SATB2 is not a reliable diagnostic marker for distinguishing between oral osteosarcoma and fibro-osseous lesions of the jaws

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Objective. Special AT-rich binding protein 2 (SATB2) is an immunohistochemical marker for osteoblast differentiation. Our aim was to investigate SATB2 expression in oral osteosarcoma and other bone-producing oral tumors/reactive lesions to evaluate its usefulness as a diagnostic marker.

Study Design. A total of 74 intraosseous and soft tissue bone-producing surgical samples and 10 samples of reactive bone tissue were stained with SATB2, including osteosarcoma/chondrosarcoma (n = 16), fibro-osseous lesions (n = 42), central giant cell granuloma (n = 6), osteoblastoma (n = 1), and gingival lesions (n = 9). Nuclear labeling of the stromal spindle cells and intensity of staining was scored and analyzed.

Results. The intraosseous (n = 65/65) and soft tissue samples (n = 9/9) diffusely expressed SATB2. The strongest expression was observed in juvenile aggressive ossifying fibroma (n = 2/2). Weak SATB2 expression was observed in the stromal spindle cells adjacent to reactive bone tissue (periosteal bone reaction).

Conclusions. Our results indicate that SATB2 is not a reliable diagnostic marker for oral osteosarcoma but has practical use in detecting cells with osteoblast differentiation in histologic samples with scant bone production or in differentiating between a periosteal bone reaction and neoplastic bone induced by the tumor mesenchymal cells. Targeting SATB2 as an alternative therapy in oral osteosarcoma, fibro-osseous lesions, and central giant cell granuloma should be further investigated. (Oral Surg Oral Med Oral Pathol Oral Radiol 2021;131:572−581)

Osteosarcoma (OS) is a group of malignant tumors where the neoplastic mesenchymal cells produce neoplastic osteoid or immature bone.[1] The etiology for OS development remains unknown, although it appears to be related to a disturbance of bone growth and maturation during periods of high osteoblastic activity.[2] Extragnathic OS is the most common primary bone malignancy in children yet infrequently arises in the oral cavity. Only 6% to 10% of all OSs present in the oral cavity.[3] OS typically affects the long bones of extremities and presents with a bimodal age distribution of 10 to 25 years and >60 years.[4] Males are affected more than females. Oral OSs present with distinct epidemiology and prognostic outcomes. Based on these differences, it has been suggested that oral OS may be a separate and specific entity.[2,5-7] Oral OS presents in an older patient population (average age, 33 years) with no sex preference and the mandible and maxilla are equally affected.[7] Oral OSs are considered less aggressive than extragnathic OSs with a lower metastatic rate.[2,5-8] Some patients report symptoms for relatively long periods before diagnosis, which indicates that some oral OSs grow rather slowly.[8] The average 5-year survival for oral OS is approximately 80%.[9] The average 5-year survival for extragnathic early stage OS is approximately 71% and drops to 41% when there is evidence of metastasis.[7,10] OS of the long bones often is accompanied by nocturnal pain,[11] an infrequent clinical finding for patients with oral OS.[2] Yet in some cases, patients with oral OS will experience symptomatic bone swelling, loosening of teeth, and/or paresthesia.[12] OS is divided into the periosteal type, which develops in the intramedullary cavity of the bone and represents approximately 75% of all OS, and the parosteal type, which develops on the cortical bone surface without evidence of medullary involvement.[4] The common microscopic features shared by all types of OS is the detection of tumor osteoid and/or osseous tissue arising directly from the sarcomatous stroma of neoplastic mesenchymal cells.[1] OS is subdivided into a variety of histologic subtypes. The most recognized subtypes include the osteoblastic type (copious amount of osteoid and immature bone), chondroblastic type (cartilaginous differentiation), and fibroblastic type (primarily mesenchymal with minimal osteoid or bone production). Less commonly observed patterns include epithelioid, giant cell rich, small cell,

Statement of Clinical Relevance

SATB2, strongly expressed in oral osteosarcoma, fibro-osseous lesions, and central giant cell granuloma, is not a reliable diagnostic marker. However, SATB2 can distinguish between induced neoplastic bone and reactive bone tissue. Targeting SATB2 as an alternative therapy should be investigated.

