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Snx10: a newly identified locus associated with human osteopetrosis

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The health and economic impact of osteoporosis, a disease characterized by excessive osteoclastic bone resorption, continues to make studies of bone resorption by osteoclasts a critically important research area. Most of what we know about the normal function of the osteoclast comes, paradoxically, from the study of osteopetrosis, a heterogeneous genetic disease characterized by osteoclast failure.[1] Osteopetrosis can be the result of reduced osteoclast numbers and/or impaired osteoclast function. While the former indicates a genetic defect that affects osteoclast differentiation, the latter suggests that the mutation affects osteoclast activity. To date, several genes have been demonstrated to be involved in the pathogenesis of the disease in humans. These mutations affect either differentiation (that is, RANKL, RANK) or activity (ATP6i, Clc-7, PLEKHM1 and OSTM1) of osteoclasts. However, approximately 25% of all human osteopetrosis cases are caused by mutations in still unidentified genes. Therefore, the identification of these new genes is critical to improve diagnosis and clinical outcomes. In addition, as each gene is expected to be required for normal osteoclast function, each discovery will lead to a clearer understanding of the molecular mechanisms underlying osteoclast formation and activity and provide new potential therapeutic targets to treat bone diseases.

Snx10: a Novel Osteopetrotic Mutation

Early in 2012, Aker *et al.*[2] found a missense mutation (R51Q) in a conserved amino acid of *Sorting Nexin 10* (*SNX10*) in four patients from two consanguineous Palestinian families. These patients had fewer and smaller osteoclasts compared with healthy controls. In addition, osteoclastic resorptive capacity and endosomal pathways were severely reduced, suggesting that SNX10 was a regulator of osteoclast activity. Simultaneously, we reported expression of mouse Snx10 to be upregulated during RANKL-induced osteoclast differentiation *in vitro* and *in vivo*. Snx10 silencing does not prevent osteoclast

Conflict of Interest
The authors declare no conflict of interest.

differentiation but inhibits osteoclastic resorption activity *in vitro*.[3] Specifically, silencing inhibits TRAP secretion. Put together, these results indicate that Snx10 has an essential role in osteoclast vesicle trafficking and osteoclastic resorption. The involvement of SNX10 in the pathogenesis of autosomal recessive osteopetrosis (ARO) was further confirmed by Mégarbané *et al.*[4] who reported the identification of a stop mutation in SNX10 (R16X) in an Iraqi boy afflicted with the disease. Finally, in the most extensive study, Pangrazio *et al.*[5] describe the identification of nine novel mutations in the *SNX10* gene in 14 subjects from 12 unrelated families in a cohort of more than 310 patients from around the world. SNX10 is now known to account for 4% of all cases of ARO, including the cases found in Västerbotten County, Sweden ('Västerbottenian osteopetrosis'), an area with a high occurrence of the disease. This frequency is comparable to that of the RANKL-, RANK- and OSTM1-dependent subsets.

Snx10, Vesicular Trafficking and Osteoclast Activity

The sorting nexin (SNX) family consists of a diverse group of cytoplasmic and membrane-associated proteins that are involved in various aspects of endocytosis and protein trafficking.[6] These proteins are unified by a common phospholipid-binding motif (the PX domain), which mediates the ability to form protein–protein complexes and protein–lipid interactions in protein sorting and membrane trafficking.[6] SNX10 overexpression induces giant vacuoles in mammalian cells.[7] Moreover, Brefeldin A, an inhibitor of protein transport from the endoplasmic reticulum to the Golgi, blocks the vacuolization process.[7] Taken together, these results suggest that Snx10 activity is involved in the regulation of membrane trafficking and endosome homeostasis. Bone-resorbing osteoclasts are highly dependent on vesicular trafficking pathways.[8] Accordingly, the disruption (genetic or pharmacological) of osteoclastic vesicle transport abolishes resorptive activity.[8] Bone resorption by osteoclasts requires the following processes: (A) adhesion and migration, which are carried out through adhesion receptors and their ability to regulate the rapid assembly and disassembly of cytoskeleton proteins at sites of adhesion; (B) secretion of proteolytic enzymes, which is carried out via vesicular transport to the secretary ruffled border, (C) internalization of vesicles from the ruffled border to the lysosomes or transcytosis, via vesicular transport in a retrograde manner; and (D) acidification via the apical vacuolar proton ATPase and the ClC7 chloride channel at the ruffled border membrane as well as homeostatic ion transport at the basolateral membrane.[9] Vacuolar and vesicular transport functions are essential to the aforementioned processes. Endocytosis and the subsequent intracellular trafficking of the endocytosed material are required for osteoclast functions.[10,11] Mutations in genes involved in osteoclastic vesicular trafficking are known to cause osteopetrosis in humans (see Table 1).

