BCOR Overexpression Is a Highly Sensitive Marker in Round Cell Sarcomas With BCOR Genetic Abnormalities

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BCOR Overexpression is a Highly Sensitive Marker in Round Cell Sarcomas with *BCOR* Genetic Abnormalities

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Abstract

With the advent of next-generation sequencing, an increasing number of novel gene fusions and other abnormalities have emerged recently in the spectrum of *EWSR1*-negative small blue round cell tumors (SBRCTs). In this regard, a subset of SBRCTs harboring either *BCOR* gene fusions (*BCOR-CCNB3, BCOR-MAML3*), *BCOR* internal tandem duplications (ITD), or *YWHAE-NUTM2B* share a transcriptional signature including high *BCOR* mRNA expression, as well as similar histologic features. Furthermore, other tumors such as clear cell sarcoma of kidney (CCSK) and primitive myxoid mesenchymal tumor of infancy (PMMTI) also demonstrate *BCOR* ITDs and high *BCOR* gene expression. The molecular diagnosis of these various *BCOR* genetic alterations requires an elaborate methodology including custom BAC FISH probes and RT-PCR assays. As these tumors show high level of *BCOR* overexpression regardless of the genetic mechanism involved, either conventional gene fusion or ITD, we sought to investigate the performance of an anti-BCOR monoclonal antibody clone C-10 (sc-514576) as an immunohistochemical marker for sarcomas with *BCOR* gene abnormalities. Thus we assessed the BCOR expression in a pathologically and genetically well-characterized cohort of 25 SBRCTs, spanning various *BCOR*-related fusions and ITDs and *YWHAE-NUTM2B* fusion. In addition we included related pathologic entities such as 8 CCSKs and other sarcomas with *BCOR* gene fusions. As a control group we included 20 SBRCT with various (non-*BCOR*) genetic abnormalities, 10 fusion-negative SBRCT, 74 synovial sarcomas, 29 rhabdomyosarcoma and other

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sarcoma types. Additionally we evaluated the same study group for SATB2 immunoreactivity, as these tumors also showed *SATB2* mRNA up-regulation. All SBRCTs with *BCOR-MAML3* and *BCOR-CCNB3* fusions, as well as most with *BCOR* ITD (93%), and all CCSKs showed strong and diffuse nuclear BCOR immunoreactivity. Furthermore, all SBRCTs with *YWHAE-NUTM2B* also were positive. SATB2 stain was also positive in tumors with *YWHAE-NUTM2B, BCOR-MAML3, BCOR* ITD (75%), *BCOR-CCNB3* (71%), and a subset of CCSKs (33%). In conclusion, BCOR immunohistochemical stain is a highly sensitive marker for SBRCTs and CCSKs with *BCOR* abnormalities and *YWHAE* rearrangements and can be used as a useful diagnostic marker in these various molecular subsets. SATB2 immunoreactivity is also present in the majority of this group of tumors.

Keywords

small blue round cell tumor; clear cell sarcoma kidney; synovial sarcoma; BCOR; SATB2; YWHAE; immunohistochemistry

INTRODUCTION

Round cell sarcomas encompass a heterogeneous group of tumors, which, despite similar cytomorphology of primitive small blue round cells, have diverse genetic abnormalities, clinical presentations and outcomes. Until recently, a substantial subset of tumors resembling Ewing sarcoma microscopically and occurring in children or young adults remained unclassified, lacking known gene fusions involving *EWSR1, FUS, SS18, FOXO1*, etc. As a result of the wide application of next generation sequencing to undifferentiated round cell sarcomas, an increasing number of novel genetic abnormalities have been identified including: CIC-DUX4, CIC-FOXO4, BCOR-CCNB3, BCOR-MAML3, ZC3H7B-BCOR, YWHAE-NUTM2B gene fusions or *BCOR* internal tandem duplication (ITD).[1-6] However, the diagnosis of these emerging entities relies largely on elaborate molecular tests, such as FISH, RT-PCR or genomic PCR, which are not widely available. Although the use of immunohistochemical markers remains a more practical approach to screen and support the diagnosis in this setting, there are only a handful of antibodies available which can be reliably used in the differential diagnosis of Ewing sarcoma-like tumors. Two such examples include the consistent nuclear expression of WT1 in *CIC-DUX4* positive small blue round cell tumors (SBRCTs)[7] and the CCNB3 nuclear overexpression in *BCOR-CCNB3* positive tumors.[4,8,9] Although most SBRCTs harboring either *BCOR* genetic abnormalities or *YWHAE* fusions show significant *BCOR* mRNA up-regulation,[6,10,11] no BCOR immunohistochemical marker has been investigated to date in this molecular subset of tumors. BCOR protein expression has been demonstrated by immunoblotting and immunohistochemistry in a small number of CCSKs.[10,11] Based on our previous findings of *BCOR* and *SATB2* up-regulation in round cell sarcomas with *BCOR* ITD, *YWHAE-NUTM2B*, and *BCOR-MAML3* fusions by RNA sequencing,[6] we sought to investigate the sensitivity and specificity of BCOR and SATB2 antibodies in this well-defined molecular cohort.