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and telangiectatic types.[1] Of the various oral OS histologic subtypes, some claim that the chondroblastic type is most common,[2,7] whereas others have found the osteoblastic subtype to be most prevalent.[13] OS is also assigned a microscopic tumor grade that has prognostic implications and is based on the cytologic nuclear and cellular atypia. Low grade OS, which has a more favorable outcome, is composed of well-differentiated spindle cells with relatively low cellularity, without significant atypia and few mitotic figures. High grade tumors have a worse outcome and present with cytologic atypia and a high number of mitotic figures.[14] Most oral OSs are microscopically low grade tumors.[7,12] The relatively well-differentiated, minimal cellular atypia and fibrous nature of oral OS make it difficult to distinguish from fibro-osseous jaw lesions and, in some small biopsy samples, a periosteal bone reaction.[7,14-17] Currently, there are no reliable diagnostic markers for either OS or fibro-osseous lesions because the immune profile lacks specificity.[18] Fibro-osseous lesions of the jaws are a heterogenous group of benign entities that microscopically are composed of cellular mesenchymal tissue that induce bone formation.[19] They include the central cemento-ossifying fibroma (COF; a benign mesenchymal odontogenic tumor), cemento-osseous dysplasia (COD; a group of reactive/dysplastic processes of unknown stimulus), and fibrous dysplasia (FD; a genetic, self-limiting dysplastic process). COD is further subtyped into the periapical, focal, and florid subtypes, depending on its location and whether it is unifocal (focal subtype) or multifocal (periapical or florid subtype).[19,20] The presenting age of patients with COF and COD is in the third to fourth decades,[19] and FD, which commonly begins to develop in childhood, is usually diagnosed at approximately 20 years of age.[21] Central giant cell granuloma (CGCG) is considered widely to be a non-neoplastic lesion of unknown pathogenesis with good prognosis and develops in a wide age range (2-80 years).[22] Hybrid tumors represent a combined lesion composed of both a CGCG and fibro-osseous lesion. They are of unknown pathogenesis and biologic behavior.[22] Several important genes have been identified in extragnathic OS, including various tumor suppressor genes, oncogenes, and genes coding for growth factors, yet due to its complex genomic background, a specific, recurrent genetic alteration has not been found that can explain tumorigenesis, used for treatment, or relied on 100% for diagnostic accuracy.[18] Nevertheless, inactivation of p53 and Rb pathways appears to be a central event in the genesis of OS.[23] Special AT-rich binding protein 2 (SATB2) is an immunohistochemical marker for osteoblast differentiation.[24] SATB2 is a transcription factor that binds DNA in the nuclear matrix attachment regions. Normally found in the branchial arches and osteoblast lineage cells, SATB2 greatly influences gene expression in several biological processes, including osteoblastogenesis and bone regeneration.[24] As a transcription factor, SATB2 can interact and enhance 2 of the most potent osteoblast master regulators: RUNX2 and ATF4 genes.[25] In addition, SATB2 imparts a negative influence on the HOX2 genes, potent bone formation inhibitors, which also results in osteoblast differentiation.[25] Since the discovery of SATB2, studies have explored the potential use of SATB2 on various human tumors with osteoblast differentiation and found SATB2 to be a sensitive diagnostic marker for sarcoma types with osteoblastic differentiation, including extragnathic OS.[10,18,26-28] Currently, SATB2 is often used routinely in pathology services as a helpful adjunct to the morphologic evaluation in diagnostically challenging cases of extragnathic OS.[10,18]

In the present study, we analyzed and compared the expression of SATB2 in a group of bone-producing intraosseous lesions and reactive soft tissue gingival lesions, including oral OS, oral chondrosarcoma (CS), fibro-osseous lesions of the jaws, CGCG, peripheral ossifying fibroma (POF), and peripheral giant cell granuloma (PGCG), in order to evaluate its usefulness as a diagnostic marker. In addition, cases of reactive bone tissue (periosteal bone reaction) were analyzed for SATB2 expression. To our knowledge, this is the first study to evaluate SATB2 in oral OS and fibro-osseous lesions of the jaws.

MATERIALS AND METHODS

Data collection

A total of 74 bone-producing lesions and tumors were evaluated for SATB2 expression. These comprised oral OS (n = 15), oral CS (n = 1), fibro-osseous lesions of the jaws (n = 42), CGCG (n = 6), osteoblastoma (n = 1), PGCG (n = 5), and POF (n = 4). In addition, 10 samples of reactive bone tissue (periosteal bone reaction) were analyzed for SATB2 expression. The fibro-osseous lesions included (1) COF (n = 14), (2) FD (n = 9), (3) COD (n = 7), (4) juvenile aggressive ossifying fibroma (n = 2), and (5) hybrid lesions (n = 10).

The inclusion criteria consisted of primary oral OS, fibro-osseous lesions, and CGCG/PGCG diagnosed and treated between 1994 and 2020 at the Oral and Maxillofacial Surgery Department and Pathology Department at Rambam Medical Center. The exclusion criteria included any case of oral OS that was treated with presurgical chemotherapy or radiation. Following a process of anonymization, all slides and tissue blocks were retrieved from the Pathology Department archives at Rambam Medical Center, Haifa, Israel, and retrospectively reviewed and analyzed. Representative hematoxylin and eosin−stained slides of all 84 samples

were retrospectively reviewed by at least 2 pathologists to confirm the diagnosis. Diagnosing a fibro-osseous lesion requires clinical, radiologic, and pathologic correlation.[19] All of the fibro-osseous cases were retrospectively analyzed by a combined staff that included an oral pathologist and oral radiologist to confirm the diagnosis. Patient medical records were retrospectively reviewed for information that related to tumor characteristics, epidemiology, and prognostic outcome. The study was approved by the institutional review board of Rambam Medical Center.

