	20 (100)
Chondroblastic osteosarcoma	15	0	15 (100)
Fibrous dysplasia	15	0	15 (100)
Extraskeletal myxoid chondrosarcoma	15	0	15 (100)
Myoepithelial tumor	13	0	13 (100)
Chordoma	10	0	10 (100)
Enchondroma	10	0	10 (100)
Clear cell chondrosarcoma	5	0	5 (100)
Phosphaturic mesenchymal tumor	5	0	5 (100)
Brown tumor	4	0	4 (100)
Chondromyxoid fibroma-like osteosarcoma	2	0	2 (100)

*Positivity was defined as the presence of cytoplasmic staining in > 5% of cells.

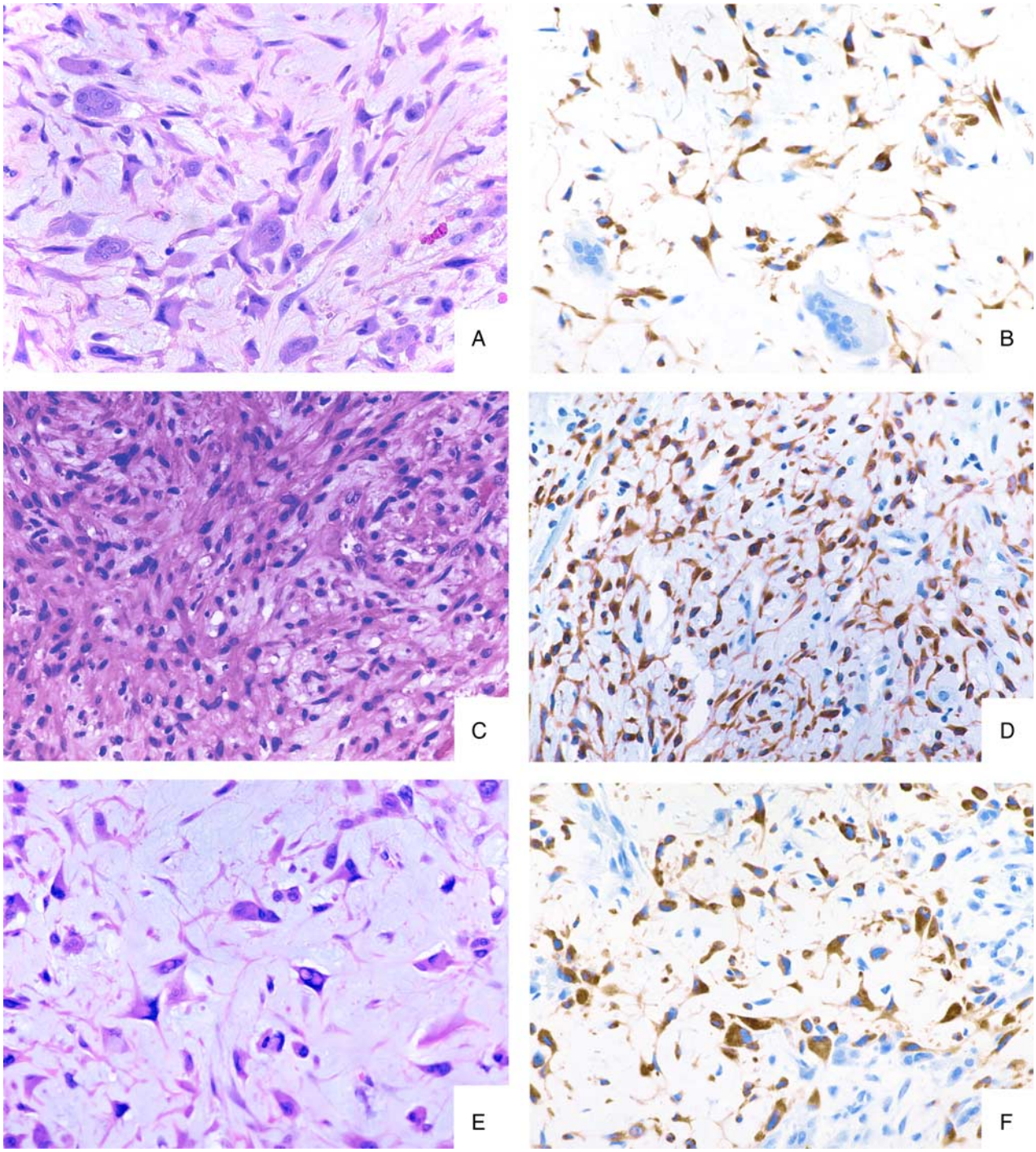

FIGURE 1. Representative photomicrographs of hematoxylin and eosin stain and GRM1 immunohistochemistry demonstrating diffuse and strong GRM1 expression in neoplastic cells of chondromyxoid fibroma, including stellate cells (A, B), spindle-shaped cells (C, D), and pleomorphic cells (E, F).