MATERIALS AND METHODS

Case selection

Round cell sarcomas with *BCOR* ITD (n=14; 8 undifferentiated round cell sarcoma, 6 primitive myxoid mesenchymal tumor of infancy), *YWHAE-NUTM2B* (n=2), *BCOR-CCNB3* (n=8), and *BCOR-MAML3* (n=1) gene fusions were collected from the consultation archives of the corresponding author (CRA), most being previously reported.[5,6] All 16 tumors with *BCOR* ITD or *YWHAE-NUTM2B* occurred in infants (age: 10 days to 11 months old) and involved abdominopelvic cavity/ retroperitoneum (n=6), trunk (n=7) and head and neck (n=3). Among the 8 *BCOR-CCNB3* tumors, 5 arose in bone (2 pelvis, 2 femur, 1 tibia) and 3 in the soft tissues (2 paraspinal, 1 chest wall), with an age range of 10-21 years. The *BCOR-MAML3* tumor occurred as a bulky intra-abdominal mass in a 44 year-old male patient. In addition, we investigated 8 CCSKs, 4 harboring *BCOR* ITD and 1 *YWHAE-NUTM2B/E* fusion. The remaining 3 CCSKs lacking both genetic abnormalities showed typical histomorphology and age at presentation (10 months, 19 months, and 5 years, respectively). Three other tumors harboring *ZC3H7B-BCOR* fusion were tested, including 2 endometrial stromal sarcomas and 1 ossifying fibromyxoid tumor. Within the control group, there were 8 Ewing sarcomas with *EWSR1-FLI1* fusion, 1 Ewing sarcoma-like with *EWSR1-NFATC2* fusion, 6 SBRCTs with *CIC* gene rearrangements, 5 desmoplastic small round cell tumors (DSRCTs) with *EWSR1-WT1* fusion, 10 SBRCTs lacking known genetic abnormalities, and 3 poorly differentiated synovial sarcomas with *SS18* gene fusions. All of the above cases were tested using whole-tissue sections from formalin-fixed paraffin-embedded (FFPE) tissues.

To further investigate the specificity of the BCOR antibody, we screened a large spectrum of soft tissue tumors, using tissue microarrays (TMA) of synovial sarcoma (n=71), rhabdomyosarcoma (n=29), angiosarcoma (n=41), low grade fibromyxoid sarcoma (n=14), chondrosarcoma (n=41), and myxofibrosarcoma (n=92). The fusion transcript types were known in a subset of synovial sarcomas, including 18 *SS18-SSX1* and 16 *SS18-SSX2* fusion cases.

Immunohistochemical BCOR protein expression analysis

Immunohistochemical staining for BCOR was performed in all cases. A commercially available antibody, clone C10 (sc-514576; Santa Cruz, Dallas, TX) was used for the immunohistochemical analysis. The antibody was generated against the N-terminus (1-300 amino acid) of BCOR. The antibody was optimized using normal and molecularly pre-typed tumor tissues. All assays were performed on a Leica Bond-3 autostainer platform (Leica, Buffalo Grioe, IL). C-10 worked best at a dilution of 1:150 (1.7ug/ml), employing heat-based antigen retrieval, using a high pH buffer solution (Leica, ER2, 30') and a primary incubation time of 30 minutes. A polymer-based secondary system (Refine, Leica) was used to detect the primary reagent. A carrier-based multi tissue block comprising of a variety of normal tissues (spleen, placenta, kidney, colon, liver, skin, pancreas, testis, and lung) as well as tumor tissue sections were used during the antibody optimization stage and as controls.[12] For example, spermatogonia in the seminiferous tubules were found to show distinct nuclear

stain in a clean background corresponding to the BCOR mRNA expression in normal tissues (Fig. 1). Other tissues were used as negative controls.

The results were evaluated based on the intensity (strong, moderate, weak, and negative) and the estimated percentage of positive tumor cells. Only nuclear stain was counted. Tumors with moderate to strong staining intensity in more than 10% of the tumor were considered positive.

Immunohistochemical SATB2 protein expression analysis

SATB2 immunoreactivity was evaluated in a subset of cases using whole sections, including round cell sarcomas with *BCOR* ITD (n=12), *YWHAE-NUTM2B* (n=2), *BCOR-MAML3* (n=1), *BCOR-CCNB3* (n=7), CCSKs (n=6), and poorly differentiated synovial sarcomas (n=2). Additionally, we tested this antibody on TMA sections of synovial sarcoma (n=73) and rhabdomyosarcoma (n=29). Monoclonal rabbit antibody clone EP281 (1:200; CellMarque, California, USA) was used for the detection of SATB2. As with C-10, the staining was performed on Leica (Bond-3) automated staining system (Leica), employing heat-based antigen retrieval (ER2, Leica, 30') and a polymer (Leica, Refine) detection system (Leica). Normal colonic mucosa served as positive control throughout. Only nuclear stain was considered positive. The results were recorded as described above.

Gene expression array and transcription factor motif analysis

To investigate the mechanism of *BCOR* up-regulation in round cell sarcomas with *BCOR* abnormalities or *YWHAE* fusions, we analyzed the overexpressed transcription factors (TFs) in these tumors for potential binding sites to the *BCOR* gene promoter area (3 KB upstream from the transcription start site). Among the 404 differentially expressed gene signature of *BCOR* ITD tumors by RNA sequencing,[6] there were 64 genes labeled as TF or TF-related genes, which were then searched for known motif sequences using available TF motifs databases (HOCOMOCO, motifDB, and factorbook tools).

Due to unexpected BCOR immunoreactivity in a subset of synovial sarcomas, we further analyzed the gene expression profiles of synovial sarcoma from publicly available array data and compared them to the signature of *BCOR-CCNB3* sarcomas and Ewing sarcomas, available on the same platform. Datasets from 34 synovial sarcomas (GSE20196), 10 *BCOR-CCNB3* sarcomas (GSE34800), and 4 Ewing sarcomas (GSE34800) on Affymetrix Human Genome U133A Plus 2 were obtained.[4,13] The data analysis of the 34 synovial sarcomas was also investigated in relationship to the histologic subtype (monophasic, biphasic and poorly differentiated) according to the information provided.[13] For comparison, we included the expression data of normal human tissues and non-sarcomatous tumoral tissues (randomly selected samples from GSE16515,[14] GSE50161,[15] GSE7307). After the signal intensities were log2-transformed, the expression levels of *BCOR* and *SATB2* in these samples were evaluated and compared to the round cell sarcomas with *BCOR* abnormalities or *YWHAE* fusions and CCSKs. Statistical t-test and false discovery rate (FDR) were applied to identify differentially expressed genes in synovial sarcomas comparing to the control group, using a threshold of log2 fold change negative 5 or positive 2 with 0.01 FDR. A similar approach was then used to identify possible TFs leading to high *BCOR* expression

in synovial sarcomas. In addition, as both *SSX1* (Xp11.23) or *SSX2* (Xp11.22) and *BCOR* (Xp11.4) are located on the p-arm of chromosome X, we investigated the expression of neighboring genes within a 21.8 MB region (chr X: 31,000,000-52,792,617) for potential coupled upregulation.

