IBMS BoneKEy. 2009 January;6(1):16-28
http://www.bonekey-ibms.org/cgi/content/full/ibmske;6/1/16
doi: 10.1138/20090358

PERSPECTIVES

New Mechanisms of Osteopetrosis

Andrea Del Fattore, Marta Capannolo, and Anna Teti

Department of Experimental Medicine, University of L'Aquila, L'Aquila, Italy

Abstract

Osteopetroses are rare genetic disorders characterized by sclerosis of the skeleton due to reduced or complete lack of osteoclast function and, as a consequence, impairment of bone resorption. Osteoclast failure causes persistence of old bone, an increase in bone density and obstruction of the internal cavities containing vital organs such as the bone marrow and the nervous system. Short stature, deformities and pathological fractures are typical symptoms, along with severe hematological and neural failures. In humans, several types can be distinguished and their classification is based on their mode of inheritance, age of onset, severity and associated clinical symptoms. The best-known osteopetroses are the severe and intermediate autosomal recessive forms and the milder autosomal dominant subtype. Besides these forms, a restricted number of cases has been reported in which patients present with additional clinical features unrelated to the skeletal phenotype. Over the last few years, molecular genetic studies have resulted in the identification of mutations in genes whose function is associated with bone resorption or with osteoclast differentiation, but about 30% of patients still remain without a recognized molecular defect. At present, therapy is unsatisfactory and effort is necessary both to unravel the gene defects yet unrecognized and to identify new treatments to improve the phenotype and save lives. Nevertheless, significant advances have been made in recent years and the disease now seems better understood both genetically and clinically. This review will summarize recent knowledge on the pathogenesis of osteopetrosis and will discuss future challenges and developments for therapy. *IBMS BoneKEy*. 2009 January;6(1):16-28.

Keywords: Osteoclasts; Osteopetrosis; Bone resorption; Osteoblasts; Bone remodeling

Definition of Osteopetrosis

The term "osteopetrosis" is used to describe a number of bone diseases ("osteopetroses") characterized by a general increase in bone mass due to reduced or a complete lack of osteoclastic bone resorption (1). The disease was identified in 1904 by Albers-Schönberg and described as "marble bone disease" due to the abnormally hard, but brittle, nature of the bone (2).

Osteoclast failure causes persistence of old bone, an increase of bone density and obstruction of the internal cavities containing vital organs such as the bone marrow and the nervous system. Short stature, deformities and pathological fractures are typical symptoms, along with severe hematological and neural failures (1;3).

Several forms are known with different modes of inheritance and severity, from asymptomatic to fatal in infancy. The osteopetroses altogether have an incidence >1:100,000 and have been reported in many ethnic populations, although certain forms of the disease appear to be more common in Arabic people from Middle Eastern or North African countries and in Costa Rica (3;4).

Presently, there is no effective treatment, except for hematopoietic stem cell transplantation (HSCT) performed only for the most severe forms. Unfortunately, HSCT is not a panacea (5), with 50% transplant failure and persistence of irreversible symptoms.

Nevertheless, because of recent genetic and clinical advances, this disease is now better

understood. This *Perspective* will summarize recent findings on osteopetrosis and will discuss future challenges and developments for therapy.

Classification

Osteopetrosis has always been (and still is) classified on the basis of its clinical and radiological appearance. This type of classification is often difficult because of the extreme variability of symptoms, disease severity, and associated complications observed in patients (1;3). However, clinically, three forms of osteopetrosis exist: autosomal recessive osteopetrosis (ARO), intermediate autosomal recessive osteopetrosis (IRO) and autosomal dominant osteopetrosis (ADO). All of these forms, although different, share some common features, including a general increase in bone mass, skeletal sclerosis, spontaneous fractures, deformities, osteomyelitis and dental problems (6).

ARO is the most severe type of osteopetrosis, with an average incidence of 1:200,000 to 1:300,000, and a peculiarly high incidence (3.4:100.000) in Costa Rica. Usually diagnosed within the first year of life, ARO is characterized by extensive sclerosis, which affects uniformly most of the skeleton, including the skull, spine, pelvis and appendicular bones. Impaired bone resorption is responsible for poor development and/or compression of vital organs, thus leading to reduction of bone marrow spaces, skull cavities and nerve foramina (Fig. 1A). The main life-threatening features, such as severe anemia, pancytopenia and hepatosplenomegaly (1;3;6), arise from the consequent bone marrow failure and impaired medullary hematopoiesis. Blindness and deafness, other typical debilitating symptoms, are generally due to cranial nerve compression. ARO patients usually have a short life expectancy of about 3-4 years and generally die because of anemia, secondary infections or sepsis (1). Moreover, a subtype of ARO is characterized by additional traits, such as primary degeneration of the brain and retina, due to lysosomal storage disease (7-9).

