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Original contribution

Sclerostin expression in skeletal sarcomas☆,☆☆

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Summary Sclerostin (SOST) is an extracellular Wnt signaling antagonist which negatively regulates bone mass. Despite this, the expression and function of SOST in skeletal tumors remain poorly described. Here, we first describe the immunohistochemical staining pattern of SOST across benign and malignant skeletal tumors with bone or cartilage matrix (n = 68 primary tumors). Next, relative SOST expression was compared to markers of Wnt signaling activity and osteogenic differentiation across human osteosarcoma (OS) cell lines (n = 7 cell lines examined). Results showed immunohistochemical detection of SOST in most bone-forming tumors (90.2%; 46/51) and all cartilage-forming tumors (100%; 17/17). Among OSs, variable intensity and distribution of SOST expression were observed, which highly correlated with the presence and degree of neoplastic bone. Patchy SOST expression was observed in cartilage-forming tumors, which did not distinguish between benign and malignant tumors or correlate with regional morphologic characteristics. Finally, SOST expression varied widely between OS cell lines, with more than 97-fold variation. Among OS cell lines, SOST expression positively correlated with the marker of osteogenic differentiation alkaline phosphatase and did not correlate well with markers of Wnt/β-catenin signaling activity. In summary, SOST is frequently expressed in skeletal bone- and cartilage-forming tumors. The strong spatial correlation with bone formation and the in vitro expression patterns are in line with the known functions of SOST in nonneoplastic bone, as a feedback inhibitor on osteogenic differentiation. With

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anti-SOST as a potential therapy for osteoporosis in the near future, its basic biologic and phenotypic consequences in skeletal tumors should not be overlooked.

1. Introduction

Sclerostin (SOST) is an extracellular Wnt signaling antagonist with high endogenous expression in osteocytes [1,2]. Human disorders of SOST expression and activity result in bone overgrowth in rare autosomal recessive syndromes, including sclerosteosis and Van Buchem disease. SOST is well described to negatively regulate osteogenesis and bone mass [1,2], and targeted *Sost* deletion in mice results in a high-Bone Mineral Density phenotype with increased bone strength [3]. Consequently, significant interests exist in the use of anti-SOST neutralizing antibodies for the clinical entity of osteoporosis, such as romosozumab (AMG 785; Amgen, Thousand Oaks, CA) [4-6] and blosozumab (LY2541546; Eli Lilly and Company, Indianapolis, IN). Preclinical studies have shown that anti-Sost antibodies inhibit bone loss in ovariectomy [7,8], in the aged skeleton, and during fracture healing [9,10]. The expression and function of SOST in skeletal tumors remain poorly understood.

The importance of avoiding tumorigenesis cannot be overlooked in the field of osteoporotic therapies. This issue has growing importance with protein-based bone anabolism. For example, the main Food and Drug Administration–approved recombinant protein for local bone formation is bone morphogenetic protein (BMP) 2. BMP ligands and BMP receptors are expressed in most osteosarcoma (OS) cell lines and OS subtypes. Moreover, although disagreement in the literature exists, the presence of BMP signaling in OS may impart a worse prognosis [11]. On the cellular level, BMP signaling appears to mediate promigratory effects in both OS and chondrosarcoma (CS) cell types. Likewise, parathyroid hormone is the main Food and Drug Administration–approved anabolic agent in the treatment of osteoporosis. Unfortunately, the clinical duration of use for parathyroid hormone is limited to 24 months, owing to the potential risk of osteosarcomagenesis (as documented in rat studies) [12]. Thus, currently approved agents for bone anabolism are not without potential risks for skeletal sarcomagenesis.

There is to date little known regarding SOST expression and function in skeletal sarcomas. Several pieces of data suggest that SOST has diverse roles in epithelial malignancies, including breast carcinoma, prostate carcinoma, thyroid carcinoma, and renal cell carcinoma. In general, studies have demonstrated that overexpression of numerous Wnt components in OS (including Wnt ligands, Frizzled, and LRP receptors), highlighting the implications of aberrant Wnt/β-catenin signaling in OS progression [13,14]. In contrast, Wnt antagonists are generally reduced in OS. For example, WIF-1 messenger RNA expression was significantly decreased in numerous OS cell lines in comparison to normal human osteoblasts, attributed to WIF-1 promoter hypermethylation [15]. Investigators found that WIF-1 down-regulates the expression of MMP-9 and 14, thereby preventing the invasion and mobility of OS cells [15]. Kansara et al [16] further confirmed that WIF-1 is epigenetically silenced in human OS, and targeted disruption of WIF-1 accelerates OS formation in mice. Likewise, expression of other Wnt/β-catenin inhibitors, such as FrzB/sFRP3, is consistently suppressed in OS [17]. Conversely, in CS, increased DKK1 expression was recently observed to correlate with high Wnt signaling activity and a poor prognosis [18]. Despite this, the expression and function of SOST in skeletal tumors is poorly understood. Here, we provide a comprehensive description of SOST expression in skeletal bone- and cartilage-forming tumors.

2. Materials and methods

2.1. Antibodies and reagents

Primary antibodies used in this study were anti-SOST (ab75914; Abcam, Cambridge, MA). All other reagents were purchased from Dako (Carpinteria, CA) unless otherwise specified.

2.2. Tissue procurement

Tumors were retrospectively collected from biopsy and resection specimens at the University of California, Los Angeles, under institutional review board number 13-897. Each tumor was re-examined by 2 blinded bone tissue pathologists to ensure accuracy of original diagnosis. When appropriate, radiographs were also examined to confirm concordance with the pathologic diagnosis. Demographic features and specific tumor measurements were recorded, including patient age, sex, anatomical location, tumor size, and history of neoadjuvant therapy (Supplementary Table 1). When available, undecalcified samples were chosen for immunohistochemical staining; 52.8% of samples were decalcified (28/53 samples), including 20 of 36 benign and malignant bone-forming tumors and 8 of 17 benign and malignant cartilage-forming tumors.

2.3. Histologic and immunohistochemical analyses

Five-micrometer-thick paraffin sections of bone and cartilage tumors were stained with hematoxylin and eosin (H&E). Using H&E sections, histomorphologic assessments were made to confirm tumor type and to determine characteristics