RESULTS

Strong and diffuse BCOR immunoexpression across all sarcomas with *BCOR*-genetic abnormalities

The results of BCOR immunohistochemical stains are summarized in Table 1. All SBRCTs with *BCOR* ITD, *YWHAE-NUTM2B, BCOR-MAML3* and *BCOR-CCNB3* fusions showed nuclear expression of BCOR (Fig. 2), except for one *BCOR* ITD-positive tumor. The staining patterns were typically strong and diffuse, except for 2 cases in the *BCOR* ITD group showing moderate intensity and patchy staining (60% in an abdominal wall mass of a 9 month-old female and 30% in a cauterized specimen from a paravertebral mass of a 10 month-old male). Regardless of the cytomorphologic features (round, stellate or spindle cells), the staining pattern was diffuse. Rich capillary network and occasional fibrous septa in these tumors were distinctively negative, serving as internal negative controls. The only *BCOR* ITD SBRCT negative for BCOR immunohistochemical stain occurred in an 11 month-old male with a thoracic mass. The presence of *BCOR* ITD was re-confirmed by PCR using genomic DNA from FFPE tissues, but no material was available for mRNA expression analysis.

Ewing sarcomas and *CIC*-rearranged tumors were negative, with only one case in each group showing patchy moderate nuclear positivity (Supplementary Fig. 1). DSRCTs were also negative. Interestingly, among the 10 undifferentiated round cell sarcomas lacking known gene fusions, 2 cases were diffusely and strongly immunopositive for BCOR (Supplementary Fig. 2). One of them occurred in a 1 year-old female presenting with an orbital mass, which was negative for all immunostains tested including CD99, desmin, myogenin, TdT, CD45, etc. Morphologically, it showed diffuse sheets of monomorphic small blue round cells in a vascular-rich background. It was negative for *EWSR1, FUS, CIC, YWHAE, BCOR* and *SS18* gene abnormalities by FISH as well as for *BCOR* ITD by PCR. The other case was a 16 year-old male patient with a retroperitoneal mass and liver metastasis. Histologically, it showed a round cell sarcoma with focal spindling and focal stromal hyalinization. It was negative for *EWSR1, FUS, CIC, YWHAE, BCOR* and *SS18* gene rearrangements by FISH.

All 8 CCSKs showed moderate to strong BCOR immunoreactivity (Fig. 2N), regardless of the genetic alterations, including *BCOR* ITD, *YWHAE* fusion or wild type for both. Cellular fibrous septa and capillaries were negative. Adjacent normal renal parenchyma was also negative.

A subset of synovial sarcoma shows BCOR immunoreactivity

We evaluated 71 synovial sarcomas on TMA and 3 additional poorly differentiated synovial sarcomas, which could mimic other round cell sarcomas, on whole-tissue sections. Overall,

49% of synovial sarcomas had moderate to strong BCOR positivity in more than 10% of the tumor (Fig. 3). All 3 poorly differentiated synovial sarcomas had diffuse and strong BCOR staining (Fig. 3C), while the 71 cases on TMA showed variable BCOR staining, ranging from negative (n=39), moderate (n=16) to strong (n=16) staining. A subset of synovial sarcomas had available information regarding the histologic subtypes as monophasic or biphasic, the fusion variants as *SS18-SSX1* or *SS18-SSX2* and patient genders. The immunoreactivity did not correlate with any of these factors (Table 1). In biphasic synovial sarcomas, the staining was noted in both the epithelial and spindle cell components (Fig. 3B, D). To investigate if the BCOR protein overexpression noted in half of the synovial sarcomas is related to transcriptional up-regulation, we analyzed the expression array data from the published synovial sarcoma dataset and found high *BCOR* mRNA levels across monophasic, biphasic and poorly differentiated subtypes, similar to other tumors harboring *BCOR* gene alterations (Fig. 4).

BCOR is not expressed in most other sarcoma types

Most other sarcoma types tested were negative for BCOR, with only 2 of 29 rhabdomyosarcomas (1 alveolar, 1 embryonal) and 1 of 92 myxofibrosarcomas showed moderate BCOR staining. No staining was found in any of the chondrosarcomas, low grade fibromyxoid sarcomas, and angiosarcomas TMAs. Furthermore, no BCOR reactivity was noted in the 3 cases harboring *ZC3H7B-BCOR* fusion (2 endometrial stromal sarcoma and 1 ossifying fibromyxoid tumor).

SATB2 overexpression in sarcomas with *BCOR* gene abnormalities

SATB2 immunoexpression was assessed in the same study group based on the significant *SATB2* mRNA upregulation in the *BCOR*-ITD genomic group and the availability of a commercial antibody against SATB2. SATB2 positivity was present in the majority of round cell sarcomas harboring *BCOR* ITD (75%), *YWHAE-NUTM2B* (100%), *BCOR-MAML3* (100%), and *BCOR-CCNB3* (71%) (Fig. 2; Table 1). However, compared to the BCOR staining pattern, SATB2 showed increased intratumoral heterogeneity in the intensity of staining. CCSK also showed variable SATB2 immunohistochemical expression. Two of 6 CCSKs tested were positive (one *BCOR* ITD and one *YWHAE* fusion), with intratumoral heterogeneity being observed.

SATB2 immunostaining is inconsistent in synovial sarcoma

As BCOR immunoreactivity was present in synovial sarcomas and rare rhabdomyosarcomas, we also evaluated SATB2 expression in these 2 additional cohorts. The results showed that only a minority of synovial sarcoma (n=9, 12%) expressed SATB2 immunohistochemically. However, both of the poorly differentiated synovial sarcomas with round cell histomorphology examined in whole-tissue sections were positive. Co-expression of BCOR and SATB2 stains was observed in 7 out of 9 cases. No *SATB2* mRNA up-regulation was noted in synovial sarcomas (Fig. 4). No SATB2 reactivity was observed in the rhabdomyosarcoma TMA.