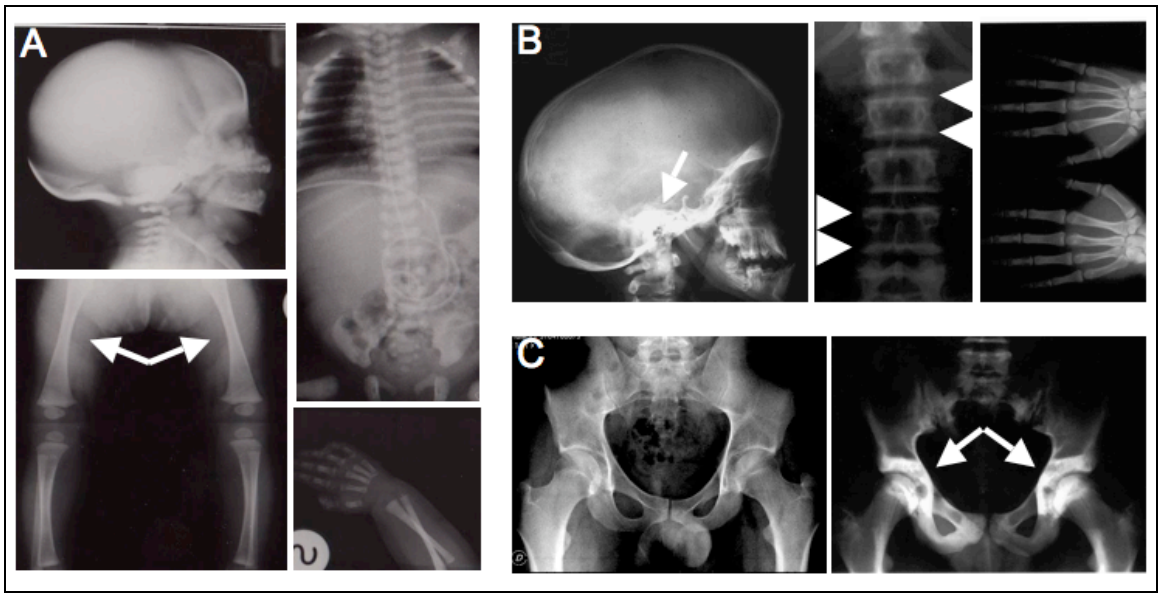

Fig. 1. X-ray analyses. (A) ARO patients showing extensive sclerosis of the skull, spine, pelvis and appendicular bones, endobone (bone within a bone) appearance and obliterated femur cavities (arrows). (B) ADO patients presenting with osteosclerosis, especially at the skull base (arrow), vertebral endplates (arrowheads) and appendicular bones. (C) Typical sclerosis at the pelvis (arrows) in an ADO patient (right panel) compared to a normal pelvis (left panel).

IRO is milder than ARO and usually compatible with long-term survival. The main IRO features include short stature, bone sclerosis (resulting in susceptibility to pathological fractures), dental malformations and jaw osteomyelitis. However, this form presents with heterogeneous clinical traits and there are also severe cases, with renal tubular acidosis, nerve compression syndromes, cerebral calcification, mental retardation, moderate anemia and psychomotor delay (10;11).

ADO is also called Albers-Schönberg disease and has an incidence of 5:100,000. It is still accepted as a "benign osteopetrosis" and presents with extreme phenotypic variability, from asymptomatic to severe disease, even within the same family and in the presence of the same mutation (12;13). ADO is typical of adults and is characterized by a "focal" sclerosis (involving mainly the skull base, pelvis and vertebral endplates), bone pain, osteomyelitis and very frequent pathologic fractures (Fig. 1B-C). In some severe cases, complications caused by cranial nerve compression can be observed. Early death is rare, but many patients have a rather poor quality of life (1).

In addition to the three classic forms of osteopetrosis, a new clinical osteopetrotic phenotype associated with ectodermal dysplasia, lymphedema and immunodeficiency (so-called OL-EDA-ID syndrome) has been described recently. Because of its mode of inheritance, this type is also termed X-linked osteopetrosis (XLO) and is very rare. Thus far, only five males have been reported with this syndrome. They presented with a severe phenotype and all died very young because of complications due to infection (14).

Genetics of Osteopetrosis

The extreme phenotypic variability of osteopetrosis arises from a remarkable genetic heterogeneity. Thus far mutations of several genes have been implicated in its pathogenesis, and osteopetrosis can be inherited in an autosomal recessive, autosomal dominant or X-linked manner. However, about 30% of patients still lack a genetic diagnosis (13). All the known genes are associated with osteoclast differentiation or activity (Table 1 and Fig. 2).