Microscopic data collection and immunohistochemical analysis

Paraffin-embedded tumor blocks of all 84 cases were retrieved from the archives of the Department of Pathology. The paraffin-embedded blocks were sectioned in 4-μm-thick slides and stained with hematoxylin and eosin. For immunohistochemical analysis of SATB2, 4-μm-thick sections were deparaffinized and immunostained with anti-SATB2 antibody (Cell Marque, Darmstadt, Germany) at a dilution of 1:30. The iView DAB detection kit was used by means of an automatic stainer (BenchMark ULTRA system, Ventana, Oro Valley, Arizona, USA). Colonic epithelium served as a positive control and nuclear staining was considered positive expression. The same tissue that was used for the positive control was used as the negative control. The variety of cell types that were present in the tissue sections offered internal negative control sites. The specimens were evaluated with an entire cross section of tumor at the area of maximum dimension submitted for histologic examination.

Scoring of immunohistochemistry

The quantity of nuclear SATB2 expression in the stroma for each stained section was evaluated blindly by 2 experienced oral pathologists. The extent of SATB2 nuclear staining in the stromal spindle cells was scored similar to previously used methods and represented the percentage of positive tumors cells (0, no staining; 1, <5%; 2, 5%-25%; 3, 26%-50%; 4, 51%-75%; 5, 76%-100%). The intensity of staining was graded as weak, moderate, and strong.

RESULTS

Patient epidemiology Table I

The age and sex were known in all cases. The overall average age at diagnosis was 37 years (range, 15-60) for oral OS/CS and 37 years (range, 10-82 years) for fibro-osseous lesions. The average age for CGCG was 28 years (range, 17-54 years). Eighty-one percent of oral OS and CS developed in men (n = 13/16) and 35% of fibro-osseous lesions and CGCG developed in men (n = 17/48). The location was known in all cases and included oral OS (mandible: n = 3, 19%; maxilla: n = 13, 81%), fibro-osseous lesions and CGCG (mandible: n = 34, 71%; maxilla: n = 14, 29%). Survival data were available for 7/16 patients with oral OS (43%), of whom 1 patient died due to disease-related causes.

SATB2 expression in malignant tumors of osteoblast differentiation

All cases of oral OS and CS (n = 16), including those with minimal osteoid production, displayed SATB2 nuclear immunoreactivity with a diffuse staining pattern in the stromal spindle cells: scores 0-1 (0), 2 (n = 1, 6%), 3 (n = 11, 69%), 4 (n = 4, 25%), and 5 (n = 0). The staining intensity was moderate or strong in 93% of cases. There were no detectable differences in SATB2 expression between the osteosarcoma histologic subtypes.

SATB2 expression in intraosseous fibro-osseous lesions, CGCG, and osteoblastoma Table II

All cases of fibro-osseous lesions of the jaws (n = 42), CGCG (n = 6), and osteoblastoma (n = 1), including those with minimal osteoid production, expressed SATB2 with a diffuse staining pattern in the stromal spindle cells: scores 0-1 (0), 2 (n = 2, 4%), 3 (n = 17, 35%), 4 (n = 26, 53%), and 5 (n = 4, 8%). The staining intensity was moderate or strong in 96% of cases.

Table I. Patient epidemiology (n = 74)

	OS and CS (n = 16)	*COF, JCOF, and OB (n = 17)*	*FD (n = 9)*	*COD (n = 7)*	*Hybrid (n = 10)*	*CGCG (n = 6)*	*Peripheral (n = 9)*
Average age (years) (range)	37 (15-60)	41 (13-66)	18 (10-26)	40 (17-50)	34 (12-82)	28 (17-54)	51 (23-68)
Gender							
Male, *n* (%)	13 (81)	7 (41)	4 (44)	2 (29)	2 (20)	3 (50)	5 (55)
Female, *n* (%)	3 (19)	10 (59)	5 (55)	5 (71)	8 (80)	3 (50)	4 (45)
Location							
Mandible, *n* (%)	3 (19)	11 (65)	5 (55)	7 (100)	7 (70)	5 (83)	NA
Maxilla, *n* (%)	13 (81)	6 (35)	4 (44)	0	3 (30)	1 (17)	NA

OS, osteosarcoma; *CS*, chondrosarcoma; *COF*, central ossifying fibroma; *JCOF*, juvenile ossifying fibroma; *OB*, osteoblastoma; *FD*, fibrous dysplasia; *COD*, cemento-osseous dysplasia; *CGCG*, central giant cell granuloma; *NA*, not applicable.

Table II. Comparative SATB2 immunohistochemistry in intraoral bone producing tumors and lesions (n = 74) and reactive bone tissue (n = 10)

Type	*Diagnosis*	*No. of cases*	*No. (%) of positive cases*					
			Score 0	*Score 1*	*Score 2*	*Score 3*	*Score 4*	*Score 5*
Malignant bone-producing tumors	OS, osteoblastic	6	0	0	1 (17)	3 (50)	2 (33)	0
	OS, fibroblastic	6	0	0	0	5 (83)	1 (17)	0
	OS, chondroblastic	3	0	0	0	2 (66)	1 (33)	0
	Chondrosarcoma	1	0	0	(0)	1 (100)	0	0
	Total	16	0	0	1 (6)	11 (69)	4 (25)	0
Intraosseous fibro-osseous lesions, CGCG, OB	COF	14	0	0	0	6 (43)	7 (50)	1 (7)
	Juv. aggress-OF	2	0	0	0	0	0	2 (100)
	COD	7	0	0	2 (29)	3 (42)	2 (29)	0
	FD	9	0	0	0	4 (44)	4 (44)	1 (12)
	Hybrid lesion	10	0	0	0	3 (30)	7 (70)	0
	CGCG	6	0	0	0	0	6 (100)	0
	OB	1	0	0	0	1 (100)	0	0
	Total	49	0	0	2 (4)	17 (35)	26 (53)	4 (8)
Soft tissue reactive lesions	PGCG	5	0	0	0	1 (20)	4 (80)	0
	POF	4	0	0	0	1 (25)	3 (75)	0
	Total	9	0	0	0	2 (22)	7 (78)	0
Reactive bone tissue	Perios. bone Rx	10	0	10 (100)	0	0	0	0
	Total	10	0	10 (100)	0	0	0	0