Gene signature and potential transcription factors regulating *BCOR* expression in tumors with *BCOR* genetic abnormalities and synovial sarcoma

As reported previously, we have obtained the gene signature of round cell sarcomas/ PMMTI with *BCOR* ITD and *YWHAE-NUTM2B* by RNA sequencing,[6] of CCSKs by Illumina HumanHT-12 V4.0 beadchip,[16] and of *BCOR-CCNB3* tumors by Affymetrix Human Genome U133A Plus 2 platform.[4] Forty-four genes, including *BCOR* and *SATB2*, were similarly up-regulated in all of them. In this study, we also investigated overexpressed TFs for potential roles in transcriptional upregulation of *BCOR*. Thus we searched the TF motif databases for known motif sequences of differentially expressed TFs in the *BCOR* ITD tumors, which could target the *BCOR* promoter region. We identified 19 TFs, each with one or more possible motifs in the *BCOR* promoter region, including *ELF2, HES7, ETV5, YWHAZ*, *INSM1, SOX9* (Supplementary Table 1; Supplementary Figure 3).

We have performed a similar analysis in synovial sarcoma. Among the 411 differentially expressed genes on U133A plus 2, *BCOR* was significantly upregulated in all synovial sarcoma subtypes (monophasic, biphasic and poorly differentiated) (Fig. 4), which correlated with the high level of immunohistochemical expression. In keeping with the inconsistent SATB2 immunoreactivity, *SATB2* mRNA expression was not up-regulated in synovial sarcoma as noted in other BCOR-positive tumors (Fig. 4). Among the 70 upregulated TF or TF-associated genes, *GTF2I, MYC, MSX2,* and *GATA6,* were found to have potential binding motifs to the *BCOR* promoter region (Supplementary table 1, Supplementary Figure 3). Interestingly, *GATA6* was up-regulated in both synovial sarcoma and *BCOR-CCNB3* tumors. Moreover, *GTF2I*, *MSX2*, and *GATA6* also had binding motif sequences on the promoter region of *TLE1* gene, another transcriptional corepressor up-regulated in synovial sarcoma. The *GATA6* motifs on *TLE1* and *BCOR* promoters were similar, while the motifs for *GTF2I* and *MSX2* were different. Although there were 12 other differentially expressed TFs in common between synovial sarcomas and *BCOR* ITD tumors, none of them had known motifs on *BCOR* promoter (Supplementary table 1). Furthermore, there were no shared patterns of mRNA expression noted in the neighboring genomic region encompassing *SSX1/2* and *BCOR* genes to suggest a common region of amplification/ up-regulation.

DISCUSSION

BCOR (BCL-6 interacting corepressor) is a transcriptional corepressor involved in suppressing gene expression by either interacting with BCL-6 or binding to PCGF1 (polycomb-group RING finger homologue 1), as part of a variant Polycomb Repressive Complex 1 (PRC1), and inducing gene silencing via histone modification.[17,18] In human tissues, *BCOR* mRNA is ubiquitously expressed in various tissues;[17] however, the BCOR protein expression in adult human tissue is largely unknown. During our antibody validation, BCOR immunoreactivity was identified mainly in the testis, with scattered weak positivity in the germinal centers of tonsil, but not in other human tissues tested (spleen, placenta, kidney, colon, liver, epidermis, and pancreas).

We have recently reported a subset of soft tissue round cell sarcomas in infants harboring *BCOR* ITD or *YWHAE-NUTM2B* fusion, occurring with predilection in the trunk and

abdominal cavity.[6] Remarkably, these tumors share similar genetic abnormalities and histologic features with CCSK.[6,19] Histologically, these lesions are characterized by uniform round cells with fine chromatin pattern and rich vascular background. Additional findings seen in a subset of cases include stellate, vacuolated or spindle cell cytomorphology, myxoid stromal change, cellular fibrous septa and rosette formation. Moreover, tumors previously designated as primitive myxoid mesenchymal tumor of infancy (PMMTI) were also shown to harbor *BCOR*-ITD and exhibit overlapping morphology and clinical presentation.[6]

An additional molecular subset of SBRCT emerging recently is characterized by *BCOR*-related fusions, either by an intra-chromosomal X inversion, *BCOR-CCNB3,* or an inter-chromosomal translocation, *BCOR-MAML3.*[4-6] These tumors share morphologic features with *BCOR*-ITD SBRCTs, including monomorphic cells with delicate chromatin and indistinct nucleoli, areas of spindling, and rich capillary network.[6] Moreover, our previous study showed an overlapping gene signature between these different SBRCT subsets harboring *BCOR* gene abnormalities. Specifically, we noted significant up-regulation of *BCOR* and *SATB2* mRNA expression in round cell sarcomas with *BCOR* alterations (ITD, *BCOR-CCNB3, BCOR-MAML3*) and *YWHAE-NUTM2B* fusion, as well as in CCSKs.[6] As the molecular investigation of each of this subset requires individual custom BAC probes for FISH or primer design for PCR assays, which are not widely available for routine diagnostic use, we assessed the specificity and sensitivity of BCOR and SATB2 antibodies for immunohistochemistry in this setting. Our results showed that the overwhelming majority of SBRCTs harboring *BCOR*-ITD, *BCOR-CCNB3, BCOR-MAML3,* and *YWHAE-NUTM2B* abnormalities demonstrate strong and diffuse nuclear immunoreactivity to BCOR. Our study also revealed strong BCOR immunostaining in CCSKs harboring *BCOR* ITD, *YWHAE-NUTM2B* fusion, or wild-type CCSKs.

In addition to *BCOR*, round cell sarcomas with *BCOR-CCNB3* fusion were also shown to have *CCNB3* mRNA up-regulation. As a consequence, CCNB3 overexpression by immunohistochemistry was demonstrated to be a useful ancillary marker in this subset.[4] No *CCNB3* mRNA up-regulation was identified in round cell sarcomas with *BCOR* ITD, *BCOR-MAML3*, or *YWHAE-NUTM2B*, by RNA sequencing and microarray data, respectively (data not shown); thus CCNB3 protein is most likely not overexpressed in these tumors.

Intriguingly, 2 of our undifferentiated round cell sarcomas without known gene fusions showed diffuse and strong BCOR positivity. The presence of *BCOR* and *YWHAE* rearrangements were excluded by FISH, and *BCOR* ITD was excluded by PCR in one of them which occurred in an infant. This finding indicated the existence of alternative genetic events in soft tissue round cell sarcomas that can drive the tumorigenesis with high BCOR expression.

