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REVIEW

Sclerosing bone disorders: a lot of knowns but still some unknowns

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In the last decade, many advances have been made in understanding how osteoclasts and osteoblasts work and communicate by elucidation of the molecular genetic causes of many rare bone dysplasias. The relationship between the clinical findings and the molecular defects underlying these aberrant bone phenotypes has given new insights into the molecular machinery of the different bone cell types, and into how they act and interact to regulate bone mass. The study of sclerosing bone dysplasias caused by a disturbance of the balance between bone formation and bone resorption has had an especially high impact. Furthermore, it has also become clear that genetic variation within several of the identified genes contributes to the risk to develop osteoporosis and that in some cases the metabolic pathways involved provide interesting targets for the development of novel treatments for osteoporosis. In this review, some of the sclerosing bone diseases are discussed, focusing on the underlying mechanisms and the broader implications of the insights gained.

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Introduction

The pathogenic mechanisms underlying rare, monogenic diseases provide, on many occasions, paradigms for more common, multifactorial diseases. This is definitely the case with sclerosing bone dysplasias and osteoporosis. The former group of conditions is clinically, genetically and pathogenically very heterogeneous.[1] The working group on nosology and classification of genetic skeletal disorders classified them into three categories: the neonatal osteosclerotic dysplasias; the increased bone density group without modification of bone shape; and the increased bone density group with metaphyseal and/or diaphyseal involvement.[2] Altogether they represent a total of about 45 different clinical entities. Especially over the last decade, the underlying molecular defects have been identified for many of them. In this review some of these will be discussed, mainly selected for their contribution to our current understanding of bone metabolism and homeostasis and their relevance to osteoporosis. The balance between bone resorption and bone formation can be disturbed on either side in sclerosing bone dysplasias. We used this as the first criterion to classify the conditions, but, as will be illustrated and discussed, the different mechanisms of bone metabolism are coupled in different ways, indicating that in many conditions both mechanisms are disturbed.

Conditions with Abnormal Bone Resorption as the Primary Defect

The osteopetroses and pycnodysostosis. This group of conditions is, by definition, caused by an impaired osteoclastic bone resorption. A possible causative mechanism is disturbed differentiation of osteoclasts from haematopoietic stem cells. However, this turned out to be the case in only a small minority of cases. Loss-of-function mutations were found in the *TNFSF11* and *TFNRSF11A* genes encoding receptor activator of NF-κB ligand (RANKL) and its receptor RANK, respectively, both key players in the NF-κB pathway, which is essential for osteoclastogenesis.[3,4] The result of these mutations is an osteoclast-poor form of osteopetrosis with almost complete absence of any functional osteoclasts. Despite this, the clinical picture is, somewhat unexpectedly, relatively mild compared to other forms of osteopetrosis. The latter are mostly caused by the presence of a defective mechanism to acidify the extracellular compartment between the functional osteoclast and the bone tissue due to deficiency of different proteins involved in this process.[5] Loss-of-function mutations in carbonic anhydrase II prevent the intracellular generation of protons in osteopetrosis with renal tubular acidosis and cerebral calcifications.[6] In other cases, the translocation of these protons over the plasma membrane is impossible, owing to mutations in a subunit of the

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vacuolar H(+)-ATPase proton pump (the *TCIRG1* gene encoding the A3 subunit).[7,8] Less frequent are loss-of-function mutations in the chloride channel ClCN7,[9] needed for the maintenance of electroneutrality, and in the *OSTM1* gene encoding a β-subunit of ClCN7.[10,11]

In general, complete loss of any of the mentioned proteins results in the most severe, autosomal recessive forms of osteopetrosis. Milder, intermediate forms are found in cases with mutations in the *ClCN7* gene that do not result in complete loss of the protein function.[5] Finally, an autosomal dominant form of osteopetrosis (ADO type II) is due to heterozygous mutations in the *ClCN7* gene causing a putative dominant-negative effect of this ion channel that functions as a dimer.[12]

