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BCOR is a robust diagnostic immunohistochemical marker of genetically diverse high-grade endometrial stromal sarcoma, including tumors exhibiting variant morphology

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Recognition of high-grade endometrial stromal sarcoma is important because of its aggressive clinical behavior. Morphologic features of *YWHAE-NUTM2* high-grade endometrial stromal sarcoma may overlap with other uterine sarcoma types. BCOR immunoexpression was studied in these tumors and their morphologic mimics to assess its diagnostic utility. BCOR immunohistochemical staining was performed on archival tissue from 28 high-grade endometrial stromal sarcomas with classic morphology (20 *YWHAE-NUTM2*, 5 *ZC3H7B-BCOR*, 3 *BCOR-ZC3H7B)*, 3 high-grade endometrial stromal sarcomas with unusual morphology and unknown gene rearrangement status, 66 low-grade endometrial stromal sarcomas, 21 endometrial stromal nodules, 38 uterine leiomyosarcomas, and 19 uterine leiomyomas. Intensity of nuclear staining and percentage of positive tumor cells were recorded. Strong diffuse nuclear BCOR staining (defined as >95% of tumor cells) was seen in the round cell component of all 20 (100%) classic *YWHAE-NUTM2* high-grade endometrial stromal sarcomas and the 3 unusual high-grade endometrial stromal sarcomas which prompted FISH studies confirming *YWHAE* rearrangement in 2 tumors. Genomic PCR confirmed the presence of *BCOR* exon 16 internal tandem duplication in the third case. Diffuse BCOR staining was strong in three and weak in one *BCOR*-rearranged high-grade endometrial stromal sarcoma while absent in the remaining four *BCOR*-rearranged tumors. BCOR staining was weakly positive in <5% of tumor cells in 4 of 66 (6%) low-grade endometrial stromal sarcomas and 1 of 18 (6%) endometrial stromal nodules and weakly to moderately positive in <5–40% of tumor cells in 6 of 31 (19%) leiomyosarcomas. No BCOR staining was seen in the remaining low-grade endometrial stromal sarcomas, endometrial stromal nodules, leiomyosarcomas, or any of the leiomyomas. BCOR immunohistochemical staining is a highly sensitive marker for *YWHAE-NUTM2* high-grade endometrial stromal sarcoma with both classic and unusual morphology and identifies a subset of high-grade endometrial stromal sarcoma with *BCOR* alterations, including *BCOR* rearrangement and internal tandem duplication.
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Endometrial stromal tumors are rare, but comprise the second most common type of uterine mesenchymal neoplasia after smooth muscle tumors, exhibiting a wide variety of morphologic patterns, genetic aberrations, and clinical outcomes. These neoplasms

are currently classified as endometrial stromal nodule, low-grade endometrial stromal sarcoma, high-grade endometrial stromal sarcoma, and undifferentiated uterine sarcoma by the World Health Organization.[1] Endometrial stromal nodules and low-grade endometrial stromal sarcomas are both characterized by small, bland, ovoid cells resembling proliferative-phase endometrial stroma and harbor rearrangement of genes often involved in transcriptional regulation with *JAZF1-SUZ12* fusion being most common.[2–4] In contrast, undifferentiated uterine sarcoma consists of highly pleomorphic cells bearing little resemblance to endometrial stroma, and most have complex karyotypes with numerous numerical and structural aberrations.[5] High-grade endometrial stromal sarcoma in the current World Health Organization classification is limited to tumors characterized by high-grade round cell morphology sometimes associated with a low-grade fibrous or fibromyxoid component and harboring t (10;17)(q22;p13) resulting in *YWHAE-NUTM2* fusion.[1,6,7] However, another recently described endometrial stromal sarcoma sharing morphologic overlap with myxoid leiomyosarcoma was found to harbor t(X;22)(p11.4;q13.2) resulting in *ZC3H7B-BCOR* fusion and has been proposed as another morphologic variant of high-grade endometrial stromal sarcoma due to its aggressive clinical behavior.[8] Other types of morphologically high-grade endometrial stromal sarcoma lacking *YWHAE*, *JAZF1*, *PHF1*, and *CCND1* rearrangements have also been described.[9] Recognition of high-grade endometrial stromal sarcoma as a distinct entity is important due to its prognosis being intermediate between low-grade endometrial stromal sarcoma and undifferentiated uterine sarcoma and different from leiomyosarcoma.[7,10]

Immunohistochemistry is often helpful in aiding the diagnosis of high-grade endometrial stromal sarcoma harboring *YWHAE-NUTM2* fusion, especially when molecular assays such as fluorescence *in situ* hybridization (FISH), reverse transcription (RT) or genomic polymerase chain reaction (PCR), and RNA sequencing are not readily available. CD10, estrogen receptor (ER), and progesterone receptor (PR) are usually negative in the high-grade round cell component, while positive in the low-grade fibrous or fibromyxoid component, if present, similar to the immunohistochemical profile seen in typical low-grade endometrial stromal sarcoma.[6] Homogeneous moderate to strong cyclin D1 staining in $\geq 70\%$ of tumor cells is also characteristic of the round cell component of *YWHAE-NUTM2* high-grade endometrial stromal sarcoma.[11] However, when present, cyclin D1 expression is variable in the low-grade fibrous or fibromyxoid component of *YWHAE-NUTM2* high-grade endometrial stromal sarcoma and can be strong and diffuse in undifferentiated uterine sarcoma and rarely in uterine leiomyosarcoma.[11] In our practice, we have also encountered examples of *YWHAE-NUTM2* high-grade endometrial stromal sarcoma in which cyclin D1 expression was weak and/or seen in $< 70\%$ of tumor cells in the round cell component, but the gene fusion was ultimately confirmed by FISH.

Recent studies have shown BCOR mRNA upregulation in most small blue round cell tumors of the soft tissues[12] and clear cell sarcomas of the kidney[13] harboring *BCOR* genetic abnormalities or *YWHAE* rearrangements. BCOR expression by immunoblotting and immunohistochemistry has also been reported in clear cell sarcoma of the kidney.[14,15] BCOR immunostaining has now emerged as a robust marker of *EWSR1*-negative small blue round cell tumors of the soft tissues harboring *YWHAE-NUTM2*, *BCOR-CCNB3*, and *BCOR-MAML3* fusions, as well as *BCOR* internal tandem duplications.[16] Its utility in the evaluation of uterine sarcomas, including *YWHAE-NUTM2* high-grade endometrial stromal sarcoma, has not yet been investigated. In this study, we assessed the sensitivity and specificity of BCOR expression in *YWHAE-NUTM2* high-grade endometrial stromal sarcoma.