RESULTS

GRM1 immunohistochemistry was tested in a cohort of 30 CMF cases (Table 1). Clinical information was available for 26 patients, including 14 females and 12 males. Patients ranged from 9 to 80 years old at presentation (median 26 y old). Tumors were localized to the tibia (n = 7), ilium (n = 6), metatarsals (n = 3), femur (n = 2), and phalanges of the foot (n = 2), with one case each involving the sternum, rib, scapula, metacarpal, radius, and the nasal septum. There were 5 patients with 2 specimens available for analysis, representing primary and recurrent tumors, yielding 35 CMF specimens altogether.

These specimens were derived from curettage (n = 21), resection (n = 8), or biopsy (n = 6) procedures.

GRM1 was positive for expression by immunohistochemistry in 29 of 30 tumors (97%) and in 34 of 35 specimens (97%). Anti-GRM1 immunostaining was invariably localized to the cytoplasm. The extent of anti-GRM1 immunoreactivity ranged from ~50% to > 95% of neoplastic cells; 25 specimens (25/35; 71%) showed staining in > 95%. Likewise, the intensity of immunoreactivity in positive tumors ranged from moderate to strong, with strong staining intensity observed in 27 specimens (27/35; 77%). GRM1 expression was present within cells across the entire spectrum of cytomorphology observed in CMF, including spindle-shaped, stellate, and pleomorphic cells (Fig. 1).

For all 5 CMF cases with 2 separate specimens available for analysis, representing primary and recurrent tumor in each case, GRM1 immunohistochemistry was positive in both specimens. While the intensity of GRM1 staining was similar when comparing each pair of specimens, 2 of the pairs showed variation in the extent of staining. In both specimen pairs, the loss of immunostaining selectively occurred in the center of tissue fragments, suggesting that the variation in the extent of staining was caused by incomplete fixation.

GRM1 RNA expression levels were previously analyzed by RT-qPCR in ten tumors.[8] Nine tumors (90%) showed high levels of *GRM1* expression, defined as more than 100-fold increased expression relative to other cartilaginous tumors. All 9 tumors (100%) with high levels of *GRM1* expression by RT-qPCR were positive for GRM1 by immunohistochemistry. The single case with low levels of *GRM1* by RT-qPCR, which involved the metatarsal of a 10-year-old female patient, was also the only tumor in the CMF cohort that was negative for GRM1 by immunohistochemistry (Fig. 2).

Records related to tissue processing were available for 24 specimens. Acid decalcification was used in nine cases (9/24; 38%; Fig. 3). The intensity of staining was strong in 4 decalcified specimens (4/9; 44%) and in all 15 nondecalcified specimens (15/15; 100%; $P = 0.003$). Similarly, whereas immunoreactivity in > 95% of neoplastic cells was observed in 13 nondecalcified specimens (13/15; 87%), such extensive staining was seen in only 4 decalcified specimens (4/9; 44%; $P = 0.06$). The single specimen that was negative for GRM1 expression by immunohistochemistry was decalcified; a nondecalcified freshly frozen sample of the same tumor showed low *GRM1* levels by RT-qPCR. There were 3 additional decalcified cases of CMF in which paired nondecalcified fresh-frozen tissue was analyzed by RT-qPCR—all 3 exhibited high *GRM1* expression at the RNA level on nondecalcified fresh tissue and were positive for GRM1 by immunohistochemistry on decalcified formalin-fixed paraffin-embedded tissue.

To determine the specificity of GRM1 immunohistochemistry, we tested 200 samples representing potential histological mimics of CMF (Table 1). Among these samples, which encompassed cartilaginous (Fig. 4), giant cell-rich (Fig. 5), and myxoid tumors (Fig. 6), we found no cases that were positive for GRM1 expression at a threshold of staining in 5% of neoplastic cells. Rare, weakly immunoreactive cells, accounting for <5% of the lesional cell population, were identified in 2 cases of chordoma, 2 cases of chondroblastoma, and one case of chondroblastic osteosarcoma. In addition, we observed no anti-GRM1 immunoreactivity in the background non-neoplastic tissue, including bone, cartilage, hematopoietic marrow, blood vessels, and adipose tissue.