SATB2, special AT-rich binding protein 2; *OS*, osteosarcoma; *CGCG*, central giant cell granuloma; *OB*, osteoblastoma; *COF*, central ossifying fibroma; *Juv. aggress-OF*, juvenile aggressive ossifying fibroma; *COD*, cemento-osseous dysplasia; *FD*, fibrous dysplasia; *PGCG*, peripheral giant cell granuloma; *POF*, peripheral ossifying fibroma; *Perios. bone Rx*, periosteal bone reaction.

SATB2 expression with a score of 5 (n = 4) was only observed in juvenile aggressive ossifying fibroma (2/2; 100%), 1 case of COF (1/14, 7%), and 1 case of FD (1/9, 7%). SATB2 expression with a score of 2 was only observed in COD (2/7, 29%).

SATB2 expression in soft tissue reactive lesions Table II

All cases of POF and PGCG (n = 9) expressed SATB2 with a diffuse staining pattern in the stromal spindle cells: scores 0-2 (0), 3 (n = 2, 22%), 4 (n = 7, 78%), and 5 (0). The staining intensity was moderate or strong in 100% of cases.

SATB2 expression in reactive bone tissue (periosteal bone reaction) Table II

The stromal spindle cells adjacent to reactive bone tissue (periosteal bone reaction) weakly and focally expressed SATB2 and were primarily detected adjacent to the osteoblasts surrounding the newly formed osteoid and woven bone: score 1 (n = 10). The staining intensity was weak in 100% of cases.

DISCUSSION

The aim of our study was to investigate the potential use of SATB2 as a diagnostic marker for differentiating between oral osteosarcoma and fibro-osseous lesions, which are a group of bone-producing lesions and tumors.

The strong influence of SATB2 on osteoblastogenesis inspired numerous investigators to evaluate its diagnostic and prognostic role in extragnathic OS. The results of these studies showed that SATB2 is a sensitive yet not specific diagnostic marker for extragnathic OS but, as an adjunct to the evaluation of the morphologic features, is practically useful in cases of diagnostic uncertainty.[10,26-29] SATB2 expression was also investigated in variety of other non-bone-producing human cancers.[18,26,29-32] In one study, a combination of CK20 and SATB2 immunohistochemistry identified 95% of all colorectal carcinomas.[33] As suggested by others, it is possible that SATB2 expression may not be limited to osteoblasts.[32,34] The oral cavity is an uncommon location of OS development. Previous studies on a large series of cases found that oral OSs are primarily low grade tumors.[7,12] A diagnosis of OS requires the identification of osteoid embedded within a matrix of neoplastic spindle cells, yet in many cases of low grade OS, the atypia is minimal and not always obvious.[7] In these cases, it may be difficult to distinguish diagnostically from fibro-osseous lesions.[2,16] Currently, there are no reliable diagnostic markers to definitively diagnose either OS or fibro-osseous lesions because their immunoprofile lacks specificity as a result of tumor heterogeneity.[35] In 25% of cases, oral OS induce a periosteal reaction characterized on the radiographs as a "sunburst" appearance or with evidence of a symmetrically widened periodontal membrane space, but these findings are not specific and present in a large array of jaw conditions, including FD and others.[2,36,37] Distinguishing oral OS from fibro-osseous lesions is essential

because OS requires a significantly more aggressive treatment regimen, including neoadjuvant chemotherapy, subsequent surgical resection with adequate margins, and postsurgical chemotherapy, adjusted according to the amount of tissue response (necrosis) from the preoperative chemotherapy.[10,29] Evidence of necrosis from the preoperative chemotherapy, in addition to the patient's metastatic status, is considered the most important prognostic variable for patients with OS.[38] It should be noted that the benefits of presurgical chemotherapy for oral OS remains unknown. A study conducted by Mardinger et al.[39] on a series of oral OS made the claim that presurgical chemotherapy did not dramatically alter the prognosis of oral OS, yet others dispute this claim.[40] In our study, the stromal spindle cells in the malignant, benign, and reactive oral bone-producing tumors were all diffusely positive with SATB2 with moderate to strong staining intensity. Fig. 1, 2, 3, 4 and 5 Table II Our findings are similar to those of Connor and Hornick,[10] who conducted a comparative SATB2 study on a group of extragnathic OS and benign bone-forming tumors (FD, osteoblastoma, and osteoid osteoma) and found nuclear activity in all cases. Machado et al.[27] found that the osteoblastic subtype of extragnathic OS expressed SATB2 more intensely than the other histologic subtypes, a finding that we could not substantiate Fig. 1. Chondrosarcoma is an exceptionally rare intraoral tumor. SATB2 expression was like intraoral osteosarcoma rendering its use as a diagnostic marker ineffective Fig. 2.