Materials and methods

Case Selection

A total of 20 high-grade endometrial stromal sarcomas with *YWHAE-NUTM2* fusion and classic histology, 8 *BCOR*-rearranged high-grade endometrial stromal sarcomas, and 3 high-grade endometrial stromal sarcomas with unusual morphology and unknown gene rearrangement status were collected from four institutions. Eight high-grade endometrial stromal sarcomas with FISH confirmation of *YWHAE* rearrangement were identified by searching the Memorial Sloan Kettering Cancer Center (New York, NY, USA) clinical database for the terms, 'high-grade endometrial stromal sarcoma,' 'undifferentiated endometrial sarcoma,' and 'undifferentiated uterine sarcoma.' *YWHAE* rearrangement was identified by FISH as part of the clinical work up in five of the cases and a previous study in the remainder (unpublished data). An additional seven and six *YWHAE-NUTM2* high-grade endometrial stromal sarcomas confirmed by FISH were obtained from Vancouver General Hospital (Vancouver, Canada) and King Edward Memorial Hospital (Perth, Australia), respectively, and were previously reported.[6,17,18] Six high-grade endometrial stromal sarcomas with *BCOR* rearrangement originated from Memorial Sloan Kettering Cancer Center, including three that were previously reported,[8] and two from Vancouver General Hospital. All eight *BCOR*-rearranged high-grade endometrial stromal sarcomas were confirmed by FISH (two cases) or next-generation sequencing platforms using Archer FusionPlex (five cases), a targeted RNA sequencing assay that detects gene fusions and oncogenic isoforms in selected protein-coding exons of 35 genes, or MSK-Integrated

Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) (one case).[19] *ZC3H7B-BCOR* fusion was present in five tumors, while its reciprocal fusion by detected in three (Table 1). Three high-grade endometrial stromal sarcomas with unusual morphology and unknown rearrangement status were contributed by Memorial Sloan Kettering Cancer Center, Vancouver General Hospital, and Massachusetts General Hospital (Boston, MA, USA). A representative hematoxylin and eosin (H&E) slide of each tumor was reviewed for independent diagnostic confirmation by at least two gynecologic pathologists (S. C., C. H. L., C. J. R. S., E. O., L. N. H.). A total of 4- or 5-μm unstained slides of formalin-fixed, paraffin-embedded tissue sections from each of 28 high-grade endometrial stromal sarcomas were obtained.

To investigate the specificity of BCOR expression, staining in additional uterine mesenchymal tumors was also assessed. This cohort included triplicate 0.6 mm diameter core tissue microarrays of 38 previously reported uterine leiomyosarcomas and 19 previously reported uterine leiomyomas from Memorial Sloan Kettering Cancer Center;[20–22] duplicate and triplicate 0.6 mm diameter core tissue microarrays and whole tissue sections of 66 previously published low-grade endometrial stromal sarcomas from Massachusetts General Hospital, Memorial Sloan Kettering Cancer Center, and King Edward Memorial Hospital;[2,17,20–22] and duplicate and triplicate 0.6 mm diameter core tissue microarrays of 21 endometrial stromal nodules from Massachusetts General Hospital and Memorial Sloan Kettering Cancer Center.[2,20–22] A subset of low-grade endometrial stromal sarcomas and endometrial stromal nodules were obtained from the consultation files of one of the authors (E. O.) and the late Dr. Robert E. Scully, while all other tumors were obtained from the surgical pathology files of the participating institutions. Gene rearrangement status by FISH was known and previously reported for 34 low-grade endometrial stromal sarcomas and 8 endometrial stromal nodules, including 24, 3, and 2 tumors with *JAZF1-SUZ12*, *JAZF1-PHF1*, and *EPC1-PHF1* fusions, respectively, and 9 and 4 tumors with *JAZF1* and *PHF1* rearrangement with no known fusion partner, respectively.[2,17] All low-grade endometrial stromal sarcomas and endometrial stromal nodules were previously screened for *YWHAE* rearrangement by FISH.[7,17] A total of 4- to 5-μm unstained slides of the tissue microarrays and whole tumor tissue sections of 17 low-grade endometrial stromal sarcomas were obtained.

Immunohistochemistry

Immunohistochemical staining for BCOR was performed using a commercially available monoclonal antibody, clone C-10 (sc-514576; Santa Cruz, Dallas, TX) at 1:150 dilution (1.7 μg/ml). Staining was performed on the Leica Bond-3 autostaining system (Leica, Buffalo Grove, IL, USA), using heat-based antigen retrieval, a high pH buffer solution (Leica, ER2, 30 min), 30 min primary incubation time, and a polymer detection system (Refine, Leica), as previously reported.[16] A carrier-based multitissue block comprising of normal skin, colon, lung, testis, spleen, placenta, pancreas, liver, and kidney served as negative controls.[23] Results were evaluated by a gynecologic pathologist (S. C.). Intensity (strong, moderate, weak, and negative) and estimated percentage of positive tumor cells (nuclear staining only) were evaluated. BCOR immunohistochemistry was repeated on 5-μm whole tumor tissue sections for cases that showed any nuclear staining on the tissue microarray.

Table 1 Fusion transcripts of *BCOR*-rearranged high-grade endometrial stromal sarcoma detected by Archer FusionPlex and correlation with BCOR expression

Fusion	*BCOR expression (intensity, % cells)*
BCOR-ZC3H7B In frame, *BCOR* exon 6 and *ZC3H7B* exon 11	Negative
ZC3H7B-BCOR[a] In frame, *ZC3H7B* exon 6 and *BCOR* exon 14	Positive (strong, >95%)
BCOR-ZC3H7B In frame, *BCOR* exon 7 and *ZC3H7B* exon 11	Positive (strong, >95%)
ZC3H7B-BCOR In frame, *ZC3H7B* exon 10 and *BCOR* exon 7	Positive (strong, >95%)
ZC3H7B-BCOR In frame, *ZC3H7B* exon 10 and *BCOR* exon 7	Positive (weak, >95%)

[a]The reciprocal fusion was also detected.