Osteopetrotic Mutations in SNX10

Pangrazio *et al.*[5] report three nonsense mutations (p.Arg16X, p.Tyr29X and p.Gln62X), which are expected to result in non-functional truncated versions of SNX10, lacking large segments of the PX and/or C-terminus domain. They also found three missense substitutions in evolutionarily conserved residues (p.Arg16Leu, p.Tyr32Ser and p.Arg51Pro), which affect function of the protein. Finally, three mutations are predicted to impair the process of

exon splicing (in particular exons 4 and 5 that code for the PX domain): c.111 + 5G>C (splicing of exon 3), c.212 + 1G>T (splicing of exon 4) and c.311 + 1G>T (splicing of exon 5).

The osteopetrotic mutations described here form a heterogeneous group; however, there is one common theme: all of them are located within the (PX) domain. The PX domain consists of three N-terminal β-strands followed by three α-helices (see Figure 1: α1, α2 and α3). Although the structural design of the PX domain is very similar across different SNX proteins, the amino-acid sequences are not well conserved. Six members of the SNX family (SNX3, SNX10, SNX11, SNX12, SNX22 and SNX24) were originally thought to contain only an N-terminal PX domain.[12] However, new evidence indicates that the PX domain of SNX10 alone is not enough for SNX10-induced vacuole formation. In fact, although the PI3P binding activity (that occurs in a positively charged pocket of the PX domain, Figure 1, *) is required for SNX10-mediated activity, both PX and the C-terminal domain (CD, in Figure 1: α4, α5 and α6) are required for the vacuolizing activity of SNX10.[7] In a later study, the same authors demonstrate that the CD, specifically a region within α4 and α5, was necessary for the vacuole-inducing activity of SNX10.[13] Very recently, a related PX-containing protein (SNX11) was shown to inhibit SNX10 vacuole-forming activity, either via direct interaction with SNX10 or via interaction with a common partner.[14] A segment of SNX11 and a segment of SNX10 CD (α4 and α5 in Figure 1) were required to mediate this interaction. The authors propose that SNX11 (and SNX10) contains an ‘extended’ PX domain, including the conventional PX (α1,α2 and α3) plus α4 and α5. This newly recognized region may regulate SNX10 activity by controlling binding to specific partners.

Final Remarks

This study clearly establishes an essential role for SNX10 in the etiology of human ARO. SNX10-dependent human ARO is a complex entity from the molecular and clinical point of view. The range of clinical severity observed in these subjects suggests that these mutations affect various functions of the protein (PI3P binding, ER targeting, endosome formation and binding to partner proteins), which will have a different net effect in the overall activity. The differences in symptoms and lethality among patients carrying mutations in SNX10 are similar to the differences that have been reported in osteopetrosis. In fact, lethality is most common during infancy, whereas those individuals who reach adulthood are more likely to have a normal life expectancy,[15,16] suggesting that the mutation(s) affects other processes that are essential during development. Craniofacial defects have been also described in ARO patients, who tend to exhibit macrocephaly, hydrocephaly and peculiar facies.[16]

The effects of these mutations in osteoclast activity are still not clearly established. The use of osteoclasts derived from circulating osteoclast precursors from these subjects will aid in determining what cellular function is affected by each specific mutation.

These findings have important clinical consequences. First, SNX10 has to be added to the panel of genes that are currently screened for mutations in newly diagnosed patients with ARO. Second, as the main site of expression is bone, SNX10 is a novel potential target for anti-resorptive therapies.

