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RREB1-MKL2 fusion in a spindle cell sinonasal sarcoma: biphenotypic sinonasal sarcoma or ectomesenchymal chondromyxoid tumor in an unusual site?

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Abstract

Biphenotypic sinonasal sarcoma (BSNS) is a rare, low grade spindle cell sarcoma, recently recognized in the WHO classification of head and neck tumors, which is characterized by a dual myogenic and neural differentiation and recurrent gene fusions, often involving *PAX3-MAML3*, and less commonly *PAX3* fusions with other partners such as *NCOA1*, *NCOA2*, or *WWTR1*. Yet, in about 4% of tumors no gene rearrangements are identified. Herein, we describe a *RREB1-MKL2* fusion in a BSNS lesion occurring in a 73-year-old female patient with a right maxillo-ethmoidal angle lesion. The polypoid, moderately cellular tumor with infiltrative submucosal growth was composed of fascicles of relatively bland spindle cells embedded in a loose collagenous matrix. The tumor cells showed moderate amounts of eosinophilic cytoplasm with indistinct borders and uniform, pale, ovoid to slender nuclei. The slowly proliferating neoplastic cells co-expressed smooth muscle actin and S100, and showed focal nuclear positivity for ß-catenin, while lacking staining for cytokeratins, desmin, myogenin, caldesmon, glial fibrillary acid protein, and SOX-10. Molecular analysis by targeted RNA-based next-generation sequencing identified an in-frame fusion between exon 8 of *RREB1* and exon 11 of *MKL2*, a genetic event that was reported to be a molecular hallmark of ectomesenchymal chondromyxoid tumor. Gene rearrangements in both genes were independently verified by fluorescence in situ hybridization (FISH). To evaluate its recurrent potential an additional group of 15 fusion negative BSNS were

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CONFLICT OF INTEREST

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tested for abnormalities in *RREB1* and *MKL2* genes by FISH, but no additional positive cases were identified.

Keywords

biphenotypic sinonasal sarcoma; gene fusion; *MKL2* ; *RREB1*

1 | INTRODUCTION

Biphenotypic sinonasal sarcoma (BSNS), an anatomically restricted low-grade sarcoma with neural and myogenic phenotype, was introduced in the recent WHO classification of head and neck tumors.[1] This tumor entity was first described by Lewis et al in 2012[2] as low-grade sinonasal sarcoma with neural and myogenic differentiation, and renamed subsequently by the same group as BSNS.[3]

Most BSNS are characterized by recurrent gene rearrangements involving the *PAX3* gene, commonly fused to *MAML3*, a coactivator of the NOTCH signaling pathway, present in approximately 70%.[3–5] Other rare fusion partners include *FOXO1*, *NCOA1*, *NCOA2*, and *WWTR1*.[4–7] In about 4% of BSNS no gene fusions can be detected.

In this study, we describe the case of a female patient with a histologically typical BSNS which showed a *RREB1-MKL2* fusion that has not been previously described in this diagnosis. The *RREB1-MKL2* fusion represents the genetic hallmark of ectomesenchymal chondromyxoid tumor, a rare benign lesion located in the glossal[8] and extraglossal regions.[9] However, the *RREB1-MKL2* fusion has also been reported in a case of oropharyngeal sarcoma with dual neural and myogenic differentiation.[10] Our case raises the hypothesis of a pathogenetic relationship between at least a rare subset of BSNS and the biphenotypic oropharyngeal sarcoma, which may belong to a spectrum of biphenotypic sarcomas of the head and neck region.

2 | METHODS AND RESULTS

2.1 | Index patient

A 73-year-old female presented with moderate nasal airway obstruction, more pronounced on the right side. Endoscopy rhinoscopy revealed a septal deviation to the left and a rounded, yellow mass within the right middle nasal meatus. Tympanic membranes showed some neglectable scars as relicts of recurrent otitis media in the childhood. A computed tomography showed a unilateral, rather sharply demarcated mass measuring 3.5 cm within the right maxillo-ethmoidal angle; the origin of the lesion was identified to be the posterior third of the right middle turbinate (Figure 1A,B). Concomitant chronic sinusitis caused a slight opacification of the adjacent dorsal ethmoidal cells. A partial endonasal endoscopic ethmoidectomy including an en bloc resection of the tumor was performed in general anesthesia. Subsequent to the rendered diagnosis of a BSNS, a complete endoscopic maxilloethmoidectomy including the resection of the middle turbinate was performed. However, after careful histological analysis of the resection specimen there was no residual tumor identified.