Fluorescence *In Situ* Hybridization

Break-apart FISH for *YWHAE*, *BCOR*, and *BCORL1* rearrangement was performed on 5-μm whole tissue sections of tumors with no known gene rearrangement status demonstrating any nuclear BCOR expression by immunohistochemistry, as previously described.[8,12] Custom probes were made by bacterial artificial chromosomes (BAC) clones flanking the *YWHAE* (RP11-105D11, RP11-1142D6, RP11-170J13, RP11-806J5), *BCOR* (RP11-21D3, RP11-1105N2, RP11-37K20, RP11-973F20), and *BCORL1* (RP11-671B10, RP11-246J10, RP11-460L15, RP11-383B16) genes and obtained from BAC/PAC Resources (Children's Hospital Oakland Research Institute, Oakland, CA, USA). BAC clones were labeled with nick translation and validated on normal metaphase chromosomes. Briefly, 5-μm whole tissue sections from formalin-fixed, paraffin-embedded tissue blocks were mounted on charged slides. Slides were deparaffinized, pretreated, and hybridized with

denatured probes overnight, followed by post-hybridization washes and counterstaining with DAPI. Slides were examined on a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany) using Isis 5 software (Metasystems). Two hundred tumor nuclei were counted, and cases with >20% of nuclei with break-apart signals were considered positive.

PCR for *BCOR* Exon 16 Internal Tandem Duplication

Formalin-fixed, paraffin-embedded tumor and adjacent normal myometrial tissue were macrodissected in tumors with positive BCOR expression and no evidence of *YWHAE*, *BCOR*, and *BCORL1* rearrangement by FISH. Briefly, tumor and normal DNA were extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen). Primer sequences targeted exon 16 of *BCOR* (Fwd-1: 5′-GTCCTCCCGCATATTT CGC-3′ or Fwd-2: 5′-GACCTGGAAGCCTTCAACCC -3′ and Rev: 5′-CAAGCTGGACCCACCATGTAC-3′). PCR was performed using Advantage 2 PCR kit at an annealing temperature of 65.2 °C (Fwd-1) or 66.5 °C (Fwd-2) for 38 cycles, and PCR products were analyzed by agarose gel electrophoresis. Amplicons larger than wild type were subjected to Sanger sequencing to confirm the presence of internal tandem duplication.

Results

Strong and Diffuse BCOR Expression is Seen in the Round Cell Component of all *YWHAE-NUTM2* High-Grade Endometrial Stromal Sarcomas

Strong nuclear BCOR staining in >95% of tumor cells was seen in the round cell component of all 20 (100%) *YWHAE-NUTM2* high-grade endometrial stromal sarcomas (Figure 1). Six of these tumors also showed a low-grade fibrous or fibromyxoid component in which BCOR staining was variable in intensity and extent, ranging from weak (two cases) to strong (one case) and expression in <5–75% (mean, 30%) of tumor cells (Figure 1).

BCOR Expression Identifies High-Grade Endometrial Stromal Sarcomas with Unusual Morphology, Including One Harboring *BCOR* Internal Tandem Duplication

BCOR immunohistochemistry was helpful in the assessment of three high-grade endometrial stromal sarcomas demonstrating unusual morphologic features. One tumor was a pulmonary metastasis from a patient with known *YWHAE-NUTM2* fusion previously confirmed in the primary uterine tumor. While the primary tumor showed typical morphologic features of *YWHAE-NUTM2* high-grade endometrial stromal sarcoma, the pulmonary metastasis was less cellular and mitotically active and consisted of spindled to stellate cells with slightly enlarged ovoid nuclei with prominent nucleoli, embedded in abundant myxoid stroma (Figure 2a and b). Strong and diffuse nuclear BCOR expression was seen throughout the metastatic tumor (Figure 2c).

The second high-grade endometrial stromal sarcoma was a primary uterine tumor that predominately consisted of highly atypical cells with marked nuclear pleomorphism and prominent nucleoli arranged in sheets and nests surrounded by hyalinized to myxoid stroma (pleomorphic undifferentiated uterine sarcoma) (Figure 2d and f). The mitotic index was 56/10 high power fields, including numerous atypical mitoses. Within the dominant pleomorphic component, there was a distinct focus of smaller, more uniform spindled cells with ovoid nuclei associated with delicate vasculature and a mitotic index of 10/10 high power fields (Figure 2d and e). While the pleomorphic sarcoma component demonstrated only weak and focal cyclin D1 positivity (Figure 2g), BCOR expression was diffuse and strong (Figure 2h), prompting FISH studies which confirmed the presence of a *YWHAE* rearrangement (Figure 2i).

The third tumor was a uterine sarcoma in a 25 year old patient that demonstrated some features similar to and other features differing from *YWHAE-NUTM2* high-grade endometrial stromal sarcoma. The tumor had a tongue-like pattern of myometrial invasion typical of stromal sarcomas (Figure 3a) and had a low-grade fibromyxoid component with small spindled cells (Figure 3b) and a high-grade round cell component with larger, round nuclei (Figure 3c). However, focal myxoid stroma within the round cell component (Figure 3d) was noted, and some spindled areas demonstrated larger cells with striking nuclear atypia (Figure 3e). The immunophenotype showed strong and diffuse cyclin D1 staining, only focal CD10 positivity, and absent ER and PR expression. BCOR expression was strong and diffuse throughout the tumor, including the round and spindled cell components (Figure 3f). This pattern of BCOR expression prompted FISH analysis for *BCOR*, *BCORL1*, and *YWHAE* rearrangement, but none were detected. However, an in-frame *BCOR* internal tandem duplication was identified by genomic PCR and targeted DNA sequencing in which the duplicated sequence from *BCOR* exon 16 spanned 86 bp with a 4-bp insertion between duplicated sequences (Figure 4). *BCOR* internal tandem duplication was absent in the adjacent normal myometrium.

A Subset of *BCOR*-Rearranged High-Grade Endometrial Stromal Sarcomas Demonstrates BCOR Immunoreactivity

We evaluated BCOR expression in whole tissue sections of five *ZC3H7B-BCOR* and three *BCOR-ZC3H7B* high-grade endometrial stromal sarcomas,