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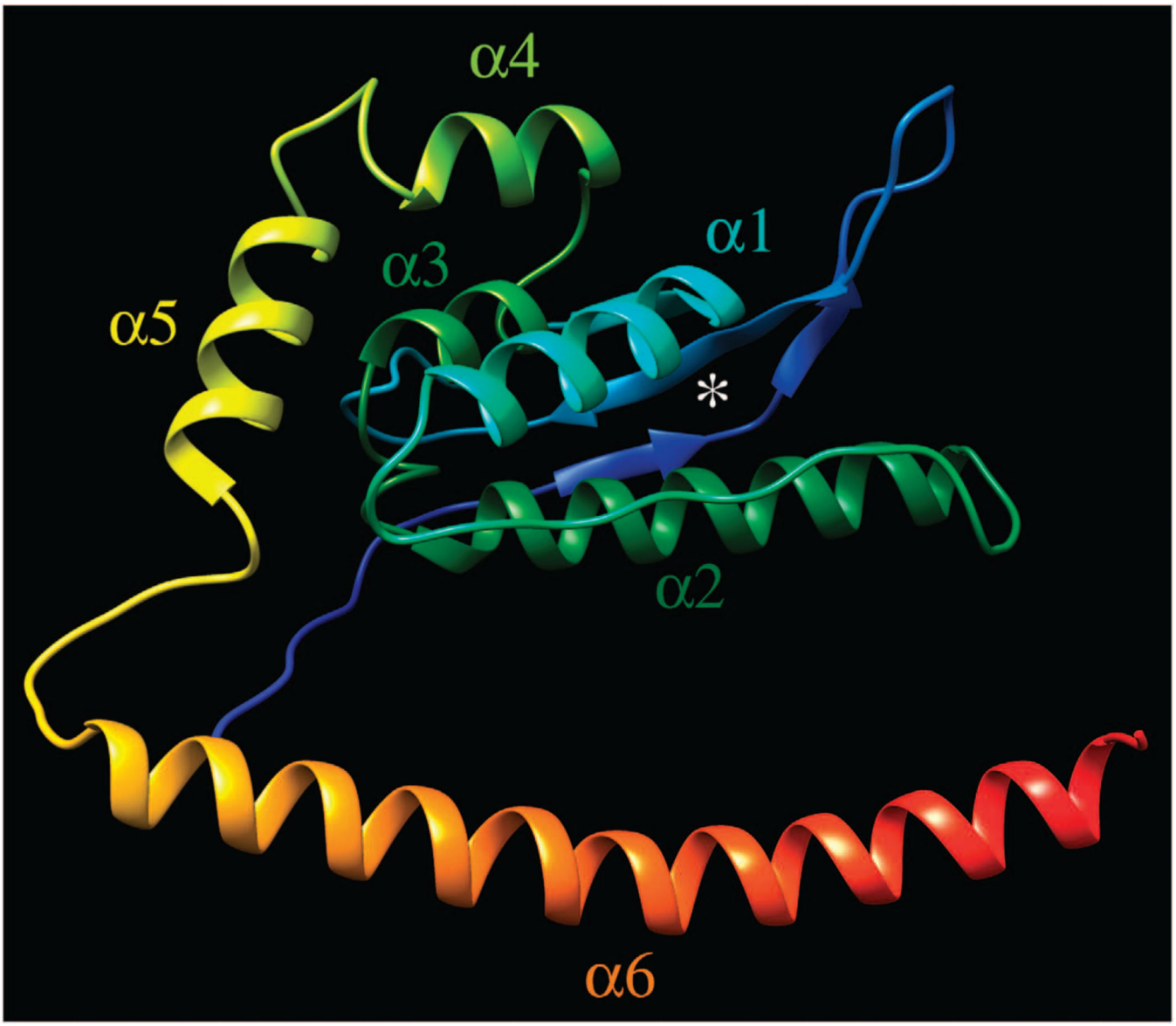

Figure 1.

A three-dimensional SNX10 model[17–19] predicts the existence of a 'conventional' PX domain that includes three β-sheets and three α-helices (α1, α2 and α3). An extended PX domain is composed of the conventional PX domain plus α-helices 4 and 5.

Table 1

Mutations in genes involved in osteoclast vesicular trafficking cause osteopetrosis in humans

Gene	Mutation	Cellular defect	References
SNX10			
Sorting Nexin 10	Loss-of-function	Defective osteoclastic vesicular trafficking	2,3
CtsK			
Cathepsin K	Loss-of-function	Failure of collagen degradation	20
TCIRG1			
a3 subunit of H+ −ATPase	Loss-of-function	Failure of extracellular acidification and ruffled border formation	21
ClCN7			
Chloride channel 7	Loss-of-function	Failure of extracellular acidification and ruffled border formation	22
CAII			
Carbonic anhydrase II	Loss-of-function	Failure of proton production	23
PLEKHM1			
Pleckstrin homology domain containing 1	Loss-of-function	Defective osteoclastic vesicular trafficking	24
OSTM1			
Osteopetrosis-associated transmembrane protein 1	Loss-of-function	Defective ruffled border, disrupted cytoskeleton, lysosomal storage	25