2.2 | Histology and immunohistochemistry of the index case

Microscopic examination of the initial resection specimen showed a moderately cellular, polypoid mesenchymal tumor with infiltrative submucosal growth and mild hyperplasia of the surface respiratory epithelium (Figure 1C). The tumor was composed of dense fascicles of relatively monotonous spindle cells, embedded in a collagenous stroma (Figure 1D). The tumor cells showed moderate amounts of eosinophilic cytoplasm with indistinct borders and uniform, ovoid to slender nuclei with open chromatin (Figure 1E). The mitotic activity was low with none to one mitosis per 10 high-power fields. Immunohistochemically, the neoplastic cells were diffusely positive for S100 (Figure 1F) and α-smooth muscle-actin (Figure 1G). Multifocal positivity was also noted with CD34 (Figure 1H) and EMA, while rare cells showed nuclear ß-catenin expression. H3K27-me3 expression was retained (Figure 1I). Tumor was negative for cytokeratins (AE1/AE3; Figure 1J), desmin (Figure 1K), myogenin, GFAP, and STAT6. A Ki67 proliferation index accounted for less than 5% (Figure 1L).

2.3 | RNA sequencing of the index case

RNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue, library preparation, and semiconductor sequencing were performed as described previously.[11] In short, extraction was conducted with the Maxwell 16 FFPE Plus LEV RNA Purification Kit. The library was generated with Archer FusionPlex Sarcoma kit.

Next generation sequencing on the IonTorrent GeneStudio S5XL/Prime platform revealed an in-frame fusion involving *RREB1* exon 8 (NM_001003698.3) and *MKL2* exon 11 (NM_014048.3) (Figure 2).

2.4 | Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) for *RREB1* and *MKL2* was performed using standard methods as previously reported.[12] Briefly, custom bacterial artificial chromosome (BAC) clone probes were designed to flank the target genes based on the UCSC genome browser (http://genome.usc.edu), and obtained from BACPAC sources of Children's Hospital of Oakland Research Institute (Oakland, CA; http://bapac.chori.org).[8] DNA from each BAC was isolated and then labeled with fluorochromes by nick translation. The slides were deparaffinized, pretreated, and then hybridized with the denatured probes. Following an overnight incubation, the slides were rinsed, stained with 4′,6-diamidino-2-phenylindole, mounted, and examined using a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany), controlled by Isis 5 software (Metasystems). A positive score was interpreted when at least 20% of the nuclei showed a break apart signal. Nuclei with incomplete sets of signals were omitted from the score.

In keeping with the RNA sequencing results, the index case showed the presence of both *RREB1* and *MKL2* gene rearrangements by FISH (Figure 3).

2.5 | Screening additional BSNS cases for *RREB1* and *MKL2* gene abnormalities

An additional group of 15 cases of BSNS with classic morphology, immunohistochemistry and clinical presentation, lacking known gene rearrangements in *PAX3* or *MAML3* genes,

were retrieved from the personal consultation files of one of the authors (CRA). Each case was re-evaluated to confirm the diagnosis according to well-defined pathologic criteria.[4,6] FISH studies were performed on these 15 additional BSNS cases, however, none showed abnormalities in *RREB1* or *MKL2* genes by FISH. The study was approved by the Institutional Review Board.

3 | DISCUSSION

Sarcomas of the head and neck are rare, accounting for approximately 5% to 10% of sarcomas and 1% to 3% of malignant head and neck tumors in adults.[13,14] They comprise a heterogeneous group of mostly aggressive mesenchymal malignancies which present a considerable diagnostic and therapeutic challenge.[13,14]