The identification of the molecular defect has important implications both for prognosis and for treatment of the disease. In many cases, bone marrow transplantation is considered in severe forms of osteopetrosis.[13] However, this will only work in cases where the primary defect is intrinsic to the osteoclast. The osteoclast-poor forms caused by mutations in the gene encoding RANKL will not benefit from this treatment. However, in these cases administration of RANKL could be the obvious replacement therapy. So far, no cases treated in this way have been reported. Furthermore, bone marrow transplantation will only partially solve the problem in *ClCN7* and *OSTM1* mutations, as these cases also suffer, besides the bone problem, from neural degeneration in the brain that cannot be solved by bone marrow transplantation.[14]

Finally, there is still some way to go in order to be able to provide the complete molecular analysis of osteopetrosis cases, as about 30% of them are not caused by a mutation in the currently known osteopetrosis genes.[14] It might be that a large number of genes underlie the remaining unsolved cases as illustrated by the fact that osteopetrosis is found in many transgenic mouse models.[15] Also, the example of the *PLEKHM1* gene, which we found to be mutated in only two osteopetrosis cases so far, might be a reflection of what can be awaited.[16] The tools of high-throughput next-generation sequencing that are currently available will be instrumental in solving these cases.

An outlier in the group of conditions with impaired bone resorption is pycnodysostosis, in which osteoclasts are formed normally and the extracellular compartment is acidified as needed. The bone matrix will, in this environment, be decalcified, but due to loss-of-function mutations no functional cathepsin K protein, the most important protease to degrade the collagen matrix.[17]

Paget disease of bone and related disorders. As an increased bone mass is present in this group of conditions they can also be classified among the sclerosing bone dysplasias. However, the primary defect is an increased bone resorption that results in osteolytic lesions. Because of the coupling between bone resorption and formation, the increased number and size of osteoclasts present in these pathologies are compensated by increased osteoblastic bone formation. The final outcome is increased bone turnover resulting in the formation of bone tissue that is disorganized and of inferior quality, resulting in deformities and low trauma fractures. Increased NF-κB signalling and consequently osteoclastogenesis seem to be a common underlying pathogenic mechanism.[18] The most severe forms of this group of conditions are caused by loss of function mutations in the *TNFRS11B* gene encoding osteoprotegerin (in juvenile Paget's disease)[19,20] or activating mutations in the gene encoding RANK (as in familial expansile osteolysis).[21] In addition, sequestosome 1[22] and valosin containing protein,[23] mutated in a subset of typical Pagetic patients or a syndromal form of Paget's disease, respectively, do play an intracellular role in this signalling pathway. However, recent insights into the function of these proteins, as well as the nature of genetic risk factors for Paget's disease recently identified by genomewide association studies,[24,25] support the hypothesis that dysfunctioning of the autophagy mechanism might also be involved. Triggered by environmental factors (such as slow virus infections), accumulation of proteins might occur in the abnormal osteoclasts and disturb normal functioning.[26]

Coupling Between Bone Resorption and Bone Formation

It has long been known that the mechanisms of bone resorption and bone formation are coupled, which is instrumental in keeping bone homeostasis in balance. This coupling takes place at different levels and in different directions. However, the pathogenic mechanisms mentioned in the conditions with abnormal bone resorption seem to indicate an additional level of coupling, as they provide evidence for osteoclastic factors with anabolic effect. In the osteopetroses with impaired bone resorption due to the incapacity to acidify the extracellular compartment, an increased number of inactive osteoclasts are present.[27] However, this seems to be enough to have an anabolic effect on osteoblasts. This can also explain the fact that these forms of osteopetrosis are in general more severe than the osteoclast-poor forms, where no increased bone formation rate is seen. Furthermore, this effect could also contribute to the impressive bone formation rate seen in Pagetoid conditions. Currently, the anabolic factors released from osteoclasts are still unidentified, but signals that increase Wnt pathway activity have been suggested.[28]

