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Review Article

Hylonome

Identification of the disease-causing gene in sclerosteosis – discovery of a novel bone anabolic target?

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Abstract

Genetic studies recently unraveled the genetic cause of sclerosteosis, a rare skeletal dysplasia characterized by a generalized increase in bone mass. Different loss-of-function mutations were identified in *SOST*, a gene with no homology to any known gene. This *SOST* gene is also involved in the pathogenesis of van Buchem disease, a disorder closely resembling sclerosteosis, since a 52-kb deletion located downstream of *SOST* is found in patients diagnosed with this condition. Molecular studies showed a very restricted expression pattern of *SOST* and its gene product, sclerostin, with areas in the bone tissue, more precisely in cells of the osteoblast lineage, being the major sites of expression. Sclerostin is a secreted protein with a cysteine knot motif. *In vitro* studies demonstrated that sclerostin acts as a modulator of BMP signaling by binding to different members of the BMP growth factor family and acting on downstream BMP signal transduction events. The important function of sclerostin in bone metabolism has also been proven *in vivo* by the osteopenic phenotype of transgenic mice overexpressing *SOST* in bone. The identification of sclerostin as an important protein in bone metabolism opens new perspectives for the development of anabolic therapeutics to prevent and treat osteoporosis.

Keywords: Sclerosteosis, *SOST*, Sclerostin, Anabolic Agent

Sclerosteosis: clinical manifestations and incidence

Sclerosteosis, a genetic condition with an autosomal recessive pattern of inheritance, was first described in 1958 by Truswell as osteopetrosis with syndactyly in two unrelated South African girls[1]. In 1967, Hansen introduced the term sclerosteosis to designate this rare but severe skeletal disorder characterized by a massive and progressive bone overgrowth[2]. The skeleton of sclerosteosis patients is radiologically virtually normal in early childhood, but thereafter bone widening and sclerosis become increasingly evident. Patients display a generalized hyperostosis of the skeleton, mainly involving the skull and mandible which results in considerable facial distortion in adulthood. The bony encroachment of the cranial nerves and the consequent narrowing of the foramina can lead to clinical complications, including facial palsy, deafness and visual disturbances.

Radiologically and clinically sclerosteosis closely resembles van Buchem disease, a rare autosomal recessive condition first described by Van Buchem in 1955 as "hyperostosis corticalis generalisata"[3]. However, in the former condition a number of additional clinical symptoms can occur allowing a differential diagnosis with van Buchem disease. First, variable syndromic components of sclerosteosis are congenital hand and foot malformations including nail hypoplasia, radial deviation of the terminal phalanges and uni- or bilateral syndactyly of the fingers in many cases and less frequently of the toes. Second, excessive height is observed in a large number of sclerosteosis patients, especially in patients from South Africa. Third, bone overgrowth of the calvarium can result in progressive diminution of cranial capacity. The consequent raised intracranial pressure often leads to severe headaches and has resulted in some cases in a sudden death of the patient.

The prevalence of sclerosteosis is very low, but it is unusually frequent in the Afrikaner population in South Africa. A total of 63 affected South African individuals have currently been described[4-6]. Additionally, several reports of affected sibs and sporadic cases from New York[7], Maryland (USA)[8-11], Switzerland[12], Japan[13], Brazil[14], Spain[15] and Senegal[16] have been published.

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Molecular genetics of sclerosteosis – identification of the disease-causing gene *SOST*

Since sclerosteosis and van Buchem disease are genetically, radiologically and clinically very similar conditions, Beighton and colleagues postulated already in 1984 that the genetic defect causing both clinical entities could be very similar, i.e., that mutations in the same gene might underlie these two conditions[4]. In 1998, we published the results of a genetic study in which a genome-wide search was carried out in an extended, highly consanguineous Dutch family with van Buchem disease indicating linkage of the van Buchem disease gene to an interval of 0.7 cM in the chromosomal region 17q12-q21[17]. Consequently, we analyzed genetic markers from this region in two consanguineous families with sclerosteosis: the Brazilian family described by Paes-Alves et al.[14] in 1982 and a tri-racial kindred from Maryland[8-11]. Linkage analysis in both sclerosteosis families provided evidence for the co-localization of the sclerosteosis and van Buchem disease gene loci[18]. Key recombinational events delineated a sclerosteosis candidate gene interval of 6.8 cM flanked by D17S927 (proximal) and D17S791 (distal), completely encompassing the van Buchem disease gene region, again suggesting that sclerosteosis and van Buchem disease could be allelic conditions. A positional cloning procedure was consequently carried out to identify the disease-causing gene(s). Following the narrowing of the candidate gene interval to approximately 1 Mb, a mutation screen was performed on known and novel genes and expressed sequences located within this minimal region. Eventually, loss-of-function mutations were identified in a previously unkown two-exon gene in patients diagnosed with sclerosteosis. The gene was designated *SOST* and the *SOST* gene product sclerostin as being causative for sclerosteosis. We found nonsense mutations in the first exon of this gene in Brazilian and American sclerosteosis patients and a splice site variant at the splice donor site of the intron in an isolated sclerosteosis case from Senegal[19]. This splice site mutation results in an alteration of mRNA transcript processing caused by the introduction of a cryptic splice site downstream of the natural splice donor site[20]. Additionally, in the South African sclerosteosis patients, a nonsense mutation in exon 2 was identified[20]. Within the genomic sequence of the *SOST* gene and in the immediate surrounding sequence, we and others failed to detect a disease-causing mutation in the Dutch van Buchem patients[19,20]. However, when scanning genomic sequence further downstream of *SOST*, a 52-kb deletion was observed in these van Buchem patients[21,22]. Since this deletion could not be observed in a control population and extensive studies did not reveal any transcribed sequence located within this deletion, it seems very likely that the presence of this chromosomal rearrangement influences the transcription of the *SOST* gene. The potential effect of the deletion on *SOST* expression levels can then be triggered by the presence of regulatory sequences within the deletion or by a position effect caused by the deletion.