BSNS is a recently recognized tumor entity of the head and neck belonging to the steadily growing group of translocation-associated sarcomas and, in contrast to most head and neck sarcomas, behaving clinically relatively indolent.[1,4,15,16] They are characterized by slowly progressive growth and typically involve multiple sites in the sinonasal tract, especially the superior aspect of the nasal cavity, and the ethmoid sinus, but may also extend to the orbit or cribriform palate.[1,4,15,16] The recurrence rate is approximately 32%, and up to now, metastatic disease has not been described in BSNS.[2–4,15,16] The only death attributable to this tumor entity was observed in a patient with two recurrences, due to persistent intracranial tumor.[17] The tumor predominantly affects females, with a female-to-male ratio of approximately 1.9:1, and the reported patient age range is 24–87 years with a median in the fifth decade.[2,4,15]

BSNS is infiltrative and composed of hypercellular fascicles of monotonous spindle cells, frequently with a herringbone pattern. The tumor cells show indistinct borders with moderate amounts of eosinophilic cytoplasm and uniform, pale, and slender nuclei. Mitotic figures are sparse, and necrosis is absent. Most cases show reactive hyperplasia of surface respiratory or squamous epithelium, and entrapped epithelial elements are common. Hemangiopericytoma-like ("staghorn") vessels are frequently found.[2,4,15,16] Focal rhabdomyoblastic differentiation may be present.[2,4,15,16] The tumors exhibit immunoreactivity for smooth muscle actin, S100, and variable staining for nuclear ß-catenin; in cases with rhabdomyoblastic differentiation, focal desmin, MyoD1 and, to a lesser degree, myogenin staining is present.[2,4,6,15–17] SOX10 staining is absent.[4,17]

The overwhelming majority of BSNS are characterized by gene fusions involving *PAX3*, most frequently with *MAML3* and, in rarer instances, with *FOXO1*, *NCOA1*, *NCOA2*, and *WWTR1* as fusion partners.[3–7] Due to common *PAX3*-related fusions, BSNS show immunopositivity for PAX3 and co-express PAX8 due to crossreactivity with other paired box transcription factor family members.[18]

The PAX3 protein is a member of the PAX family of transcription factors. It is expressed during development of skeletal muscle, central nervous system, and neural crest derivatives, and regulates expression of target genes that impact on proliferation, survival, differentiation, and motility in these lineages.[19] In alveolar rhabdomyosarcomas, the *PAX3*-

FOXO1 gene fusion, which results from the translocation t(2;13) (q36;q14), generates a potent transcription factor, and also results in high-level expression of the PAX3-FOXO1 fusion protein.[19]

The sequence of the *PAX3-MAML3* fusion in BSNS predicted a PAX3-MAML3 chimeric protein consisting of the highly conserved paired-box DNA-binding domain and the paired-type homeodomain of *PAX3* fused to the transactivation domain of *MAML3*, and it was shown that the *PAX3-MAML3* fused BSNS showed altered expression of several genes and signaling networks involved in neural crest, skeletal system and general embryonic development.[3] These observations suggested that the phenotype of most BSNS simulates the developmental roles of *PAX3* and that the differentiation of BSNS might be modulated by *PAX3-MAML3* fusion.

RREB1 (*RAS*-responsive element binding protein 1), located on chromosome 6q24.3, is an alternatively spliced transcription factor implicated in RAS signaling, chromatin modification, and various tumors including, for example, pancreatic and colorectal adenocarcinomas, medullary thyroid carcinomas, and malignant melanomas (reviewed in Ref. 20). Furthermore, it was shown to bind the p53 promoter and transactivate p53 expression on DNA damage in osteosarcoma cells.[21] Recently, *RREB1* was identified as the key molecular integrator of Ras and TGF-ß signals to induce epithelial-tomesenchymal transitions.[22]

MKL2 (myocardin like 2), also known as *MRTFB* (myocardin-related transcription factor B), located on chromosome 16p13.12, is a transcription factor that is involved in smooth and skeletal muscle differentiation, and neural development.[23,24] In addition, *MRTFB* (*MKL2*) was shown to be a human colorectal cancer tumor-suppressor gene that functions in part by inhibiting cell invasion and migration.[25] Together with MKL1 (MRTFA, MAL) and MYOCD (myocardin) it belongs to the MRTF (myocardin-related transcription factors) protein family.[23–25]

The *RREB1-MKL2* fusion has not only been detected in 90% of ectomesenchymal chondroid tumors (ECMT), all located in the tongue,[8] but also in a biphenotypic “oropharyngeal” sarcoma.[10] Interestingly, Siegfried et al[10] showed in their case of biphenotypic “oropharyngeal” sarcoma that the RREB1-MKL2 chimeric transcription factor encoded by this fusion gene produced an increase in MKL2 expression, thus mimicking the role of PAX3 in BSNS tumorigenesis. Just recently, a *RREB1-MKL2* fusion transcript was described in two cases of mesenchymal tumors involving the mediastinum.[26] These data suggest a potential role of RREB1 and MKL2 activation in oncogenesis leading to effects in the corresponding downstream pathways.