DISCUSSION

Our data suggest that GRM1 immunohistochemistry is a useful ancillary technique for the diagnosis of CMF, serving as a surrogate marker of recurrent promotor-swapping *GRM1* rearrangements. As with other immunohistochemical surrogates of recurrent molecular

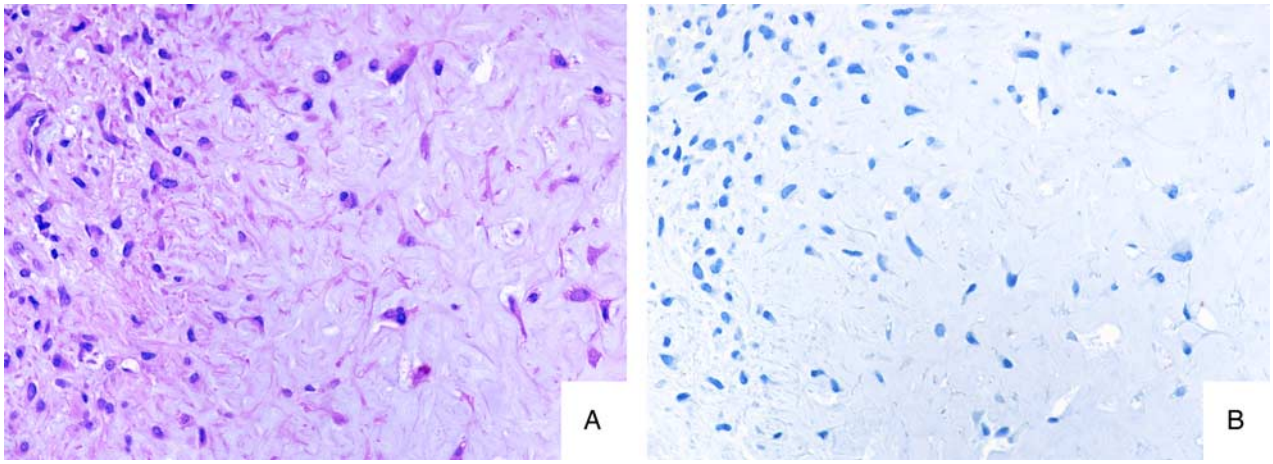

FIGURE 2. Representative photomicrographs of hematoxylin and eosin stain (A) and GRM1 immunohistochemistry (B) in a case of GRM1-negative chondromyxoid fibroma. The tumor involved the first metatarsal in a 10-year-old female patient, and it showed a low level of *GRM1* expression by RT-qPCR.

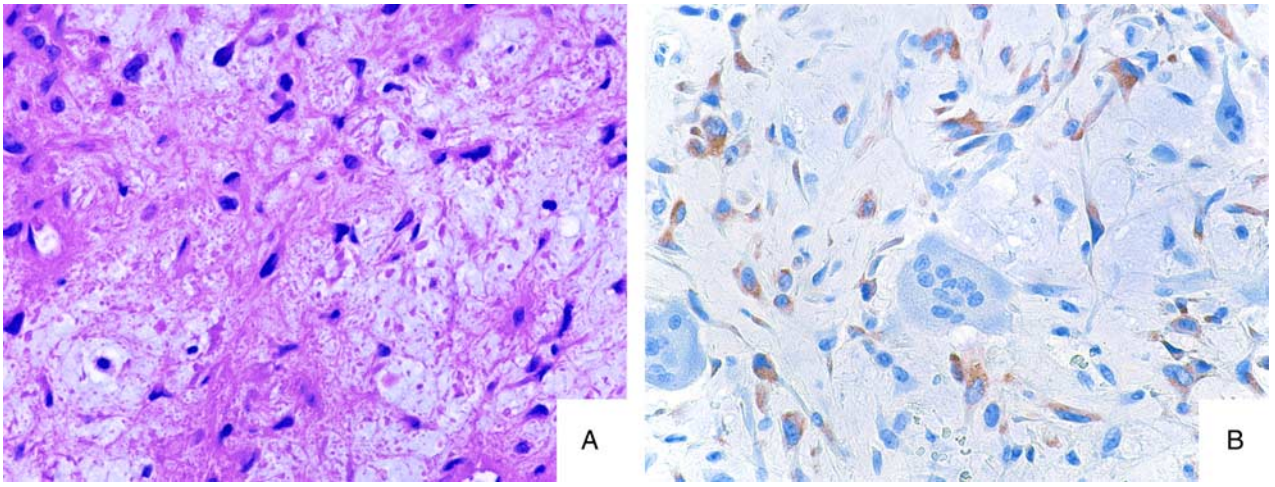

FIGURE 3. Representative photomicrographs of hematoxylin and eosin stain (A) and GRM1 immunohistochemistry (B) in a case of chondromyxoid fibroma exhibiting moderate anti-GRM1 staining intensity. This specimen underwent acid decalcification.

alterations, GRM1 immunohistochemistry offers practical advantages relative to alternative cytogenetic and molecular techniques, including accessibility, cost, and turnaround time. We anticipate that GRM1 immunohistochemistry may have particular utility in scant or fragmented biopsy specimens in which characteristic morphologic features,