Fibro-osseous lesions all share common microscopic features that include a fibrocellular stroma with induction of calcified tissue without obvious osteoblastic rimming. Their distinction requires microscopic, clinical, and radiologic correlation, which is unique for each entity.[19] CGCGs are composed of a fibrocellular stroma that contains multinucleated giant cells and, in some cases, bone induction.[41] In our study, the stromal spindle cells in all of the fibro-osseous lesions, CGCGs, and hybrid lesions diffusely expressed SATB2, independent of the amount of osteoid produced. Fig. 3 These findings support the claim that the stroma in these entities is rich in differentiating osteoblasts. The practical implication of this finding is that SATB2 immunohistochemistry may be used to detect a fibro-osseous lesion in small biopsy specimens or specimens with minimal bone production. In the realm of extraoral oncology, SATB2 is being investigated as a target for treating a variety of different neoplasms.[42,43] Based on the results of our study, it may be possible to target SATB2 receptors as an alternate treatment option for fibro-osseous lesions or CGCG, especially in young patients, where surgery is undesired. Further studies are warranted.

FD presents in an age group similar to that for oral OS. FD is a self-limiting process with apparent responsiveness to the hormonal changes of puberty. Following diagnostic confirmation with an incisional biopsy and clinical/radiologic correlation, no further treatment is required.[19] Yet according to previous studies, 0.4% to 2.0% cases of FD undergo malignant transformation to a low grade osteosarcoma, with or without a history of irradiation.[44-47] These cases often pose a significant diagnostic challenge. Though the detection of GNAS mutations is diagnostic for FD,[19,21] this approach is costly, laborious, and unavailable in many centers. Therefore, immunohistochemistry is an appealing substitute. In our study, SATB2 was diffusely expressed in 97% of the FD cases and therefore cannot be used to distinguish FD from oral OS. There were no differences in SATB2 expression between the monostotic and polyostotic FD types. Our results differ from those of Li et al.,[48] who found negative SATB2 expression in their series of fibrous dysplasia cases.

COF is considered a benign tumor and is treated conservatively with enucleation. In our study, all cases diffusely expressed SATB2. Juvenile aggressive COF is a distinct clinical subtype of COF that develops in young patients, has a high propensity for recurrence, and exhibits aggressive clinical behavior.[49] Microscopically, the stroma in juvenile COF is particularly cellular and may be confused with a low grade oral OS.[50] In our study, the stromal cells in juvenile aggressive COF were exceptionally immunoreactive to SATB2, which implies that the stroma is highly rich in osteoblasts. Utilizing SATB2 to differentiate juvenile COF from oral OS would require a further study on a larger series of cases.

COD, a reactive/dysplastic process of unknown etiology, is treated conservatively.[50] Compared to the other fibro-osseous lesions, COD showed weaker SATB2 expression, which may imply that, in some cases, COD shares more features with reactive bone than an actual fibro-osseous process.

The fibrocellular stroma of CGCG, PGCG and juvenile aggressive CGCG diffusely expressed SATB2, even though the presence of calcified material was usually scant. Fig. 3 and 4 All of the above subtypes are considered benign processes of unknown etiology or pathogenesis.[50] Juvenile aggressive CGCG is an aggressive subtype of CGCG that occurs in young patients. Currently, alternative medical therapies to surgery for CGCG and juvenile aggressive CGCG are 'being investigated,[50] and we propose,based on the strong SATB2 expression, that additional studies regarding its potential use as an adjunct treatment option are warranted.

Hybrid tumors, composed of both a COF and CGCG component, diffusely expressed SATB2. It has been