Given the broad spectrum of differential diagnosis, which ranges from cellular schwannomas, solitary fibrous tumors and sinonasal glomangiopericytomas, to highly aggressive sarcomas such as synovial sarcoma, a molecular genetic verification of BSNS should be attempted, albeit the co-expression of myogenic and neural markers in a cytomorphologically rather bland spindle cell tumor of the sinonasal tract is a strong clue to the diagnosis of a BSNS. The detection of a *RREB1-MKL2* fusion transcript in the current

case suggests that molecular analysis for rearrangements of the *RREB1* and/or the *MKL2* genes, which was previously reported to be a genetic feature in ECMT,[8] or for alternative gene fusions should be performed in presumed BSNS without detectable *PAX3* or *MAML3* rearrangements. Our data are in line with the notion that fusions, which are highly prevalent or even pathognomonic in a specific histological setting, can occur outside their primary histological context as evidenced, for example, for *ALK*[27] or *NTRK*[28] gene fusions. In the future, more comprehensive studies are warranted to unravel the relation between the spectrum of tumor types primarily identified by histological features and *RREB1-MKL2* fusions.

In summary, we report a case of spindle cell sinonasal sarcoma with an *RREB1-MKL2* gene fusion. Our data suggest that this is a BSNS with an *RREB1-MKL2* fusion known to be highly prevalent in ectomesenchymal chondromyxoid tumor.

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DATA AVAILABILITY STATEMENT

Additional data will be made available upon reasonable request.

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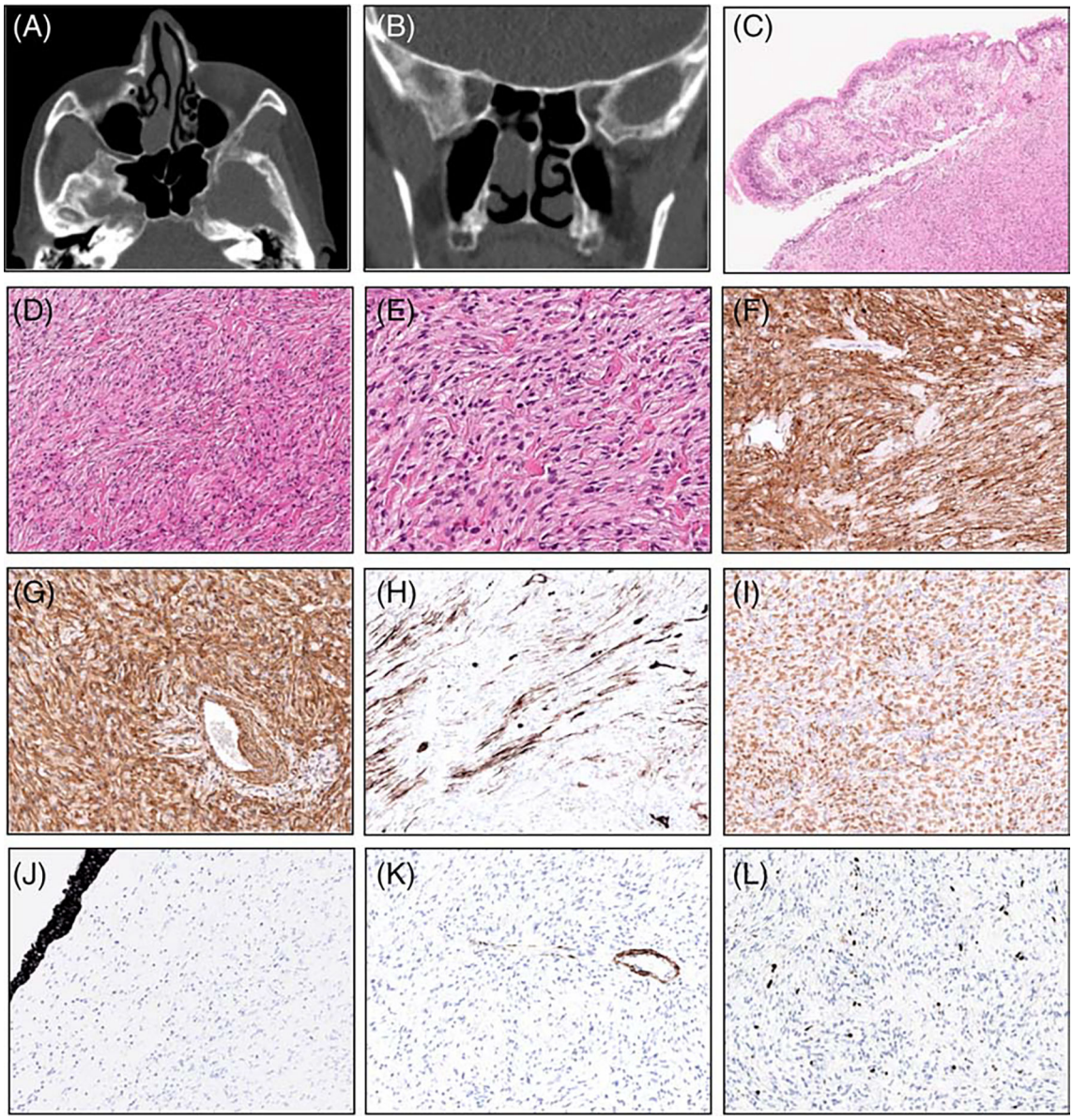