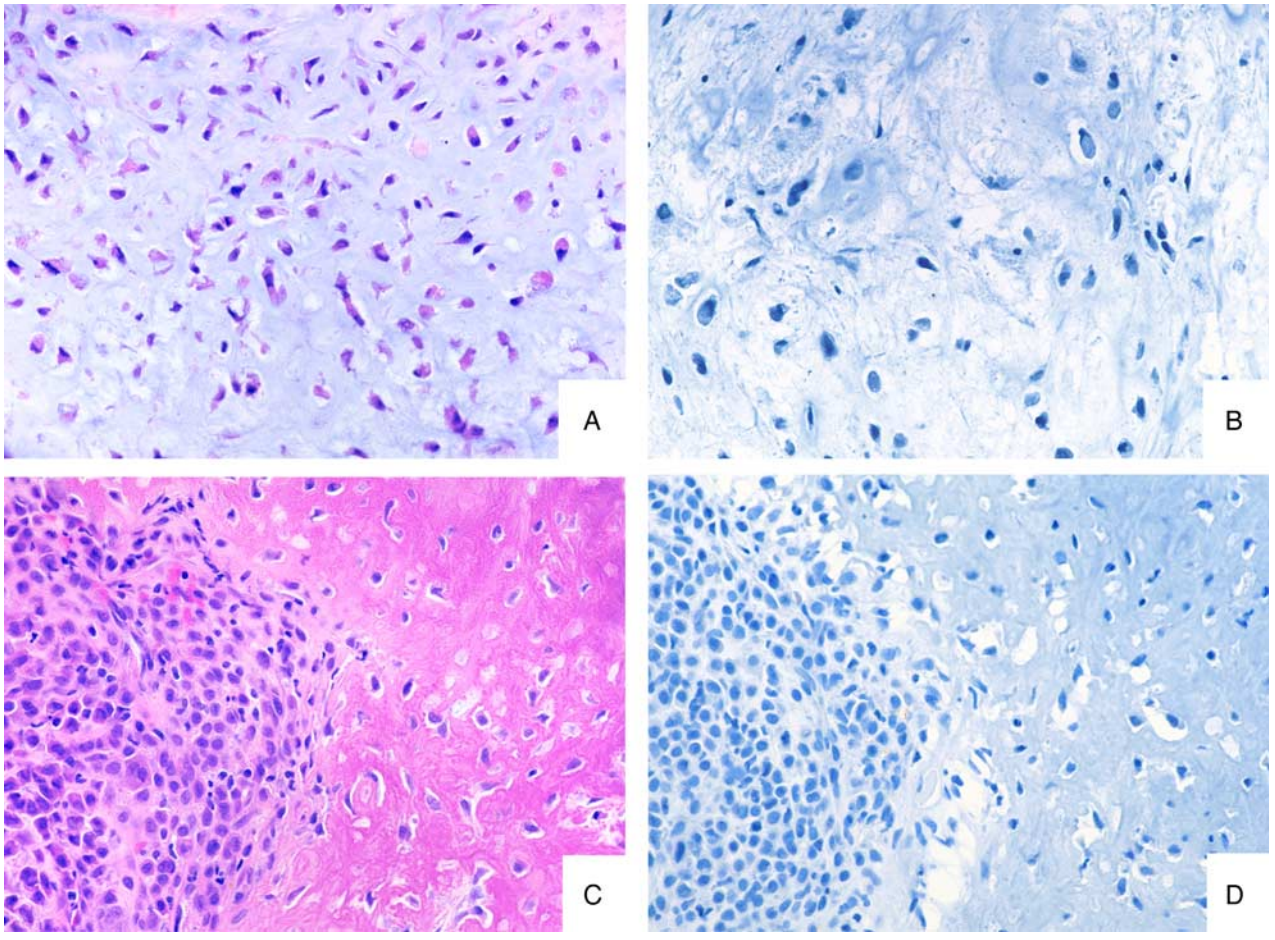

FIGURE 4. Representative photomicrographs of hematoxylin and eosin stain and GRM1 immunohistochemistry demonstrating lack of GRM1 expression in conventional chondrosarcoma with myxoid stroma (A, B) and chondroblastoma (C, D).