Localization of *SOST* mRNA and sclerostin protein in human bone

The *SOST* gene has a very restricted pattern of expression. Human *SOST* expression was quantified using real time quantitative RT-PCR analysis revealing low but significant levels in whole long bone, cartilage, primary osteoblasts differentiated for 21 days, kidney, liver and bone marrow[19,20]. Although an initial report by Kusu et al.[23] suggested that the *SOST* gene is expressed in the bone-resorbing osteoclast, a more recent and currently more generally accepted study demonstrated the presence of *SOST* mRNA transcripts in cells of the osteoblast lineage (primary human osteoblasts, human mesenchymal cells differentiated to osteoblasts and hypertrophic chondrocytes in cartilage tissue)[24]. Moreover, a strong positive sclerostin immunostaining was observed in osteocytes and osteocytic canaliculi in normal adult human bone sections. Chondrocytes and other osteoblastic cells were also stained, but less intensive[24].

Sclerostin as a modulator of BMP signaling

Sclerostin is a secreted monomer[21] with the characteristics of a cysteine knot. The spacing of the conserved cysteines is highly homologous to that of the Bone Morphogenetic Protein (BMP) antagonists of the DAN/Cerberus family[19,20,25], suggesting a potential BMP modulatory effect of sclerostin. BMPs are secreted growth factors exerting a very broad spectrum of biological activities during embryogenesis, organogenesis and during adult life[26-29], with important roles in the regulation of osteoblast differentiation[30-32]. Sclerostin is able to physically bind different members of the BMP family, including BMP-2, -4, -5, -6 and –7, but not TGF-β1, -β2 and -β3[23,24]. Winkler et al.[24] demonstrated a specific binding of sclerostin to BMP-6 which most likely results in competition for interaction of BMP-6 with type I and II BMP receptors. Additionally, early BMP signal transduction was impaired in mouse mesenchymal C3H10T1/2 cells whereas BMP-6-induced Smad phosphorylation was partially blocked by sclerostin[24]. The effect of sclerostin on BMP-mediated osteoblast differentiation was studied in the mouse pre-osteoblast MC3T3-E1 cell line. The activity of BMP-6 and -7, but not BMP-2 and -4, was inhibited by sclerostin as shown by a significant dose-dependent decrease in alkaline phosphatase activity[23].

Osteopenic phenotype in *SOST* transgenic mice

A transgenic mouse model has been generated in which the human *SOST* gene, under control of the mouse osteocalcin promoter, is selectively expressed in bone[24]. These mice display with an osteopenic phenotype with more fragile bones compared to wild type littermates. Histologically, lumbar vertebrae sections showed a disorganized bone architecture, thinner cortices, reduced amounts of trabecular bone,

impaired lamellar bone formation and chondrodysplasia. Additionally, histomorphometric measurements of calvarial bone sections illustrated a decreased osteoblast surface and bone formation rate in transgenic mice compared to wild type littermates. The alkaline phosphatase activity in mesenchymal cells isolated from bones of transgenic mice and grown in osteogenic medium was significantly reduced in comparison with cells from wild type littermates. Additionally, long-term cultures of these mesenchymal cells from transgenic mice showed a reduced level of mineralization. There were no significant changes in bone resorption parameters.

Concluding remarks

The molecular genetics of sclerosteosis and van Buchem disease, two similar sclerosing bone dysplasias in which a progressive overgrowth of the bones is manifested throughout the entire life, has recently been elucidated by the cloning of the disease-causing gene *SOST*. The localization of this gene is almost completely restricted to areas in the bone tissue where osteogenesis is actively taking place, more precisely in cells from the osteoblast lineage. Osteocytes and osteocytic canaliculi are sites within bone where sclerostin is most abundantly present. Therefore, the current hypothesis of sclerostins' mechanism of action is as an osteocytic-mediated regulator of bone remodeling. Once sclerostin is secreted by osteocytes, the protein could control on the one side proliferation and differentiation of osteoblastic cells and on the other side the activity of mature osteoblasts by modulating BMP activity.

Based on the sclerosteosis and van Buchem disease phenotypes with a continuous deposition of normal bone tissue together with the biological function of sclerostin as a regulator of bone homeostasis, the protein is considered a very strong target in the development of anabolic (bone-forming) agents to prevent and treat osteoporosis. Osteoporosis, a multifactorial disorder, is nowadays a major public health problem characterized by low bone mass and microarchitectural deterioration resulting in bone fragility and increased fracture risk. The mainstay of current therapies used in the treatment of osteoporosis is anti-resorptive agents. These drugs inhibit osteoclast-mediated bone loss, reduce bone turnover and only modestly increase bone mineral density. They reduce, but do not eliminate fracture risk and do not restore lost bone structure. On the contrary, anabolic agents directly stimulate bone formation by augmenting osteoblast proliferation and/or inhibiting osteoblast apoptosis. They have the potential to increase bone mass, restore skeletal microarchitecture and reduce fracture risk to a greater extent than anti-resorptives. Having seen the restraints of anti-resorptives, there is an unmet need for anabolic agents which might compensate these limitations, with sclerostin emerging as a very promising bone-forming agent.

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