FIGURE 1.

Computed tomographic (CT), histopathological, and immunohistochemical findings. A, Axial CT and B, coronal CT shows a unilateral, rather sharply demarcated polypoid mass located within the right maxillo-ethmoidal angle and the nasal cavity; the base of the lesion was in the posterior third of the middle right turbinate. C, Polypoid tumor with infiltrative submucosal growth and slight reactive hyperplasia of surface respiratory epithelium. D, Moderately dense intersecting fascicles of monomorphic spindle cells intermingled by a loose network of collagen fibers. E, The tumor cells show moderate amounts of pale eosinophilic cytoplasm with indistinct borders and uniform, ovoid to slender nuclei. F, Diffuse expression for S100 and G, α-smooth muscle actin, and H, multifocal positivity for CD34-positive. I, H3K27-me3 expression is retained. J, In contrast to slightly hyperplastic surface respiratory epithelium, the neoplastic cells are cytokeratin (AE1/AE3)-negative. K,

The tumor is desmin-negative throughout. L, The Ki67 labeling index accounts for less than 5%

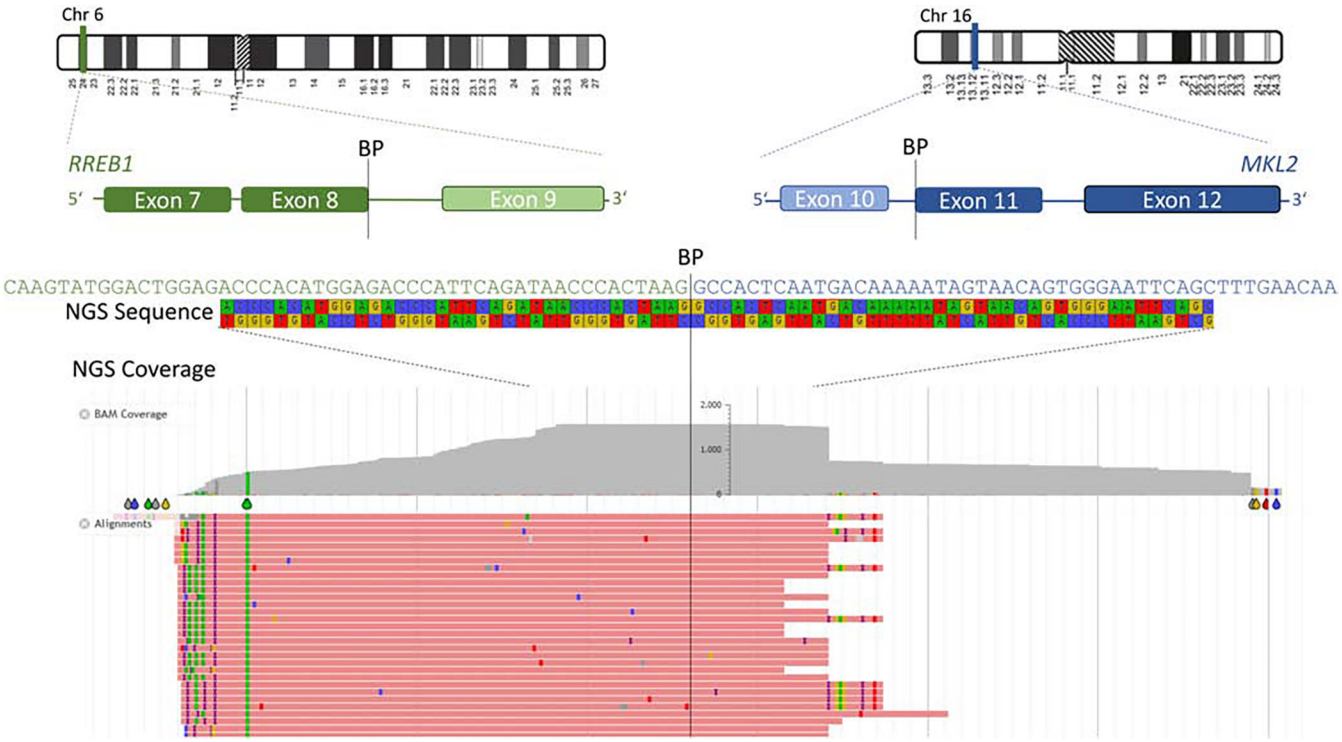

FIGURE 2.

Schematic representation of the *RREB1* (green)–*MKL2* (blue) fusion gene detected in our patient. Given are the positions on chromosome 6 (*RREB1*) and 16 (*MKL2*) as well as the break point (BP) of the gene fusion in the exon-intron sequence of the mRNA of the respective genes. In contrast to the case described by Siegfried et al,[10] the break point is located intronically and the exon boundaries in both genes (*RREB1* exon 8 and *MKL2* exon 11) remain intact. The consensus sequence of the NGS is shown underneath the hg38 human genome sequence for both genes. Underneath, the coverage of the gene fusion and representative NGS reads are depicted

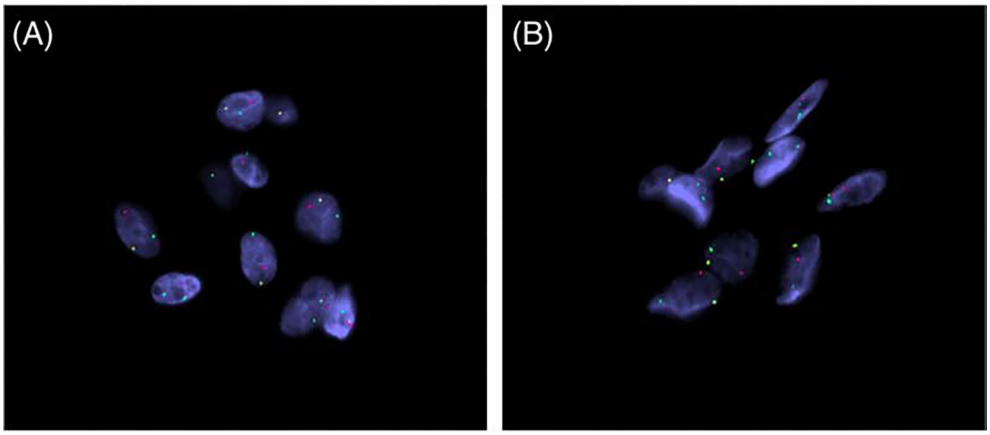

FIGURE 3.

Fluorescence in situ hybridization (FISH) in the index biphenotypic sinonasal sarcoma (BSNS) case showing break-apart signals in keeping with gene rearrangements in *MKL2* (A) and *RREB1* (B) (both images: red, centromeric; green, telomeric)