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Original article

The utility of SATB2 immunohistochemical expression in distinguishing between osteosarcomas and their malignant bone tumor mimickers, such as Ewing sarcomas and chondrosarcomas

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ABSTRACT

SATB2 is commonly expressed in osteosarcomas. Although apparently being a valuable diagnostic marker for differentiating between small cell osteosarcoma (SCO) and other small round cell tumors of bone, for instance Ewing sarcoma family of tumors (ESFT), it has not been tested in a large series of ESFT and chondrosarcomas so far. We studied the immunohistochemical expression of SATB2 in 42 osteosarcomas, 31 chondrosarcomas, and 371 genetically confirmed ESFT. SATB2 positivity was detected in 90.4% of osteosarcomas, 87.5% of SCO, 91.3% of osteoblastic osteosarcomas, and in all chondroblastic and parosteal osteosarcomas. The osteoblastic and SCO subtypes expressed SATB2 more intensely than other histological types. SATB2 was expressed in 46.6% of chondrosarcomas, and in 1.3% of ESFT. Sensitivity and specificity of SATB2 immunoexpression were 90.4% and 95.3%, respectively. The positive and negative predictive values in osteosarcoma diagnosis were 66.6% and 98.9%, respectively.

In chondrosarcoma, SATB2 immunoexpression was more frequent and intense in high-grade chondrosarcoma (Grade III) and uncommon in chondrosarcoma grade I. SATB2 positivity was detected in 55.6% of chondrosarcomas grade II. SATB2 apparently cannot distinguish between chondroblastic osteosarcoma and high-grade chondrosarcoma. Nevertheless, SATB2 is frequently expressed in osteogenic tumors, but is rarely positive in ESFT, and with the support of CD99 expression and specific molecular studies, it is very useful for distinguishing between these two lesions. Although SATB2 immunoexpression helps to distinguish osteosarcoma from their mimickers, the identification of malignant osteoid matrix formation and the integration of clinical and radiological data remain the corner stone of osteosarcoma diagnosis and as yet no antibody has equalled the diagnostic value of this important morphologic hallmark.

1. Introduction

Although the identification of osteoid matrix is elemental for osteosarcoma diagnosis, in some cases, immunohistochemistry (IHC) can offer additional information to support the final diagnosis, especially in tumors with minimal or scant osteoid matrix formation [1–8]. Particularly in small biopsies, the osteoid matrix is usually scant, and the distinction between tumor osteoid matrix and hyalinized collagen/fibrin, sclerosing stromal tissue or reactive bone formation is complex [1,2,4–8]. The accurate distinction between osteosarcoma and other bone sarcomas is very important since treatment protocols depend mainly on the histopathological diagnosis [1–9]. Scant osteoid matrix production complicates the differential diagnosis mainly in two scenarios: 1-"chondroblastic osteosarcoma with scant osteoid matrix formation" versus "high grade chondrosarcoma", 2-"small cell osteosarcoma (SCO) with scant osteoid matrix in a small biopsy" versus "Ewing sarcoma family of tumor (ESFT) with bone infiltration and reactive/metaplastic bone formation". IHC studies have explored the utility of antibodies directed against proteins involved in bone matrix production (osteonectin, osteocalcin, DMP-1) [1,2,6], or other membranous and/or cytoplasmic proteins, for instance ezrin or GAL-1 [3,5,9,10] that apparently, when expressed, support a diagnosis of osteosarcoma, although the results did not support their efficacy in the differential diagnosis.

Regulation of osteoblast differentiation by nuclear matrix protein SATB2 and osteoblast positivity in osteosarcomas has been

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Table 1
SATB2 immunohistochemical expression in bone tumors.

SATB2	Total	Positives	Negatives
Ewing sarcoma	371	5 (1.3%)	366 (98.7%)
Osteosarcomas	42	38 (90.4%)	4 (9.6%)
Chondrosarcomas	30	14 (46.6%)	16 (43.4%)
Total	443	57	386

well documented by Conner and Hornick [11]. However, SATB2 is not completely specific for osteoblastic differentiation since it is also expressed in colon epithelium and colorectal, squamous and breast carcinomas [12–19]. SATB2 was a very sensitive marker for osteosarcoma: 100% on whole slides, and 94%, including tissue micro-array (TMA) data, as reported by David and Horvai [8]. These results are also analogous to the findings published previously by Connor and Hornick [11], who report a 98% sensitivity of this marker for staining osteosarcomas.

The aims of this study were to evaluate SATB2 IHC expression in a large series of bone tumors, including osteosarcomas, ESFT and chondrosarcomas in order to confirm the potential diagnostic utility of this marker in distinguishing osteosarcomas from other non-osteogenic primary bone sarcomas.