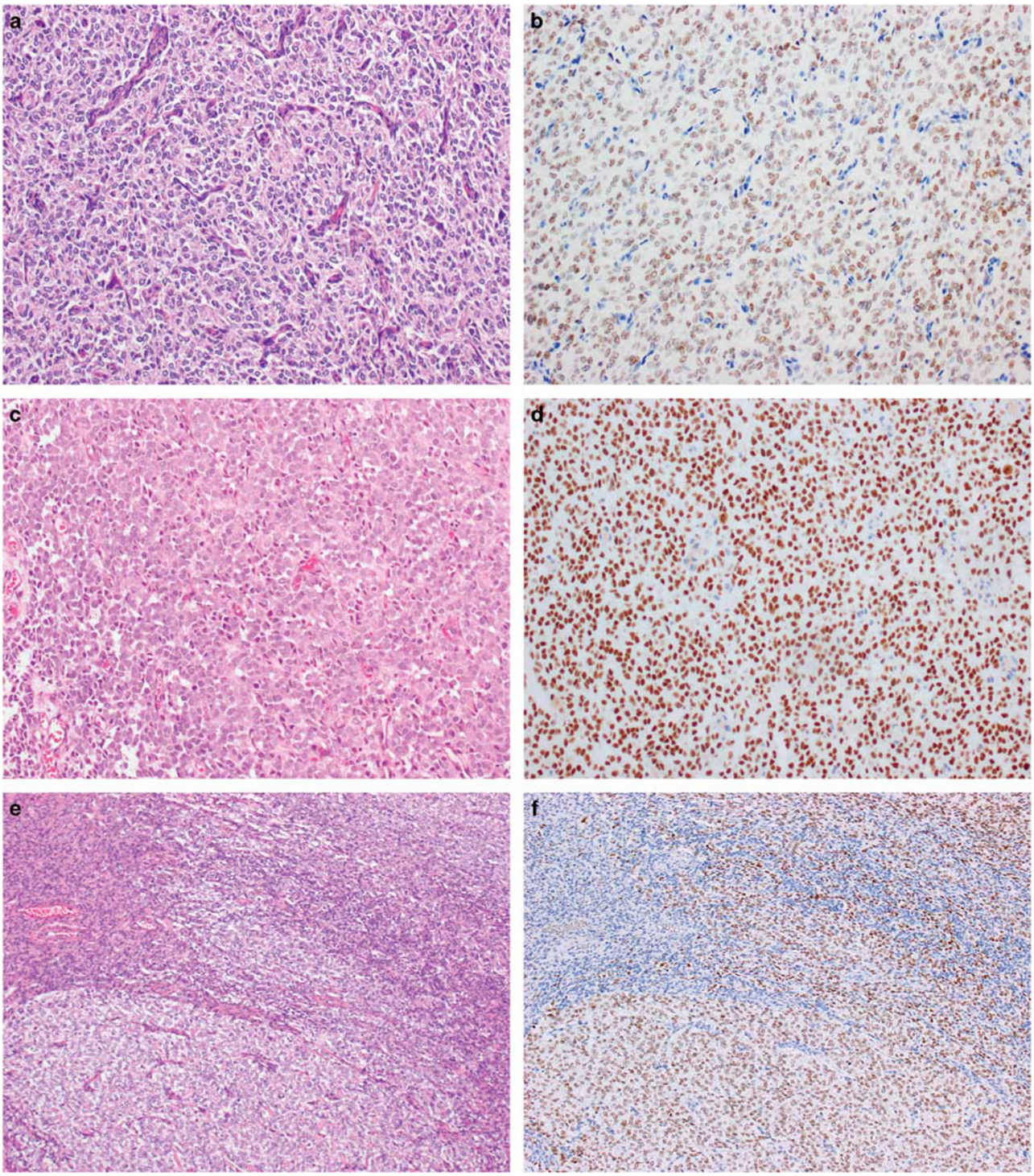

Figure 1 Distinct BCOR expression in the low-grade spindled cell and round cell components of *YWHAE-NUTM2* high-grade endometrial stromal sarcoma. Representative H&E (**a**, **c**) and immunohistochemical stains (**b**, **d**) demonstrating diffuse nuclear BCOR staining that may be moderate (**b**) or strong (**d**) in intensity in the round cell component. The low-grade component (**e**, top left) shows focal, weak BCOR immunostaining (**f**, top left) compared to strong and diffuse BCOR expression (**f**, bottom right) in the round cell component (**e**, bottom right).

and found nuclear staining in >95% of tumor cells that was strong in three cases (two *ZC3H7B-BCOR* and one *BCOR-ZC3H7B*) (Figure 5a and b) and weak in another (*ZC3H7B-BCOR*) (Figure 5c and d) (Table 1). BCOR staining was absent in the remaining four tumors. Among the three *ZC3H7B-BCOR* high-grade endometrial stromal sarcomas with fusion transcriptome data, the reciprocal fusion was detected in only one tumor that showed strong and diffuse BCOR expression.