As SBRCTs with *BCOR* genetic abnormalities can show focal areas of spindling, the differential diagnosis often includes a poorly differentiated or monophasic synovial sarcoma. Thus our investigation of BCOR immunoreactivity included a large number of synovial sarcomas, spanning all histologic subtypes and fusion transcripts. Somewhat unexpectedly, our results revealed that half of synovial sarcomas had variable degree of BCOR

immunoreactivity. In contrast to the diffuse and strong staining pattern seen in SBRCTs with *BCOR*-gene alterations, BCOR immunoreactivity in synovial sarcomas was more variable in intensity and extent. There was no correlation between the BCOR staining pattern and histologic subtype or transcript variant of synovial sarcomas. Although all 3 poorly differentiated synovial sarcomas examined in whole sections showed diffuse and strong BCOR staining, further studies with larger case numbers are needed to determine whether the frequency of BCOR expression in poorly differentiated synovial sarcomas is different from monophasic and biphasic variants. At gene expression level, there was no significant difference in *BCOR* mRNA among the 3 different histologic subtypes of synovial sarcomas. It is intriguing to speculate that the histologic overlap with mixed round and spindle cell phenotype and BCOR immunoreactivity seen in both synovial sarcoma and SBRCT with *BCOR*-gene alterations might be related to the *BCOR* gene upregulation through different pathogenetic mechanisms.

In translocation-related mesenchymal tumors, *BCOR* gene has been involved both as 5' (*BCOR-CCNB3* and *BCOR-MAML3* in SBRCT) and 3' partners (*ZC3H7B-BCOR* in endometrial stromal sarcoma, ossifying fibromyxoid tumor and SBRCT).[5,20,21] Our results showed that only tumors harboring fusions with *5'BCOR* partner had BCOR protein overexpression by immunohistochemistry, while tumors with 3'*BCOR* fusions did not. All 3 tumors harboring *ZC3H7B-BCOR* fusion (2 endometrial stromal sarcomas and 1 ossifying fibromyxoid tumor) were negative for BCOR immunostaining. One explanation is that the BCOR monoclonal antibody sc-514576(c-10) used is against the first 300 amino acids of BCOR protein, which is not represented in the chimeric protein of ZC3H7B-BCOR. Furthermore, in contrast to the *BCOR-CCNB3* or *BCOR-MAML3* positive SBRCTs, our RNA sequencing data showed that tumors with *ZC3H7B-BCOR* fusion had low levels of *BCOR* mRNA expression.

Our study also attempted to establish the relevance of BCOR protein expression in the context of round cell sarcomas/SBRCT, as well as in the broader spectrum of different sarcoma types. In contrast to the diffuse and consistent staining results in SBRCTs with *BCOR*-gene abnormalities, BCOR reactivity was present with patchy moderate intensity only in one of the 8 Ewing sarcoma tested. Similarly, only 2/29 rhabdomyosarcomas and 1/6 round cell sarcomas with *CIC* rearrangements showed BCOR immunoreactivity with moderate intensity. Our results suggest that BCOR immunostaining can be used as a reliable marker in the differential diagnosis of various molecular subsets of SBRCTs, triage for further molecular studies if needed and avoid extensive immunohistochemical and molecular work-ups, especially in limited specimens. Aside from SBRCTs, BCOR expression was not found in other sarcomas examined, including angiosarcomas, chondrosarcomas, low grade fibromyxoid sarcomas, endometrial stroma sarcomas, and ossifying fibromyxoid tumor with *ZC3H7B-BCOR,* except for one myxofibrosarcoma (1%) showing moderate staining.

In search of possible mechanisms of *BCOR* up-regulation, we identified 4 differentially expressed TFs (*GTF2I, MYC, MSX2,* and *GATA6*) in synovial sarcomas that have binding motifs to the promoter region of *BCOR*. However, whether these TFs are involved in *BCOR* up-regulation requires further experimental work. In contrast, SBRCTs with *BCOR* ITD showed 19 differentially expressed TFs with motif sequences potentially binding to the

BCOR promoter; however, none overlapped with the TFs found in synovial sarcoma. Other mechanisms of *BCOR* overexpression may involve epigenetic regulations. The *SS18-SSX* fusion transcript in synovial sarcoma is involved in chromatin modification through interaction with polycomb and SWI/SNF complexes.[22,23] For *BCOR* ITD tumors, the duplicated *BCOR* sequences reside in the region encoding the PUFD (PCGF ubiquitin-like fold discriminator) domain of BCOR, which is responsible for the binding with PCGF1 (polycomb group ring finger 1) and the formation of a variant PRC1, involved in histone modification.[6,18,24,25]

Another marker evaluated in this study, SATB2 (special AT-rich sequence-binding protein 2), is known as an osteoblastic as well as colorectal or appendiceal differentiation marker.[26-28] *SATB2* encodes a DNA-binding protein that also interacts with chromatin remodeling molecules, such as histone deacetylase 1 and metastasis-associated protein 2.[29] Overall, SATB2 was positive in the majority (11/14; 79%) of round cell sarcomas with *BCOR* anomaly or *YWHAE* fusion. However, a greater degree of intratumoral heterogeneity with variations in the extent and intensity of staining was observed. Hence, SATB2 stain should be used with caution, especially in limited biopsy specimens. Of note, among the SATB2-positive round cell sarcomas, *BCOR-CCNB3* tumors often arise as bone tumors in adolescent patients.[4] SATB2 immunoreactivity, when interpreted as an osteoblastic marker, could lead to the misdiagnosis of a small cell osteosarcoma, as seen in one of our cases. Unlike BCOR stain, SATB2 expression was only seen in two out of six CCSKs tested. However, the gene expression data of CCSK revealed up-regulated *SATB2* expression in the majority of cases irrespective of the genotype being *BCOR* ITD or *YWHAE* fusion. This result may reflect the greater variability of SATB2 protein expression identified by immunohistochemistry. Synovial sarcoma infrequently showed SATB2 immunoreactivity, which correlated to the low levels of *SATB2* mRNA expression.