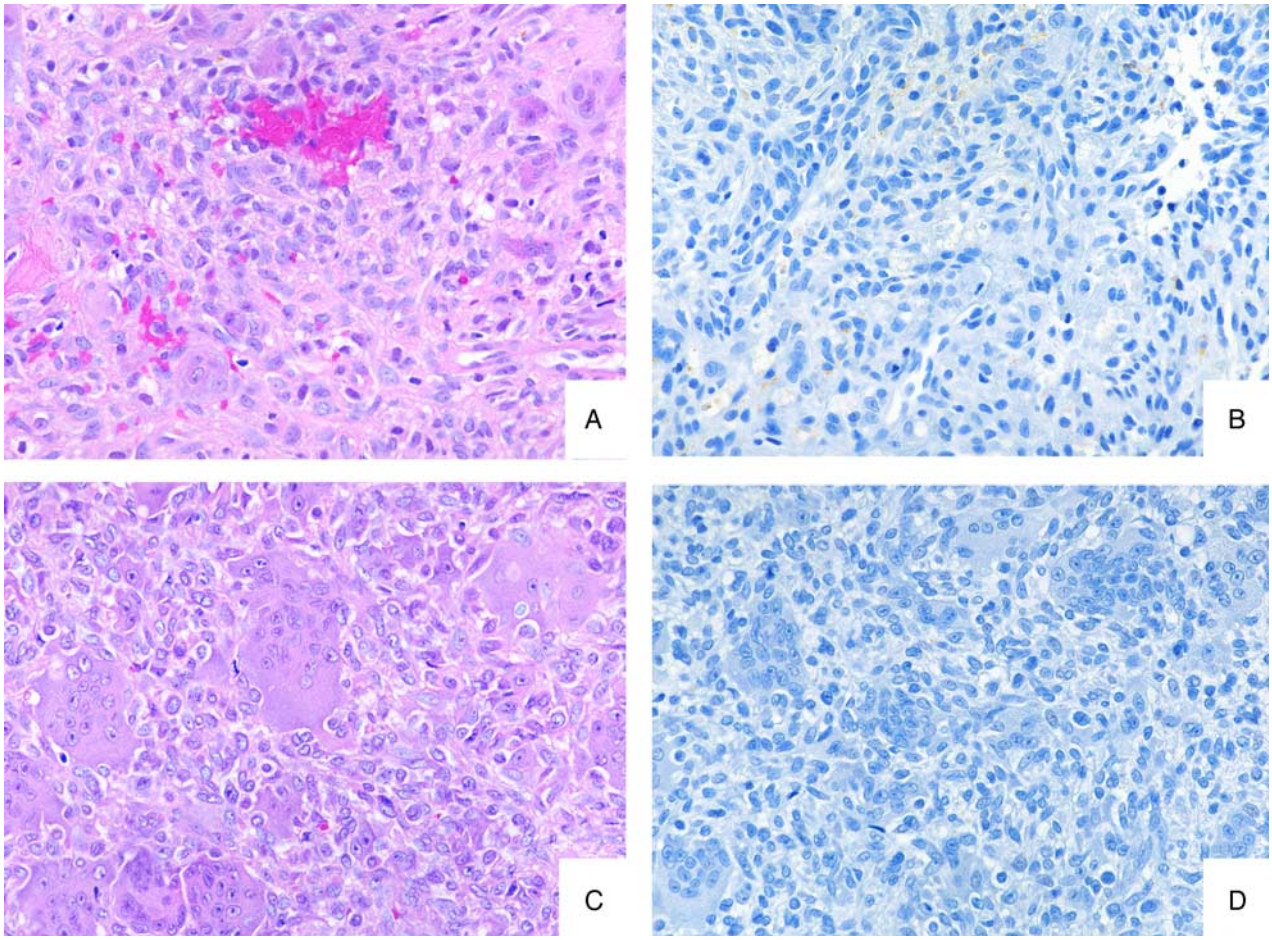

FIGURE 5. Representative photomicrographs of hematoxylin and eosin stain and GRM1 immunohistochemistry demonstrating lack of GRM1 expression in primary aneurysmal bone cyst (A, B) and giant cell tumor of the bone (C, D).

such as distinctive lobular architecture, are difficult to discern, or when there is a discrepancy with the radiologic imaging. Assessment of GRM1 may also prove to be useful in tumors with unusual features, such as cytologic atypia. Thus, GRM1 may complement other immunohistochemical markers for recurrent molecular alterations in primary bone tumors, such as histone H3.3 G34W for giant cell tumor of the bone and H3.3 K36M for chondroblastoma.[22–25]

CMF can be difficult to distinguish from its histologic mimics, especially chondrosarcoma of bone with myxoid changes. Previous studies have used immunohistochemistry to compare CMF with high-grade chondrosarcoma, showing decreased expression of *CCND1* (67% vs. 20%) and p16INK4A (67% vs. 27.5%) in chondrosarcoma.[26] However, these results could not be easily translated to routine diagnostics. Here, we use GRM1 immunohistochemistry as a surrogate histologic tool to detect *GRM1* rearrangement in CMF, and show GRM1 expression in 29 of 30 CMF (97%) versus 0 out of 20 (0%) conventional chondrosarcomas with myxoid stroma. Therefore, GRM1 immunohistochemistry is a highly specific and sensitive tool to make this distinction.