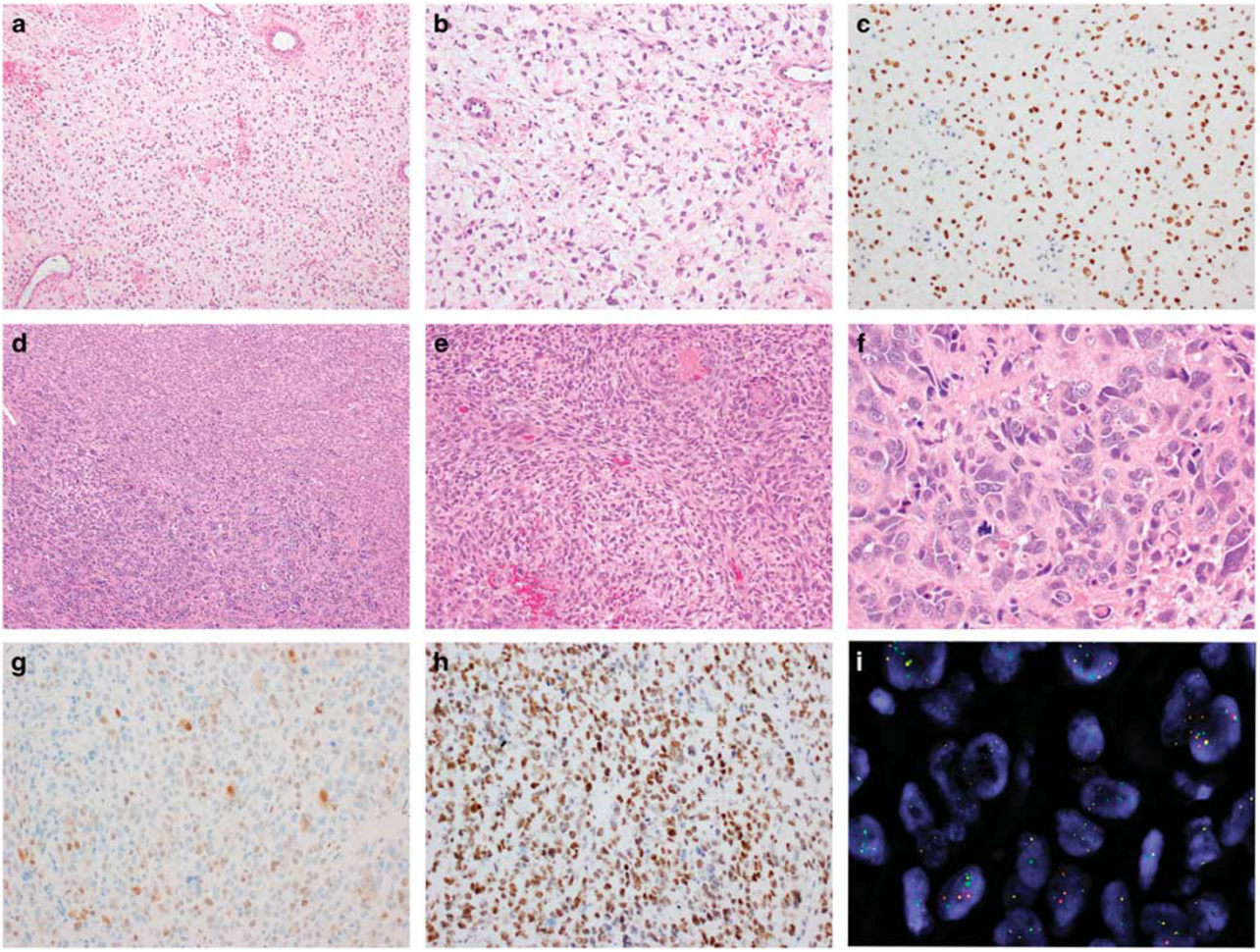

Figure 2 BCOR expression in two cases of *YWHAE-NUTM2* high-grade endometrial stromal sarcoma with unusual histology. A pulmonary metastasis composed of intermediate-grade fibromyxoid spindled cells with low mitotic index (**a**, **b**) exhibits strong and diffuse BCOR immunoexpression (**c**). Pleomorphic undifferentiated sarcoma (**d**, bottom left, **f**) composed of cells with marked nuclear pleomorphism, brisk mitotic activity, and atypical mitoses in a background of hyalinized stroma adjacent to high-grade endometrial stromal sarcoma (**d**, top right, **e**) demonstrating spindled cells with ovoid nuclei and prominent nucleoli vaguely whorling around delicate arterioles. The pleomorphic sarcoma component shows only focal weak to moderate cyclin D1 staining (**g**), but diffuse and strong BCOR expression (**h**). Separation of green 5′ and red 3′ signals and single green 5′ signals (**i**) confirm *YWHAE* rearrangement by break-apart FISH.

BCOR Expression is not Seen in Most Other Uterine Mesenchymal Tumors

BCOR immunohistochemical staining was evaluated in 31 of 38 (82%) leiomyosarcomas, 18 of 19 (95%) leiomyomas, all 66 (100%) low-grade endometrial stromal sarcomas, and 18 of 21 (86%) endometrial stromal nodules. Staining was not evaluable in the remaining tumors in which tissue cores were no longer present on the tissue microarray slides (Figure 6). Weak BCOR staining in $<5\%$ of tumor cells was seen in 4 (6%) low-grade endometrial stromal sarcomas and 1 of 18 (6%) endometrial stromal nodules. Gene rearrangement status was known in two of the low-grade endometrial stromal sarcomas exhibiting focal, weak BCOR expression with *JAZF1-SUZ12* fusion in one and *JAZF1* rearrangement with no known partner in the other. Weak to moderate BCOR staining was seen in <5–40% (mean, 22%) of tumor cells in 6 of 31 (19%) leiomyosarcomas. Among four leiomyosarcomas for which whole tissue sections were available, weak to moderate BCOR expression was seen in $<5\%$ of cells in three tumors and 50% of cells in one tumor. Genomic PCR was performed on the leiomyosarcomas demonstrating more extensive BCOR staining, and *BCOR* exon 16 internal tandem duplication was not detected. BCOR staining was not seen in any of the leiomyomas.

Discussion

Identification of *YWHAE-NUTM2* high-grade endometrial stromal sarcomas is important due to its different prognosis and management from low-grade endometrial stromal sarcomas and undifferentiated uterine sarcomas, and novel commercially available

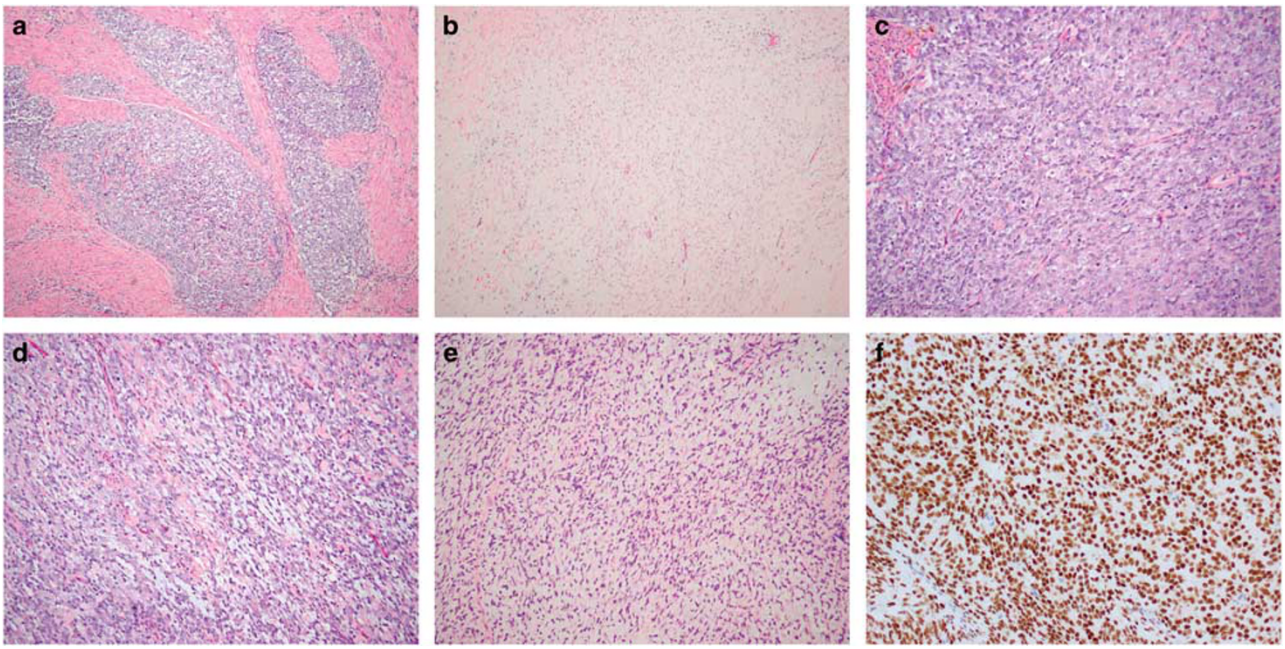

Figure 3 Morphologic features and BCOR staining pattern of high-grade endometrial stromal sarcoma harboring *BCOR* internal tandem duplication. (**a**) Tongue-like myometrial invasion. Low-grade fibromyxoid (**b**) and high-grade round cell (**c**) components similar to features seen in *YWHAE-NUTM2* high-grade endometrial stromal sarcoma. (**d**) Foci of myxoid stroma within the round cell component. (**e**) More extensive nuclear atypia within the fibromyxoid component. (**f**) Diffuse and strong BCOR expression.