This anabolic effect coming from osteoclasts themselves adds up to the release of anabolic factors secondary to the bone resorption process itself. One well established factor in this context is transforming growth factor β-1 (TGFβ1), stored at high concentration in the bone matrix. Recently Tang *et al.*[29] nicely illustrated that a gradient of TGFβ1 is essential at bone resorption sites to recruit mesenchymal stem cells for osteoblastogenesis and bone formation. This is in line with the picture seen in Camurati-Engelmann disease showing irregular hyperostosis mainly affecting the diaphyses of the long bones. We and others were able to show that this condition is caused by activating mutations in the *TGFβ1* gene.[30,31] Most likely the same pathway is disturbed in osteopoikilosis, characterized by spots of increased bone density, as in such patients mutations are found in the *LEMD3* gene, encoding an inner membrane protein involved in TGFβ1 signalling.[32]

Abnormal Bone Formation Underlying Sclerosing Bone Dysplasias

One of the milestones in our understanding of bone homeostasis is the identification of mutations in the low-density lipoprotein receptor-related protein 5 (LRP5) underlying, on the one hand, a high bone mass phenotype and, on the other hand, osteoporosis-pseudoglioma syndrome.[33–35] The latter is an autosomal recessive condition caused by complete loss-of-function mutations, the former by an autosomal dominant phenotype due to

one amino-acid substitution with an assumed activating effect. These findings made the Wnt/β-catenin signalling pathway one of the most studied areas of bone research in the last decade, as LRP5 was known to play a role in this pathway. Numerous genetic and cell biology studies have been performed and many animal models have been generated, which together suggest a key role for Wnt signalling in osteoblast differentiation, proliferation and activation, and in suppressing osteoblast apoptosis, as reviewed in detail on many occasions.[36,37]

In 2008, a paper by Yadav *et al.*[38] put question marks behind many of the proposed mechanisms. Rather than a local effect of the *LRP5* mutations on Wnt signalling in bone, they provided support for a systemic effect generated by the role of LRP5 in the synthesis of serotoninin gut enterochromaffin cells by regulating the expression of tryptophan hydroxylase 1 (Tph1), the rate-limiting biosynthetic enzyme for serotonin. The gut-derived serotonin could subsequently inhibit osteoblast proliferation through the 5-HT receptor (Htr 1b) on osteoblasts.[38] More recently, Cui *et al.*[39] tested this hypothesis by making a set of transgenic animals, some of them similar to those described by Yadav *et al.*[39] However, they could not confirm the serotonin hypothesis, but rather obtained support for a local role of LRP5 in bone. The putative explanations for these conflicting data and their implications were recently elegantly discussed by Johnson in *Bonekey*.[40]

Findings in other sclerosing bone dysplasias may provide us with some arguments in this ongoing debate. Strong support for a local bone effect of the *LRP5* mutations on bone formation comes from sclerostin, a transmembrane protein. We and others identified this protein encoded by the *SOST* gene as the causative gene for sclerosteosis and Van Buchem disease, two allelic, autosomal recessive conditions characterized by hyperostosis mostly affecting the long bones and the skull.[41–43] Clinically, the main characteristics are an enlarged mandible, facial nerve palsy and hearing loss due to cranial nerve encroachments. Different modes of action have been suggested on how sclerostin exerts its effect on bone, but it is clear that the anabolic effect is mostly explained by the inhibition of Wnt signalling.[44] Sclerostin can bind LRP5, thus inhibiting Wnt/β-catenin signalling. The different missense mutations found in the *LRP5* gene in different cases of craniotubular hyperostosis, including the high bone mass phenotype, are clustered extracellularly in the first propeller domain.[45] Interestingly, all these mutations disrupt the binding between sclerostin and LRP5.[46–48] This provides a clear explanation for the clinical and radiological similarities between sclerosteosis/Van Buchem disease and high-bone-mass phenotypes. Very recently, the genetic study of two sclerosteosis families provided evidence that another member of the LRP gene family, *LRP4*, plays a role as a receptor for sclerostin and facilitates sclerostin action.[49] In these patients, we identified two missense mutations in the same domain of LRP4, and *in vitro* evidence was obtained that these mutations disrupt the binding with sclerostin and subsequently the inhibitory effect of sclerostin on Wnt signalling. Sclerostin has a very restricted expression pattern, only being detected in cementocytes in teeth, mineralized hypertrophic chondrocytes and mature osteocytes embedded in mineralized bone. All of these support the idea that interaction at the bone surface between sclerostin and LRP5 is an important modulator of bone formation. Furthermore, sclerostin is also linked both with the anabolic effect of parathyroid hormone and with mechanical loading.[50] Both parathyroid hormone treatment and mechanical loading suppress sclerostin expression, thus increasing Wnt/β-catenin signalling. In addition, catabolic activity has been associated with sclerostin action by way of increasing the ratio between RANKL and OPG secreted from osteoblasts.[51] This leads to increased osteoclastogenesis and bone resorption. The latter mechanism also provides an explanation for the fact that some patients with an activating mutation in LRP5 and subsequent increased Wnt signalling were shown to have a reduced number of osteoclasts and were therefore diagnosed with autosomal dominant osteopetrosis type I.[52]