In conclusion, we demonstrated BCOR immunohistochemistry as a highly sensitive marker in identifying round cell sarcomas with *BCOR* abnormality, namely *BCOR* gene rearrangement or ITD, and *YWHAE* fusion. CCSK shares with these soft tissue tumors not only the histomorphology, genetic changes, but also BCOR immunoreactivity. Synovial sarcoma should also be included in the differential diagnosis in round cell sarcomas positive for BCOR stain. SATB2 immunoreactivity is also expressed in the majority of these tumors, although with lower sensitivity and greater variation of staining.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Italiano A, Sung YS, Zhang L, et al. High prevalence of CIC fusion with double-homeobox (DUX4) transcription factors in EWSR1-negative undifferentiated small blue round cell sarcomas. Genes Chromosomes Cancer. 2012; 51:207–218. [PubMed: 22072439]
2. Graham C, Chilton-MacNeill S, Zielenska M, et al. The CIC-DUX4 fusion transcript is present in a subgroup of pediatric primitive round cell sarcomas. Hum Pathol. 2012; 43:180–189. [PubMed: 21813156]
3. Sugita S, Arai Y, Tonooka A, et al. A novel CIC-FOXO4 gene fusion in undifferentiated small round cell sarcoma: a genetically distinct variant of Ewing-like sarcoma. Am J Surg Pathol. 2014; 38:1571–1576. [PubMed: 25007147]
4. Pierron G, Tirode F, Lucchesi C, et al. A new subtype of bone sarcoma defined by BCOR-CCNB3 gene fusion. Nat Genet. 2012; 44:461–466. [PubMed: 22387997]
5. Specht K, Zhang L, Sung YS, et al. Novel BCOR-MAML3 and ZC3H7B-BCOR Gene Fusions in Undifferentiated Small Blue Round Cell Sarcomas. Am J Surg Pathol. 2016
6. Kao YC, Sung YS, Zhang L, et al. Recurrent BCOR Internal Tandem Duplication and YWHAE-NUTM2B Fusions in Soft Tissue Undifferentiated Round Cell Sarcoma of Infancy: Overlapping Genetic Features With Clear Cell Sarcoma of Kidney. Am J Surg Pathol. 2016
7. Specht K, Sung YS, Zhang L, et al. Distinct transcriptional signature and immunoprofile of CIC-DUX4 fusion-positive round cell tumors compared to EWSR1-rearranged Ewing sarcomas: further evidence toward distinct pathologic entities. Genes Chromosomes Cancer. 2014; 53:622–633. [PubMed: 24723486]
8. Peters TL, Kumar V, Polikepahad S, et al. BCOR-CCNB3 fusions are frequent in undifferentiated sarcomas of male children. Mod Pathol. 2015; 28:575–586. [PubMed: 25360585]
9. Puls F, Niblett A, Marland G, et al. BCOR-CCNB3 (Ewing-like) sarcoma: a clinicopathologic analysis of 10 cases, in comparison with conventional Ewing sarcoma. Am J Surg Pathol. 2014; 38:1307–1318. [PubMed: 24805859]
10. Ueno-Yokohata H, Okita H, Nakasato K, et al. Consistent in-frame internal tandem duplications of BCOR characterize clear cell sarcoma of the kidney. Nat Genet. 2015; 47:861–863. [PubMed: 26098867]
11. Roy A, Kumar V, Zorman B, et al. Recurrent internal tandem duplications of BCOR in clear cell sarcoma of the kidney. Nat Commun. 2015; 6:8891. [PubMed: 26573325]
12. Frosina D, Jungbluth AA. A Novel Technique for the Generation of Multitissue Blocks Using a Carrier. Appl Immunohistochem Mol Morphol. 2015
13. Nakayama R, Mitani S, Nakagawa T, et al. Gene expression profiling of synovial sarcoma: distinct signature of poorly differentiated type. Am J Surg Pathol. 2010; 34:1599–1607. [PubMed: 20975339]
14. Pei H, Li L, Fridley BL, et al. FKBP51 affects cancer cell response to chemotherapy by negatively regulating Akt. Cancer Cell. 2009; 16:259–266. [PubMed: 19732725]
15. Griesinger AM, Birks DK, Donson AM, et al. Characterization of distinct immunophenotypes across pediatric brain tumor types. J Immunol. 2013; 191:4880–4888. [PubMed: 24078694]
16. Karlsson J, Holmquist Mengelbier L, Ciornei CD, et al. Clear cell sarcoma of the kidney demonstrates an embryonic signature indicative of a primitive nephrogenic origin. Genes Chromosomes Cancer. 2014; 53:381–391. [PubMed: 24488803]
17. Huynh KD, Fischle W, Verdin E, et al. BCoR, a novel corepressor involved in BCL-6 repression. Genes Dev. 2000; 14:1810–1823. [PubMed: 10898795]
18. Gearhart MD, Corcoran CM, Wamstad JA, et al. Polycomb group and SCF ubiquitin ligases are found in a novel BCOR complex that is recruited to BCL6 targets. Mol Cell Biol. 2006; 26:6880–6889. [PubMed: 16943429]
19. Argani P, Perlman EJ, Breslow NE, et al. Clear cell sarcoma of the kidney: a review of 351 cases from the National Wilms Tumor Study Group Pathology Center. Am J Surg Pathol. 2000; 24:4–18. [PubMed: 10632483]