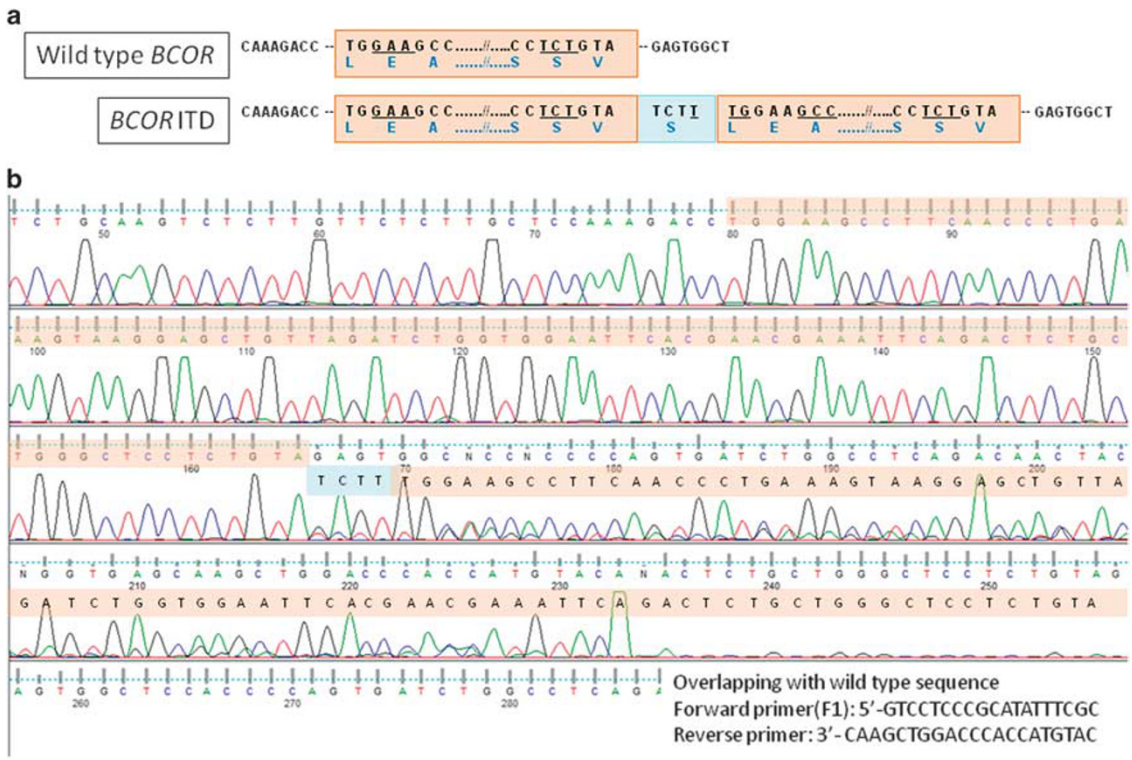

Figure 4 *BCOR* exon 16 internal tandem duplication in high-grade endometrial stromal sarcoma. (**a**) Schematic demonstrating wild type *BCOR* (top) and *BCOR* internal tandem duplication (bottom) sequences. (**b**) Duplicated region (highlighted in beige) overlapping with the *BCOR* wild type sequence with a 4-bp insertion (highlighted in blue) in between duplicated regions.

biomarkers such as BCOR may help in distinguishing high-grade endometrial stromal sarcomas from these and other types of uterine sarcomas potentially in the differential diagnosis. Herein, we showed that BCOR nuclear immunoexpression was strong and diffuse in the round cell component of all 20 *YWHAE-NUTM2* high-grade endometrial stromal sarcomas of classic histology studied. A similar