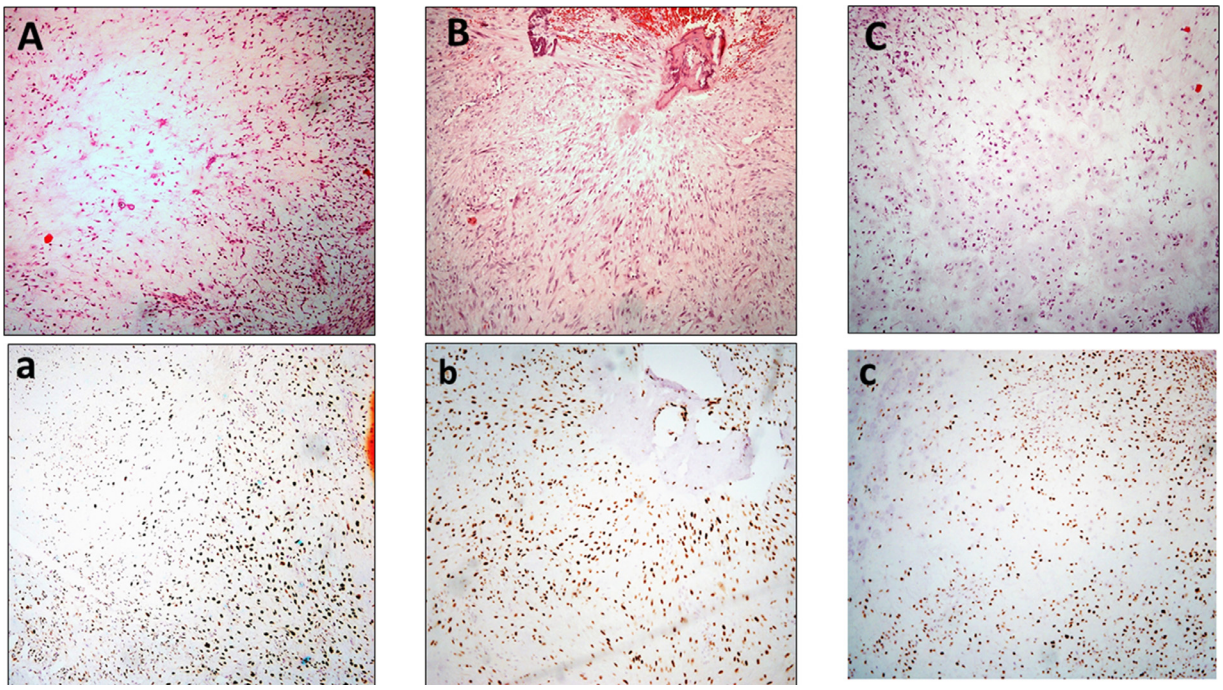

Fig. 1. All osteosarcomas, irrespective of morphologic pattern, were immunoreactive to SATB2: **(A)** fibroblastic type (hematoxylin and eosin × 100); **(B)** osteoblastic type (hematoxylin and eosin × 100); **(C)** chondroblastic type (hematoxylin and eosin × 100). Positive SATB2 immunohistochemical staining of the spindle cells for the cases depicted in **(A),** (B), and (C), respectively. **(a)**-**(c)** Score 3.

speculated that the primary lesion of a hybrid tumor is a fibro-osseous lesion with induction of a CGCG compartment. Our findings were unable to either support or deny that claim because SATB2 was diffusely expressed in both compartments Fig. 3.

Osteoblastoma is a true benign tumor that usually presents in the spine or long bones, with rare cases developing in the oral cavity. Microscopically, they contain proliferating osteoblasts and bone trabeculae.[1,51] Our single case of oral osteoblastoma expressed SATB2 with a pattern like fibro-osseous lesions and CGCG Fig. 5.

An important discovery from our study was that SATB2 showed potential in the microscopic distinction between oral OS/fibro-osseous lesions and the cellular stromal spindle cells often found adjacent to reactive bone tissue (periosteal bone reaction). Periosteal bone reactions occur secondary to a variety of oral pathologic conditions, including traumatic, inflammatory, and neoplastic conditions.[52] Microscopically, a periosteal bone reaction is composed of trabeculae of newly formed woven bone and osteoid that may mimic woven bone or osteoid induced by tumor mesenchymal cells, especially in small biopsies

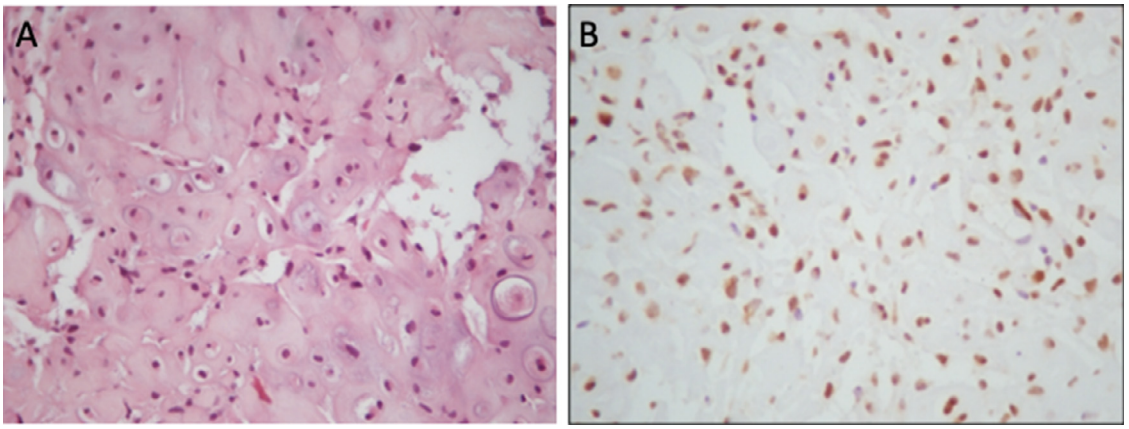

Fig. 2. An example of chondrosarcoma: **(A)** hematoxylin and eosin × 200 and **(B)** SATB2 stain (score 3).