The histologic differential diagnosis of CMF also includes the extremely rare CMF-like variant of osteosarcoma, which is characterized by spindle-shaped or stellate tumor cells in a background of myxoid stroma.[27–29] We observed no GRM1 expression in 2 cases of CMF-like osteosarcoma, suggesting that GRM1 immunohistochemistry may aid in this challenging diagnostic distinction. However, given the limited number of CMF-like osteosarcoma cases in this series, additional studies are warranted. Thorough morphologic examination remains the key to diagnosing CMF-like osteosarcoma, which shows more cytologic atypia and infiltrative growth than CMF. CMF-like osteosarcoma also characteristically exhibits osteoid production by the malignant cells, albeit inconspicuous in some cases.[27–29] In addition, correlation with imaging studies is required when considering this differential diagnosis, as CMF-like osteosarcoma will be more aggressive-appearing radiographically with ill-defined margins.

One case of CMF (1/30; 3%) was negative for GRM1 by immunohistochemistry. This tumor involved the first metatarsal in a 10-year-old female patient. It also was the only 1 of the 10 examined cases that had low levels of *GRM1* mRNA expression by RT-qPCR.[8] From a practical perspective, the finding of this GRM1-negative case indicates that an absence of anti-GRM1 immunoreactivity

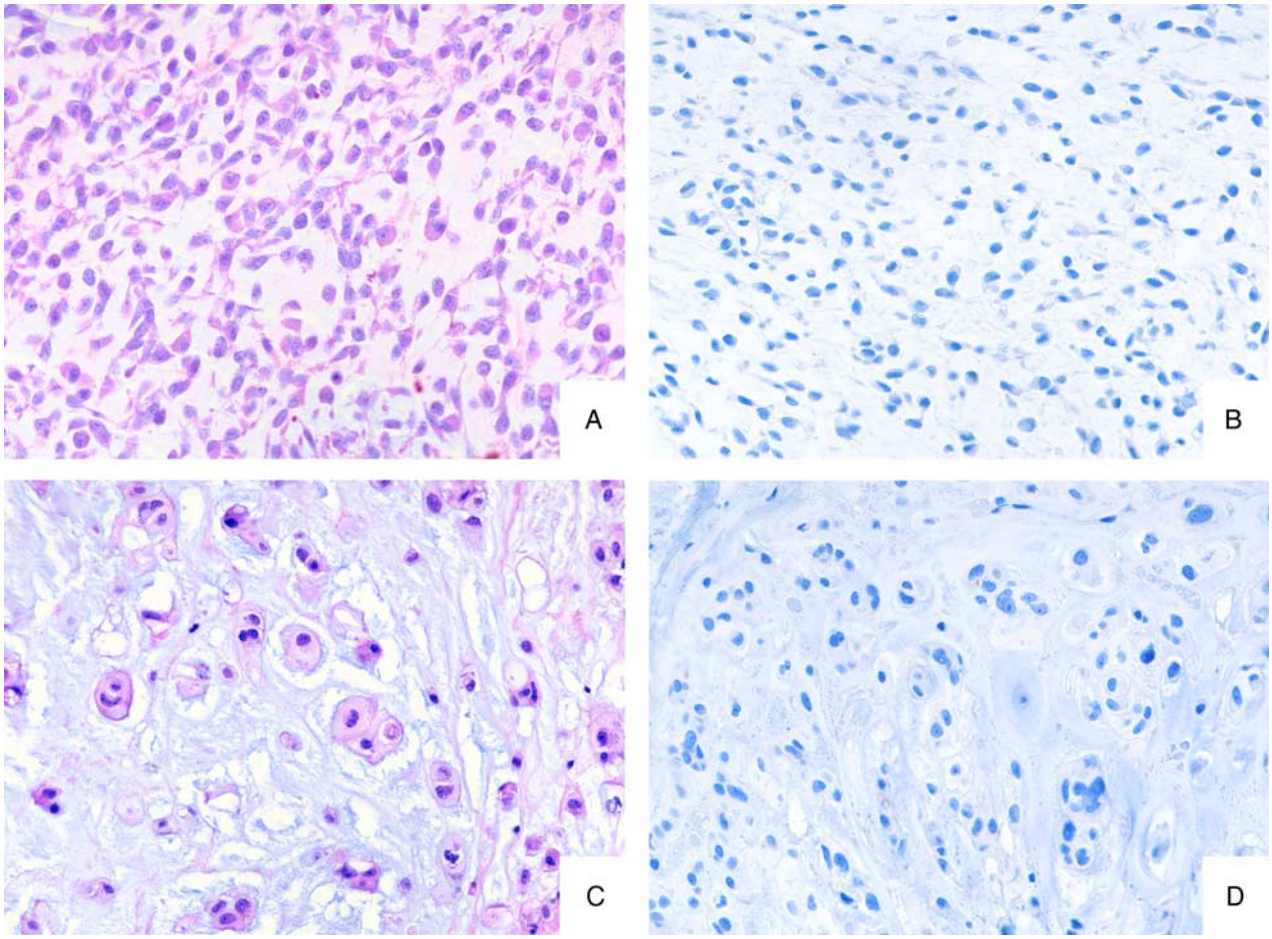

FIGURE 6. Representative photomicrographs of hematoxylin and eosin stain and GRM1 immunohistochemistry demonstrating lack of GRM1 expression in extraskeletal myxoid chondrosarcoma (A, B) and chordoma (C, D).

does not entirely exclude a diagnosis of CMF. This finding also raises the possibility that there is a small subset of CMF that arises through pathogenic mechanisms other than *GRM1* promoter-swapping rearrangements. Alternatively, given the substantial morphologic similarity between CMF and several other cartilaginous and myxoid neoplasms, it may be that rare GRM1-negative CMF cases in fact represent "CMF-like" variants of another entity. However, a review of the histology of the GRM1-negative case in our series did not reveal any unusual histologic features (Fig. 2). Identification and analysis of additional GRM1-negative CMF cases will allow for a better understanding of the molecular pathogenesis of this rare subset of tumors.