2. Material and methods

A retrospective study was performed on 443 osteogenic and non-osteogenic bone sarcomas, including tumor material from two pathology departments (Valencia and Bologna). Forty-two osteosarcomas (23 osteoblastic, 5 chondroblastic, 4 parosteal, 8 small cell osteosarcoma (SCO), 1 poorly differentiated and 1 telangiectatic), 31 chondrosarcomas (12, grade I; 9, grade II; 10, grade III), and 371 genetically confirmed ESFTs were included in the study using TMAs. Representative areas of each tumor were selected for TMA production by first observing the hematoxylin-eosin stained biopsy tumor slide and then sampling the tissue from the corresponding paraffin blocks. A tissue microarray instrument (Beecher Instruments, Sun Prairie, WI) was used for TMA assembly. From the TMA blocks, 4-μm-thick sections were selected for IHC staining using a rabbit polyclonal antibody SATB2 (Sigma-Aldrich) at 1:1000 dilution with pressure cooker antigen retrieval in citrate buffer (low pH from DAKO). The percentage of SATB2 positive cells and the staining intensity were scored semiquantitatively, and four groups were formed according to the percentage and intensity of nuclear stained cells. Cases were scored as negative (mild <5%); 1+ (mild staining in 5–10% of cells); 2+ (moderate staining in 10–50% of cells); or 3+ (strong staining in >50% of cells). Three pathologists independently evaluated the percentage and intensity of stained cells (IM, SN, ALB).

3. Results

Table 1 summarizes the IHC findings in osteosarcomas, chondrosarcomas and Ewing sarcoma family of tumors.

3.1. SATB2 expression in osteogenic bone tumors

SATB2 was expressed in 90.4% of osteosarcomas, almost always with strong, diffuse nuclear staining (Fig. 1A,B). Virtually all cases of osteoblastic osteosarcomas and SCO expressed SATB2 (Fig. 1C,D). All chondroblastic and parosteal osteosarcomas were also positive. The osteoblastic and SCO subtypes revealed strong SATB2 immunoexpression, which was more intense than in other histological types. Table 2 summarizes the IHC findings in osteosarcomas subtypes.

Table 2
SATB2 immunohistochemical expression in osteosarcoma subtypes.

SATB2 Osteosarcomas	Total	Positives	Negatives
Osteoblastic	23	21 (91.3%)	2 (8.7%)
Chondroblastic	5	5 (100%)	0
Parosteal	4	4 (100%)	0
SCO	8	7 (87.5%)	1 (12.5%)
Others	2	1 (50%)	1 (50%)
Total	42	38 (90.4%)	4 (9.6%)

Table 3
SATB2 immunohistochemical expression in chondrosarcomas.

SATB2 Chondrosarcomas	Total	Positives	Negatives
Grade 1	12	1 (8.3%)	11 (91.7%)
Grade 2	9	5 (55.6%)	4 (44.4%)
Grade 3	10	8 (80%)	2 (20%)
Total	31	14 (46.6%)	17 (53.4%)

Sensitivity and specificity of SATB2 immunoexpression were 90.4% and 95.3% respectively. The positive and negative predictive values in osteosarcoma diagnosis were 66.6% and 98.9% respectively.

3.2. SATB2 expression in non-osteogenic bone tumors

SATB2 was expressed in 46.6% of chondrosarcomas, and in1.3% of ESFT. In chondrosarcoma, SATB2 expression was similar to osteosarcoma, with a clear nuclear pattern (Fig. 2), being more frequent and intense in high-grade chondrosarcoma (Grade III) (Fig. 3) and uncommon in chondrosarcoma grade I. SATB2 positivity was detected in 55.6% of chondrosarcoma grade II. Table 3 summarizes the IHC findings in chondrosarcomas. SATB2 was expressed in a very low proportion of ESFT cases (Fig. 4A–D) as compared with osteosarcomas and chondrosarcomas. Heterogeneous SATB 2 expression was detected in one ESFT (Fig. 4E, F). SATB2 positivity was observed in 4 conventional Ewing sarcomas and 1 PNET. All atypical ESFT were negative for SATB2.

Reactive woven bone in chondroblastic and Ewing tumors with bone involvement showed intense SATB2-positive osteoblastic rimming. Furthermore, SATB2 was also expressed in endothelial cells in stromal tissue in osteo-chondrosarcomas and ESFT.

4. Discussion

Our results regarding SATB2 expression in osteosarcomas confirm the previous results obtained by Conner and Hornick [14] and Davis and Horvai [8], demonstrating that many osteosarcomas reveal SATB2 immunoexpression independently of malignant osteoid quantity.

Despite the high sensitivity of SATB2 expression in osteosarcoma diagnosis, the results obtained by Davis and Horvai [8] suggested that SATB2 positivity is not specific for osteosarcoma as compared with other primary bone sarcomas. They observed SATB2 immunoexpression in some undifferentiated pleomorphic sarcoma (UPS) and primary fibrosarcoma of bone. These results are parallel to the present results since we observed strong nuclear SATB2 positivity in many high-grade chondrosarcomas. Such SATB2 expression in chondrosarcomas has not been reported previously. A possible alternative diagnosis for UPS, fibrosarcoma [8] or primary chondrosarcoma of bone with strong SATB2 positivity is that in fact they represent osteosarcomas with very scant or minimal malignant osteoid material, but which is difficult to confirm or exclude since as yet there are no highly specific genetic alterations that characterize any of these tumors. Specifically, all chondrosarcomas that belong to the present series showed at least