20. Panagopoulos I, Thorsen J, Gorunova L, et al. Fusion of the ZC3H7B and BCOR genes in endometrial stromal sarcomas carrying an X;22-translocation. Genes Chromosomes Cancer. 2013; 52:610–618. [PubMed: 23580382]
21. Antonescu CR, Sung YS, Chen CL, et al. Novel ZC3H7B-BCOR, MEAF6-PHF1, and EPC1-PHF1 fusions in ossifying fibromyxoid tumors--molecular characterization shows genetic overlap with endometrial stromal sarcoma. Genes Chromosomes Cancer. 2014; 53:183–193. [PubMed: 24285434]
22. Garcia CB, Shaffer CM, Eid JE. Genome-wide recruitment to Polycomb-modified chromatin and activity regulation of the synovial sarcoma oncogene SYT-SSX2. BMC Genomics. 2012; 13:189. [PubMed: 22594313]
23. Kato H, Tjernberg A, Zhang W, et al. SYT associates with human SNF/SWI complexes and the C-terminal region of its fusion partner SSX1 targets histones. J Biol Chem. 2002; 277:5498–5505. [PubMed: 11734557]
24. Gao Z, Zhang J, Bonasio R, et al. PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes. Mol Cell. 2012; 45:344–356. [PubMed: 22325352]
25. Junco SE, Wang R, Gaipa JC, et al. Structure of the polycomb group protein PCGF1 in complex with BCOR reveals basis for binding selectivity of PCGF homologs. Structure. 2013; 21:665–671. [PubMed: 23523425]
26. Conner JR, Hornick JL. SATB2 is a novel marker of osteoblastic differentiation in bone and soft tissue tumours. Histopathology. 2013; 63:36–49. [PubMed: 23701429]
27. Dragomir A, de Wit M, Johansson C, et al. The role of SATB2 as a diagnostic marker for tumors of colorectal origin: Results of a pathology-based clinical prospective study. Am J Clin Pathol. 2014; 141:630–638. [PubMed: 24713733]
28. Strickland S, Parra-Herran C. Immunohistochemical characterization of appendiceal mucinous neoplasms and the value of special AT-rich sequence-binding protein 2 in their distinction from primary ovarian mucinous tumours. Histopathology. 2016; 68:977–987. [PubMed: 26542609]
29. Gyorgy AB, Szemes M, de Juan Romero C, et al. SATB2 interacts with chromatin-remodeling molecules in differentiating cortical neurons. Eur J Neurosci. 2008; 27:865–873. [PubMed: 18333962]

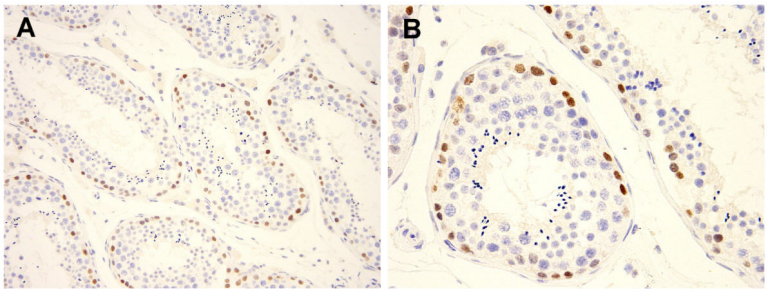

Figure 1. BCOR immunostaining positive control in testis

BCOR nuclear positivity in the spermatogonia within the seminiferous tubules (A, 200X; B, 400X).

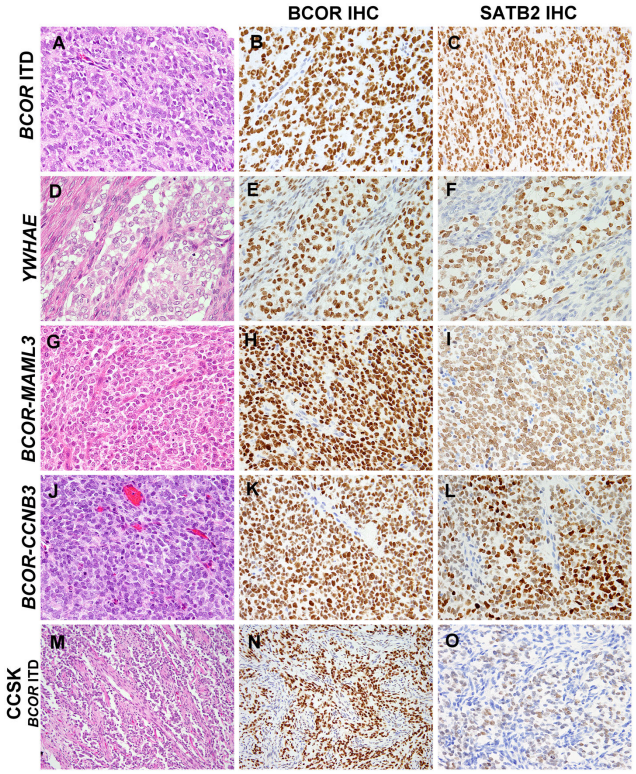

Figure 2. Diffuse BCOR and SATB2 immunoreactivity in round cell sarcomas with *BCOR* abnormalities, *YWHAE-NUTM2B* fusion, and CCSK

Round cell sarcomas with *BCOR* ITD and *YWHAE-NUTM2B* show uniform round cells with fine chromatin pattern and rich fibrovascular background (A, D) and exhibit diffuse and strong nuclear expression of BCOR (B, E), and strong (C) to moderate (F) SATB2 staining. SBRCT with *BCOR-MAML3* and *BCOR-CCNB3* fusions show diffuse sheets of round to ovale cells with fine chromatin (G, J) and demonstrate strong nuclear expression of BCOR (H, K), while SATB2 had strong to moderate (I) intensity and greater degree of intratumoral heterogeneity (L) compared to BCOR stain. CCSK harboring *BCOR* ITD has similarly uniform round cells (M) and displayed strong BCOR staining (negative fibrovascular septa) (N), whereas SATB2 expression was weak (O).

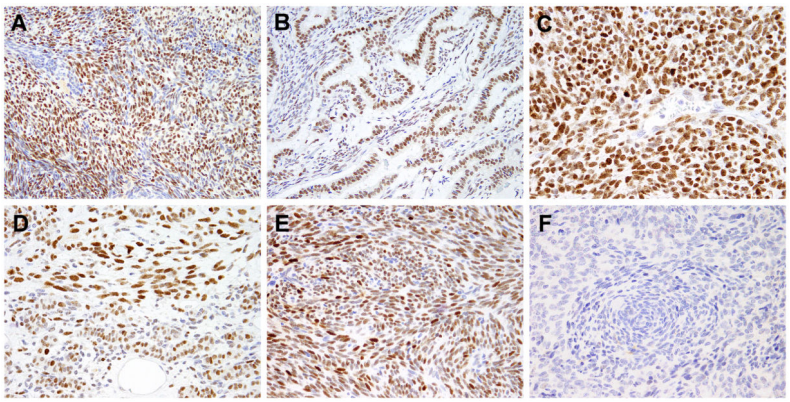

Figure 3. BCOR staining in synovial sarcomas

BCOR nuclear immunoreactivity was present in all histologic subtypes of synovial sarcoma, including monophasic (A), biphasic (B) and poorly differentiated (C), and fusion types, including *SS18-SSX1* (D) and *SS18-SSX2* (E). In biphasic tumors, BCOR expression was stronger in either epithelial (B) or spindle cell component (D). About half of synovial sarcoma cases are negative for BCOR (F).