We found that GRM1 is susceptible to diminished immunoreactivity secondary to acid decalcification, similar to other immunohistochemical markers. Overall, the effect of decalcification on GRM1 immunoreactivity was fairly modest, with all decalcified specimens exhibiting at least moderate staining intensity in $>50\%$ of neoplastic cells, except for the one case that was completely negative for GRM1. Given that the GRM1-negative CMF case also showed low expression of *GRM1* by RT-qPCR in a nondecalcified fresh-frozen sample, the lack of GRM1 staining in this case is likely to reflect the absence of an underlying *GRM1* rearrangement, rather than an effect of decalcification. Still, we recommend that the results of GRM1 immunohistochemistry be interpreted with caution in the setting of a previously acid-decalcified specimen. Whenever possible, decalcification should be avoided in order to optimize epitope preservation.

Our findings suggest that GRM1 expression by immunohistochemistry is a specific feature that characterizes the vast majority of CMFs. Although our study aimed to assess the specificity of GRM1 immunohistochemistry by evaluating a wide variety of tumors that reasonably could be included in a broad differential diagnosis of CMF, we would recommend caution when implementing and interpreting GRM1 immunostaining, given that this marker has yet to be applied extensively to the vast landscape of human neoplasms. Moreover, while we relied mostly on whole tissue sections to analyze 230 unique tumors, the use of TMAs to test a subset of tumors represents a limitation of our study. Reassuringly, our GRM1 immunohistochemistry data corroborate previous RT-qPCR data demonstrating that increased *GRM1* expression is highly sensitive and specific for CMF.[8] Therefore, we conclude that GRM1 immunohistochemistry, when combined with

both careful histomorphologic assessment and consideration of radiographic imaging features, may be a useful ancillary tool for the diagnosis of CMF.