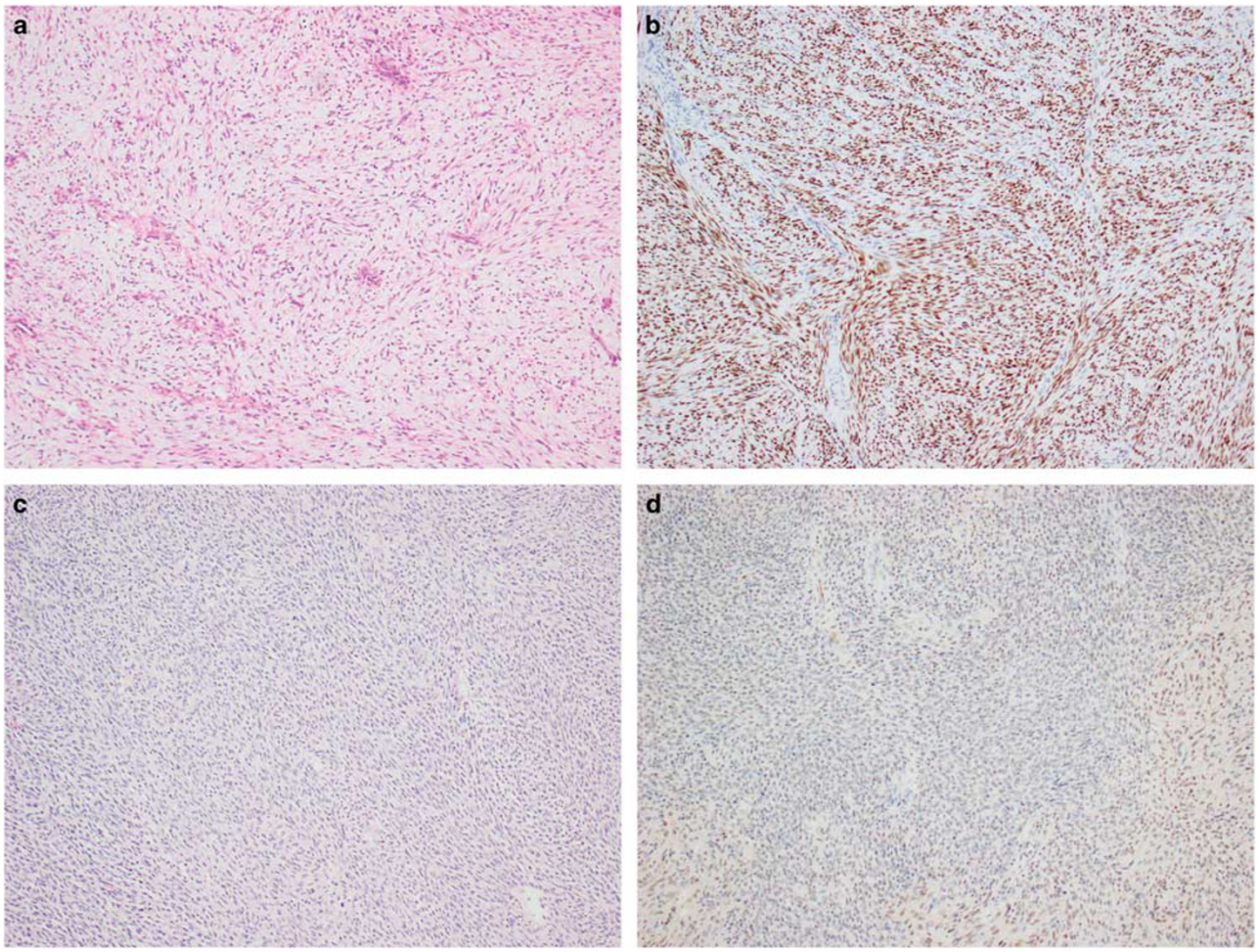

Figure 5 BCOR expression in *ZC3H7B-BCOR* high-grade endometrial stromal sarcoma. (**a** and **b**) Myxoid spindled cell sarcoma demonstrating strong and diffuse BCOR staining. (**c** and **d**) Spindled cell sarcoma with less obvious myxoid features showing only weak BCOR expression.

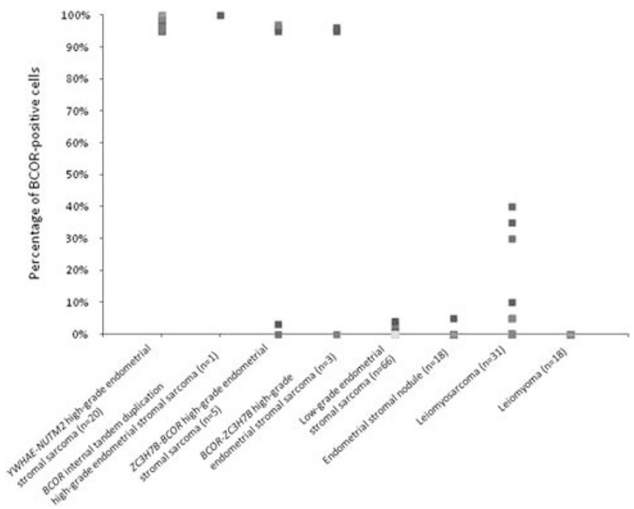

Figure 6 Percentage of BCOR-positive tumor cells in high-grade endometrial stromal sarcoma harboring *YWHAE-NUTM2*, *ZC3H7B-BCOR*, and *BCOR-ZC3H7B* fusions and *BCOR* internal tandem duplication, low-grade endometrial stromal sarcoma, endometrial stromal nodule, leiomyosarcoma, and leiomyoma.

staining pattern was seen in three high-grade endometrial stromal sarcomas of unusual morphology which prompted FISH confirmation of *YWHAE* rearrangement in two and identification of a novel somatic *BCOR* in-frame internal tandem duplication by genomic PCR and targeted DNA sequencing of BCOR exon 16 in the third tumor. Diffuse BCOR expression was also seen in 50% (four of eight) of *BCOR*-rearranged high-grade endometrial stromal sarcomas, including tumors harboring *ZC3H7B-BCOR* and its reciprocal fusion. Staining was largely negative in other uterine mesenchymal tumors, with only limited, weak to moderate reactivity seen in 6% (4 of 66) of low-grade endometrial stromal sarcomas, 6% (1 of 18) of endometrial stromal nodules and 19% (6 of 31) of leiomyosarcomas.

Since the recent histologic description of *YWHAE-NUTM2* high-grade endometrial stromal sarcomas,[6] cyclin D1 along with CD10, ER, and PR have so far served as the most helpful immunohistochemical markers in the diagnosis of these tumors.[11] Strong and diffuse cyclin D1 staining coupled with absent CD10, ER, and PR expression is highly characteristic

of the round cell component of *YWHAE-NUTM2* high-grade endometrial stromal sarcomas, while variable cyclin D1 and positive CD10, ER, and PR staining is seen in the low-grade component if present.[11] On the basis of our study, BCOR appears to be at least equally robust in identifying the round cell component of these tumors, while the staining varies in the low-grade component, similar to what has been reported with cyclin D1.[11] However, our findings also suggest that the utility of BCOR immunohistochemistry may surpass that of cyclin D1 particularly in *YWHAE-NUTM2* tumors demonstrating unusual morphology or in the minority of classic *YWHAE-NUTM2* high-grade endometrial stromal sarcomas that fail to demonstrate diffuse cyclin D1 expression. While we did not perform a direct comparison of cyclin D1 and BCOR in our study, at least one of our *YWHAE-NUTM2* high-grade endometrial stromal sarcomas with classic histology had only focal, weak cyclin D1 staining, while BCOR was strongly positive. BCOR expression was also strong and diffuse in a metastatic paucicellular *YWHAE-NUTM2* high-grade endometrial stromal sarcoma with a myxoid background, as well as a pleomorphic undifferentiated sarcoma showing only focal cyclin D1 expression of variable intensity, but demonstrating *YWHAE* rearrangement by FISH. The latter tumor is particularly noteworthy given that de-differentiation or transformation into undifferentiated sarcoma may occur in *YWHAE-NUTM2* high-grade endometrial stromal sarcomas similar to what has rarely been reported in low-grade endometrial stromal sarcomas.[24,25] Therefore, BCOR immunohistochemistry may be informative regarding the histogenesis of some pleomorphic undifferentiated uterine sarcomas.