Another sclerosing bone dysplasia that has been linked with Wnt signalling is osteopathiastriata with cranial sclerosis. This is an X-linked dominant condition that presents in females with sclerosis of the long bones and skull and longitudinal striations visible on radiographs of the long bones, pelvis and scapulae. Clinically, macrocephaly, cleft palate and mild learning disabilities are noticed. In males this entity is often lethal, but surviving males show cardiac, intestinal and genitourinary malformations in addition to hyperostosis. Jenkins *et al.*[53] were able to identify mutations in the gene encoding WTX (Wilms tumour on the X-chromosome), a repressor for Wnt signalling. Despite an extended mutation spectrum, it was not possible to establish a clear genotype–phenotype correlation explaining the clinical variability seen in this condition.[54] The linear striations in the metaphyseal region of the long bones and pelvis can be explained by a local increased bone formation due to a mixture of affected and non-affected osteoblasts under the growth plate associated with random X-inactivation in females. Corroborating this hypothesis was the finding of longitudinal striations in one male patient showing somatic mosaicism for a mutation in the *WTX* gene.[55]

Conclusions

The unravelling of the underlying molecular genetic mechanisms in sclerosing bone disorders obviously has direct implications for the conditions themselves, as confirmation of a clinical diagnosis can be obtained and genetic counselling can be offered. Despite the fact that for many of these conditions gene identification has not resulted directly in novel therapeutic approaches, in some cases (as in the group of the osteopetroses), the precise gene defect is currently critical to choosing the optimal treatment.

The relevance of the insights generated over the last decade goes decidedly beyond the mostly very rare sclerosing bone dysplasias, and has clear implications to our understanding of osteoporosis. This is illustrated by two facts. First, many of the genes identified in sclerosing bone dysplasias are currently among the top hits in genome-wide association studies, indicating that besides the large effect of some mutations in these genes, other variants within the same genes can also have an effect, albeit smaller, on bone mineral density in the general population.[56] Second, sclerosing bone dysplasias can definitely be considered as models for the development of therapeutic strategies to prevent and treat osteoporosis. Efforts are currently ongoing to use some of the genes (cathepsin K, *ClCN7*, and so on) identified in conditions with impaired bone resorption as potential drug targets. Furthermore, several clinical trials with novel anabolic therapies are ongoing, mainly focused on the inactivation of Wnt antagonists such as sclerostin, and the results obtained are very promising.[57]

Although many major breakthroughs have been realized, it is likely that a great deal of significant information lies hidden in these pathologies, as for many of these genes and newly identified pathways the function is not yet completely understood. Furthermore, not all genes responsible for these rare bone dysplasias have been identified, and additional key players in the processes of bone homeostasis are awaiting their discovery. With the currently available tools of next-generation DNA sequencing, we have entered an area in which the unravelling of the molecular defects underlying even almost unique genetic cases has become possible. Since there is, as illustrated in this review, no direct correlation between the prevalence of a disease and the relevance and applicability of studying it, new, interesting, and clinically relevant data can be expected from studies on sclerosing bone dysplasias.

Conflict of Interest

The author declares no conflict of interest.

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