REFERENCES

1. WHO Classification of Tumours Editorial Board. *Soft Tissue and Bone Tumours (Vol 3)*. Lyon, France: International Agency for Research on Cancer; 2020.
2. Nielsen GP, Keel SB, Dickersin GR, et al. Chondromyxoid fibroma: a tumor showing myofibroblastic, myochondroblastic, and chondrocytic differentiation. *Mod Pathol*. 1999;12:514–517.
3. Wu CT, Inwards CY, O'Laughlin S, et al. Chondromyxoid fibroma of bone: a clinicopathologic review of 278 cases. *Hum Pathol*. 1998;29:438–446.
4. Konishi E, Nakashima Y, Iwasa Y, et al. Immunohistochemical analysis for Sox9 reveals the cartilaginous character of chondroblastoma and chondromyxoid fibroma of the bone. *Hum Pathol*. 2010;41:208–213.
5. Bleiweiss IJ, Klein MJ. Chondromyxoid fibroma: report of six cases with immunohistochemical studies. *Mod Pathol*. 1990;3:664–666.
6. Shon W, Folpe AL, Fritchie KJ. ERG expression in chondrogenic bone and soft tissue tumours. *J Clin Pathol*. 2015;68:125–129.
7. Romeo S, Duim RAJ, Bridge JA, et al. Heterogeneous and complex rearrangements of chromosome arm 6q in chondromyxoid fibroma: delineation of breakpoints and analysis of candidate target genes. *Am J Pathol*. 2010;177:1365–1376.
8. Nord KH, Lilljebjörn H, Vezzi F, et al. GRM1 is upregulated through gene fusion and promoter swapping in chondromyxoid fibroma. *Nat Genet*. 2014;46:474–477.
9. Anderson ND, de Borja R, Young MD, et al. Rearrangement bursts generate canonical gene fusions in bone and soft tissue tumors. *Science*. 2018;361:eaam8419.
10. Shibuya R, Matsuyama A, Shiba E, et al. CAMTA1 is a useful immunohistochemical marker for diagnosing epithelioid haemangioendothelioma. *Histopathology*. 2015;67:827–835.
11. Doyle LA, Fletcher CDM, Hornick JL. Nuclear expression of CAMTA1 distinguishes epithelioid hemangioendothelioma from histologic mimics. *Am J Surg Pathol*. 2016;40:94–102.
12. Cheah AL, Billings SD, Goldblum JR, et al. STAT6 rabbit monoclonal antibody is a robust diagnostic tool for the distinction of solitary fibrous tumour from its mimics. *Pathology*. 2014;46:389–395.
13. Doyle LA, Vivero M, Fletcher CD, et al. Nuclear expression of STAT6 distinguishes solitary fibrous tumor from histologic mimics. *Mod Pathol*. 2014;27:390–395.
14. Yoshida A, Tsuta K, Ohno M, et al. STAT6 immunohistochemistry is helpful in the diagnosis of solitary fibrous tumors. *Am J Surg Pathol*. 2014;38:552–559.
15. Yoshida A, Ushiku T, Motoi T, et al. Immunohistochemical analysis of MDM2 and CDK4 distinguishes low-grade osteosarcoma from benign mimics. *Mod Pathol*. 2010;23:1279–1288.
16. Yoshida A, Ushiku T, Motoi T, et al. MDM2 and CDK4 immunohistochemical coexpression in high-grade osteosarcoma: correlation with a dedifferentiated subtype. *Am J Surg Pathol*. 2012;36:423–431.
17. Dujardin F, Binh MBN, Bouvier C, et al. MDM2 and CDK4 immunohistochemistry is a valuable tool in the differential diagnosis of low-grade osteosarcomas and other primary fibro-osseous lesions of the bone. *Mod Pathol*. 2011;24:624–637.
18. Amary F, Markert E, Berisha F, et al. FOS expression in osteoid osteoma and osteoblastoma: a valuable ancillary diagnostic tool. *Am J Surg Pathol*. 2019;43:1661–1667.
19. Fittall MW, Mifsud W, Pillay N, et al. Recurrent rearrangements of FOS and FOSB define osteoblastoma. *Nat Commun*. 2018;9:2150.
20. Lam SW, Cleven AHG, Kroon HM, et al. Utility of FOS as diagnostic marker for osteoid osteoma and osteoblastoma. *Virchows Arch*. 2020;476:455–463.
21. Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med*. 1998;4:844–847.
22. Kerr DA, Brcic I, Diaz-Perez JA, et al. Immunohistochemical characterization of giant cell tumor of bone treated with denosumab: support for osteoblastic differentiation. *Am J Surg Pathol*. 2021;45:93–100.
23. Amary F, Berisha F, Ye H, et al. H3F3A (Histone 3.3) G34W immunohistochemistry: a reliable marker defining benign and malignant giant cell tumor of bone. *Am J Surg Pathol*. 2017;41:1059–1068.
24. Schaefer I-M, Fletcher JA, Nielsen GP, et al. Immunohistochemistry for histone H3G34W and H3K36M is highly specific for giant cell tumor of bone and chondroblastoma, respectively, in FNA and core needle biopsy. *Cancer Cytopathol*. 2018;126:552–566.
25. Amary MF, Berisha F, Mozela R, et al. The H3F3 K36M mutant antibody is a sensitive and specific marker for the diagnosis of chondroblastoma. *Histopathology*. 2016;69:121–127.
26. Romeo S, Oosting J, Rozeman LB, et al. The role of noncartilage-specific molecules in differentiation of cartilaginous tumors. *Cancer*. 2007;110:385–394.
27. Chow LT, Lin J, Yip KM, et al. Chondromyxoid fibroma-like osteosarcoma: a distinct variant of low-grade osteosarcoma. *Histopathology*. 1996;29:429–436.
28. Derqaoui S, Marbouh O, Madhi T, et al. Chondromyxoid fibroma-like osteosarcoma in a 13 years old girl: a report of a new case. *Clin Pathol*. 2021;14:2632010X211057555.
29. Zhong J, Si L, Geng J, et al. Chondromyxoid fibroma-like osteosarcoma: a case series and literature review. *BMC Musculoskelet Disord*. 2020;21:53.