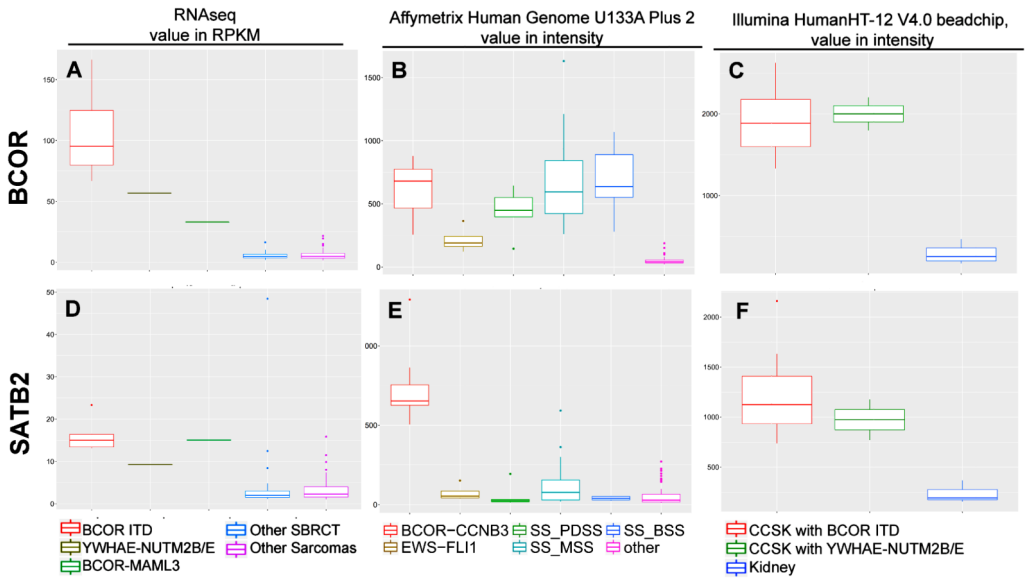

Figure 4. *BCOR* and *SATB2* transcriptional up-regulation is a consistent feature of SBRCT with *BCOR* gene abnormalities

By RNA sequencing, SBRCTs with *BCOR* ITD, *YWHAE-NUTM2B,* and *BCOR-MAML3* showed BCOR mRNA upregulation compared to other SBRCT and other sarcomas (A).[6] Using different publicly available datasets, *BCOR* mRNA expression is also up-regulated in *BCOR-CCNB3* tumors as well as in all synovial sarcomas histologic subtypes (PDSS, poorly differentiated; MSS, monophasic; BSS, biphasic) by Affymetrix Human Genome U133A Plus 2 platform, as compared to Ewing sarcoma (*EWSR1-FLI1*) and other non-sarcomatous tumors and normal tissues (other)(B).[4,13-15] By Illumina HumanHT-12 V4.0 beadchip, CCSK with *BCOR* ITD and *YWHAE-NUTM2B* fusion also showed high BCOR mRNA compared to normal kidney tissues.[16] In contrast, *SATB2* mRNA up-regulation was seen in all SBRCTs with *BCOR* genetic abnormalities and CCSK, but not in synovial sarcomas (D-F).

Table 1

Immunohistochemical protein expression analysis of BCOR (clone C-10) and SATB2 (clone EP281)

Tumor type	BCOR	SATB2
Round cell sarcoma		
BCOR ITD	13/14 (92.9%)	9/12 (75%)
YWHAE-NUTM2B	2/2 (100%)	2/2 (100%)
BCOR-MAML3	1/1 (100%)	1/1 (100%)
BCOR-CCNB3	8/8 (100%)	5/7 (71.4%)
CIC rearrangement	1/6 (16.7%)	ND
EWSR1-FLI1/NFATC2	1/9 (11.1%)	ND
DSRCT	0/5 (0%)	ND
Unknown/Fusion neg.	2/10 (20%)	ND
Clear cell sarcoma of kidney		
Total	8/8 (100%)	2/6 (33.3%)
BCOR ITD	4/4 (100%)	1/3 (33.3%)
YWHAE-NUTM2B	1/1 (100%)	1/1 (100%)
Wild type for both	3/3 (100%)	0/2 (0%)
Synovial sarcoma		
Overall (TMA*+PD)	35/74 (49.3%)	9/75 (12%)
Monophasic*	7/20 (35%)	2/20 (10%)
Biphasic*	5/14 (35.7%)	1/15 (6.7%)
*SYT-SSX1**	5/18 (27.8%)	3/19 (15.8%)
*SYT-SSX2**	7/16 (43.8%)	0/16 (0%)
Poorly differentiated	3/3 (100%)	2/2 (100%)
Rhabdomyosarcoma*	2/29 (6.9%)	0/29 (0%)
Angiosarcoma*	0/41 (0%)	ND
Myxofibrosarcoma*	1/92 (1.1%)	ND
Chondrosarcoma*	0/41 (0%)	ND
LGFMS*	0/14 (0%)	ND
ESS with *ZC3H7B-BCOR*	0/2 (0%)	ND
OFMT with *ZC3H7B-BCOR*	0/1 (0%)	ND

ITD: internal tandem duplication; DSRCT: desmoplastic small round cell tumor; neg.: negative; TMA: tissue microarray; PD: poorly differentiated; LGFMS, low grade fibromyxoid sarcoma; ESS, endometrial stromal sarcoma; OFMT, ossifying fibromyxoid tumor; ND, not done.

*Immunohistochemical stains performed on tissue microarray.