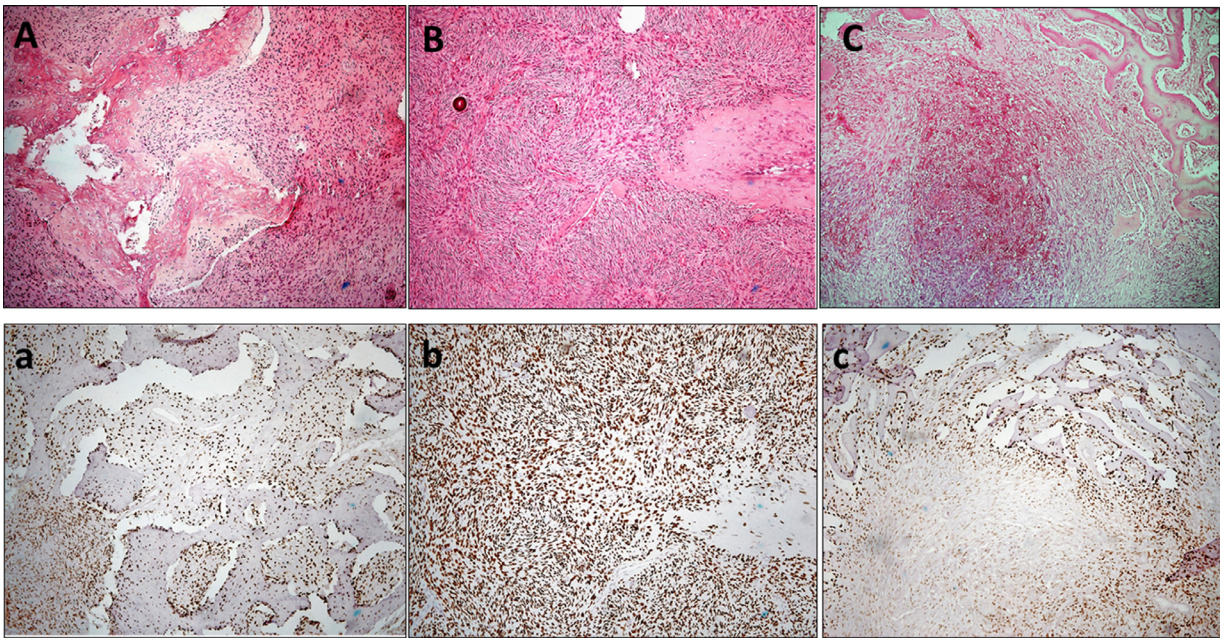

Fig. 3. All fibro-osseous lesions were immunoreactive to SATB2. Sample case of **(A)** fibro-osseous lesion (hematoxylin and eosin × 100), **(B)** juvenile aggressive ossifying fibroma (hematoxylin and eosin × 100), and **(C)** hybrid tumor (hematoxylin and eosin × 100). Positive SATB2 immunohistochemical staining of the spindle cells: **(a)** fibro-osseous lesion (score 3), **(b)** juvenile aggressive ossifying fibroma (score 5), **(c)** hybrid lesion (score 3).

or in cases with significant bone deposition and scant intervening stroma.[17,52] The SATB2 expression pattern in OS, fibro-osseous lesions, CGCG, and hybrid tumors was distinctly diffuse and expressed in both the stromal spindle cells and osteoblasts surrounding the newly induced osteoid and bone.

In our study, the stromal spindle cells adjacent to reactive bone tissue (periosteal bone reaction) weakly and focally expressed SATB2 and were primarily detected adjacent to the osteoblasts surrounding the newly formed osteoid and woven bone: score 1 (n = 10). The staining intensity was weak in 100% of cases Fig. 6.

These findings seem to provide evidence that SATB2 may be used to distinguish between the stromal spindle cells in a reactive bone process and tumor mesenchymal tissue that induces bone. Further studies are warranted.

The impact of SATB2 on tumor progression and prognosis was investigated in a variety of human

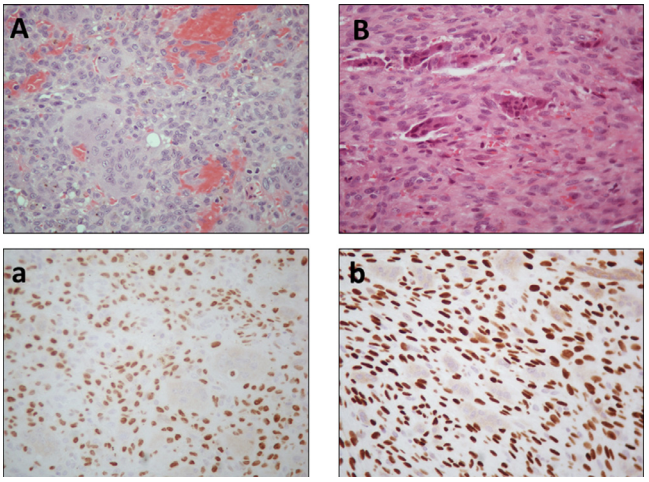

Fig. 4. All giant cell granulomas were immunoreactive to SATB2. Sample case of **(A)** CGCG (hematoxylin and eosin × 200) and **(B)** PGCG (hematoxylin and eosin × 200). Positive SATB2 immunohistochemical staining of the spindle cells: **(a)** CGCG (score 3) and **(b)** PGCG (score 4).