In addition to identifying the round cell component of *YWHAE-NUTM2* high-grade endometrial stromal sarcomas, BCOR expression led to the discovery of a novel in-frame *BCOR* internal tandem duplication in one of our high-grade endometrial stromal sarcomas sharing both morphologic and immunophenotypic features with *YWHAE-NUTM2* high-grade endometrial stromal sarcomas, but lacking *YWHAE*, *BCOR*, and *BCORL1* rearrangement. Highly recurrent *BCOR* internal tandem duplications have recently been discovered in clear cell sarcoma of the kidney and small blue round cell tumors of the soft tissues in infants.[12,14,15,26,27] The discovery of *BCOR* internal tandem duplication in one of our high-grade endometrial stromal sarcomas now expands the spectrum of cancers in which *BCOR* internal tandem duplication appears to be a genetic driver of tumorigenesis. While *BCOR* internal tandem duplication is present in the vast majority of clear cell sarcomas of the kidney,[14,15,27] its prevalence among uterine sarcomas is currently unknown. However, with the application of BCOR immunohistochemistry in the routine evaluation of uterine sarcomas, particularly those that are morphologically reminiscent of *YWHAE-NUTM2* high-grade endometrial stromal sarcomas or undifferentiated uterine sarcomas of uniform type lacking *YWHAE* or other gene rearrangements more common among endometrial stromal sarcomas, additional examples of high-grade endometrial stromal sarcomas with this unusual genetic abnormality may be identified. *BCOR* internal tandem duplication and *YWHAE-NUTM2* fusion appear mutually exclusive in clear cell sarcoma of the kidney.[26,27] Future studies confirming this finding in high-grade endometrial stromal sarcomas should be undertaken.

Our findings demonstrate that BCOR expression is present in 50% of *BCOR*-rearranged high-grade endometrial stromal sarcomas and *ZC3H7B-BCOR* fusion was recently described in an uncommon group of endometrial stromal sarcomas.[7,28] In a previous study, we described the unique histologic features of this tumor which significantly overlap with myxoid leiomyosarcoma. While our cohort was small, the clinical data suggested that *ZC3H7B-BCOR* endometrial stromal sarcoma was clinically aggressive compared to low-grade endometrial stromal sarcoma which typically follows an indolent course.[8] Compared to the three *ZC3H7B-BCOR* high-grade endometrial stromal sarcomas that were previously published,[8] the five additional *BCOR*-rearranged high-grade endometrial stromal sarcomas in our current study displayed similar morphologic and immunohistochemical features, including extensive myxoid change, focal fascicular architecture, brisk mitotic activity of at least 10 mitoses/10 high power fields, diffuse CD10 expression, variable ER and PR staining, and either negative or only focal desmin or SMA positivity. While BCOR expression was absent in two previously reported *ZC3H7B-BCOR* high-grade endometrial stromal sarcomas,[16] we have shown here for the first time that diffuse BCOR staining may be seen in other high-grade endometrial stromal sarcomas harboring *ZC3H7B-BCOR* or its reciprocal fusion. The BCOR antibody clone C-10 detects the epitope encoded by exons 1, 2, and 3 and part of exon 4 of *BCOR*. On the basis our findings, BCOR immunoexpression does not appear to correlate with *BCOR* breakpoints in the fusion transcripts. Among the three *ZC3H7B-BCOR* high-grade endometrial stromal sarcomas with fusion transcriptome data, the reciprocal fusion was observed only in one case which demonstrated strong BCOR expression. Given the identification of *ZC3H7B-BCOR* and *BCOR-ZC3H7B* fusions among our cohort of high-grade endometrial stromal sarcomas and lack of correlation between BCOR expression pattern and either gene fusion, it remains uncertain whether *ZC3H7B* or *BCOR* constitutes the genetic driver of these tumors.

Our results also suggest that BCOR expression is a reliable marker that can distinguish high-grade endometrial stromal sarcomas with *YWHAE-NUTM2*, *ZC3H7B-BCOR*, *BCOR-ZC3H7B*, and *BCOR* internal tandem duplication from other uterine mesenchymal tumors. In contrast to strong and

diffuse BCOR staining in high-grade endometrial stromal sarcomas harboring *YWHAE-NUTM2* fusion or *BCOR* internal tandem duplication and a subset of *BCOR*-rearranged high-grade endometrial stromal sarcomas, BCOR immunoreactivity of only weak to moderate intensity was present in <5–40% of tumor cells among $<6\%$ of low-grade endometrial stromal sarcomas and endometrial stromal nodules and $\sim 20\%$ of leiomyosarcomas. The presence of focal BCOR staining in a subset of leiomyosarcomas may be a potential caveat since this tumor type is in the differential diagnosis of high-grade endometrial stromal sarcomas, particularly those harboring *BCOR* rearrangements. BCOR expression was not found in the remaining tumors or any of the leiomyomas tested.

The mechanism by which BCOR expression is upregulated in tumors harboring *BCOR* genetic aberrations and *YWHAE-NUTM2* gene fusions is unclear. BCOR is encoded by the BCL-6 corepressor (*BCOR*) gene located on Xp11.4 and interacts with PCGF1 within a variant polycomb repressive complex (PRC1) that suppresses gene expression by histone modification.[29,30] Ubiquitous expression of BCOR mRNA is seen in a variety of normal human tissues,[29] and among tumors, high expression of BCOR transcripts and protein have been found in most round cell sarcomas of the bone and soft tissues and clear cell sarcomas of the kidney with *BCOR* genetic aberrations or *YWHAE-NUTM2* fusion.[12,14,15] In tumors with *BCOR* internal tandem duplication, the duplicated *BCOR* region resides within a PUFD domain that facilitates binding with PCGF1, another member of the variant PRC1, and may disrupt the structure and/or function of the PRC1 in epigenetic modification.[14,15] Further studies are needed to investigate the impact of *BCOR* internal tandem duplication, *BCOR* and *YWHAE* rearrangements on PRC1 function, and the mechanism of high-grade endometrial stromal sarcoma oncogenesis.

In summary, BCOR expression is highly sensitive in identifying high-grade endometrial stromal sarcomas harboring *YWHAE-NUTM2* fusion and *BCOR* internal tandem duplication as well as a subset of *BCOR*-rearranged high-grade endometrial stromal sarcomas. Diffuse and strong expression should prompt FISH, RT-PCR, or RNA sequencing confirmation of *YWHAE* and *BCOR* rearrangement or targeted DNA sequencing for *BCOR* internal tandem duplication if those rearrangements are not detected. Identification of *BCOR* internal tandem duplication and similar BCOR immunophenotype in high-grade endometrial stromal sarcomas adds to the growing body of histologic, immunophenotypic, and genetic evidence uniting these tumors with clear cell sarcoma of the kidney and soft tissue round cell sarcomas.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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