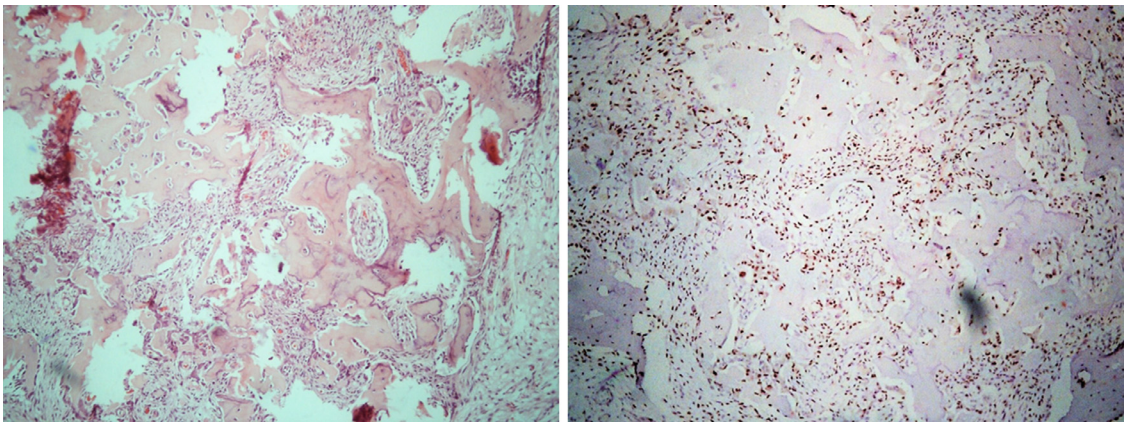

Fig. 5. A case of osteoblastoma with score 3 SATB2 expression: **(A)** hematoxylin and eosin × 100 and **(B)** SATB2 stain.

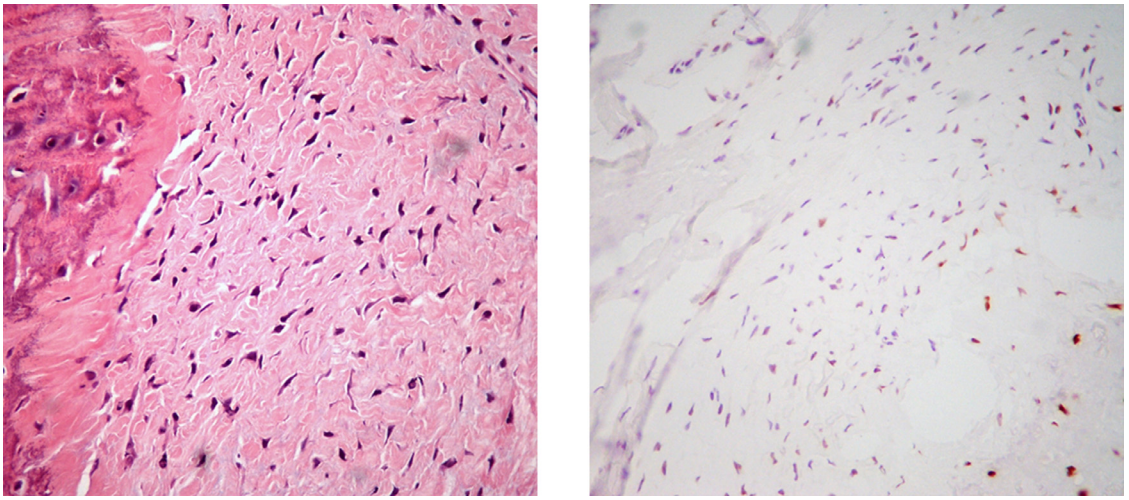

Fig. 6. An example of periosteal bone reaction: **(A)** hematoxylin and eosin × 200 and **(B)** SATB2 stain (score 1). Note the weak SATB2 expression of the stromal spindle cells.

cancers, including osteosarcoma, lung, renal, colorectal, gastric, breast, colorectal, pancreatic, hepatocellular, and head and neck cancers, with conflicting results. Some previous studies found that SATB2 imparts positive prognostic characteristics, including reduced tumor migration, invasion, and metastasis.[28,31] In our study, strong SATB2 expression was more common in the benign tumors, which may suggest, in agreement with these previous studies,[28,31] that SATB2 may have a positive influence on tumor characteristics. Further studies on a larger series of cases are warranted to substantiate these findings.

CONCLUSION

Our study shows that SATB2 cannot be used as a reliable diagnostic marker to distinguish between oral OS and fibro-osseous lesions of the jaws. The mesenchymal tissue of OS, fibro-osseous lesions, and CGCG is composed of stromal spindle cells that diffusely and strongly expressed SATB2. These findings confirm that the stromal spindle cells are primarily composed of cells with osteoblast lineage. Compared to fibro-osseous lesions, reactive bone tissue (periosteal bone reaction) weakly expressed SATB2. The practical implications of our study include the following: (1) SATB2 staining can be used in histologic samples with scant bone production or in small biopsy samples to detect osteoblast lineage cells for diagnostic purposes, (2) SATB2 staining may be a useful diagnostic tool to distinguish between reactive bone formation and the tumor-induced calcified tissue, (3) further studies should explore SATB2 as a potential target for therapy in fibro-osseous and CGCG cases where an alternative therapy to surgery may be desired.

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