Clinically, the most severe form is ARO, which is due to loss-of-function mutations of the *TCIRG1* gene in more than 50% of cases (15;16). This gene encodes the osteoclast-specific a3 subunit of the vacuolar H^+-ATPase (V-H^+-ATPase) (17-19) responsible for the resorbing lacuna acidification (pH≈4.5). In rare cases, double mutations of the *TCIRG1* and *ATP6V1B1* genes, the latter encoding the B1 H^+-ATPase subunit, were found in patients with severe ARO that, due to renal tubular acidosis, was described as a phenocopy of carbonic anhydrase II deficiency, the latter typically observed in IRO forms (20).

There are four other genes known thus far to be responsible for ARO. One gene is *CLC7*, which is involved in 10-15% of ARO cases (7;21). *CLC7* encodes a chloride/proton antiporter, with a stoichiometric ratio of $2Cl^-$:$1H^+$ (22). ARO patients who carry loss-of-function mutations of this gene present with impairment of bone resorption because Clc7 is essential for ruffled border membrane charge balance during the acidification process.

Mutations in the *OSTM1* (OSteopetrosis associated TransMembrane protein 1) gene have been described thus far in only five ARO patients (23;24) carrying single-point mutations or deletions. According to protein prediction, the *OSTM1* gene encodes a protein with a single transmembrane domain. It localizes in the cytoplasm, most likely attached to intracellular membranes, and co-localizes with Clc7. Its specific role in bone resorption is still unknown, but it is probably related to Cl^- conductance (25). Recently, it has been shown that mutations in the *OSTM1* gene cause deregulation of the canonical Wnt/β-catenin signaling pathway (26). Moreover *CLC7*- and *OSTM1*-dependent AROs are characterized by primary brain and retinal degeneration, likely due to a lack of still unknown specific functions of *CLC7* and *OSTM1* in the

Table 1.
Genetic defects in human osteopetroses

Genes involved	Protein function	Type of mutation	Clinical features	Severity
TCIGR1	a3 subunit of the vacuolar H^+-ATPase, essential for proton transport to the resorbing lacuna	Loss-of-function	ARO	Severe
CLC7	Cl^-/H^+ antiport, crucial for charge balance in the acidification process	Loss-of-function	ARO	Severe with neuro-degeneration
		Dominant negative	ADO	Hetero-geneous
OSTM1	Transmembrane protein probably associated with CLC7 function	Loss-of-function	ARO	Severe with neuro-degeneration
RANKL	Essential osteoclastogenic cytokine	Loss-of-function	ARO	Severe
RANK	RANKL receptor	Loss-of-function	ARO	Severe with B-cell impairment
CAII	Carbonic anhydrase type II, enzyme that catalyzes the reaction between CO_2 and H_2O to form H_2CO_3	Loss-of-function	IRO	Intermediate with tubular acidosis and cerebral calcifications
PLEKHM1	Protein probably involved in vesicular trafficking and acidification	Loss-of-function	IRO	Intermediate
NEMO	Protein involved in activation of the NF-kB transcription factor	Loss-of-function	XLO	Severe with ectodermal dysplasia, lymphedema, and immuno-deficiency

nervous system, leading to lysosomal storage disease (7;21;23;24).

Besides the most frequent osteoclast-rich AROs described above, characterized in this way because in these forms osteoclasts form normally or are even increased in number, there is also a particularly rare, osteoclast-poor form of this pathology, characterized by the impairment of osteoclast formation. Subsets of osteoclast-poor ARO patients have been found to harbor mutations in the *TNFSF11* or in the *TNFRSF11A* (27;28) genes. These two genes encode the most potent osteoclast inducer, receptor activator of NFκB ligand (RANKL) and its receptor RANK, respectively; impairment of the RANKL/RANK interaction clearly explains the lack of osteoclast formation.

To date, only six patients have been described as carrying mutations of *RANKL*. Nevertheless, the importance of this finding lies not only in its diagnostic aspects, but also in its therapeutic implications, since these patients could not be effectively treated with HSCT but may be cured by soluble RANKL administration or by transplantation of bone marrow mesenchymal stem cells (27). RANK-dependent ARO had been described initially in two siblings from a Turkish family in which the bone phenotype was associated with hypogammaglobulinemia due to impairment in immunoglobulin-secreting B cells. Further investigation of other patients revealed a

IBMS BoneKEy. 2009 January;6(1):16-28
http://www.bonekey-ibms.org/cgi/content/full/ibmske;6/1/16
doi: 10.1138/20090358

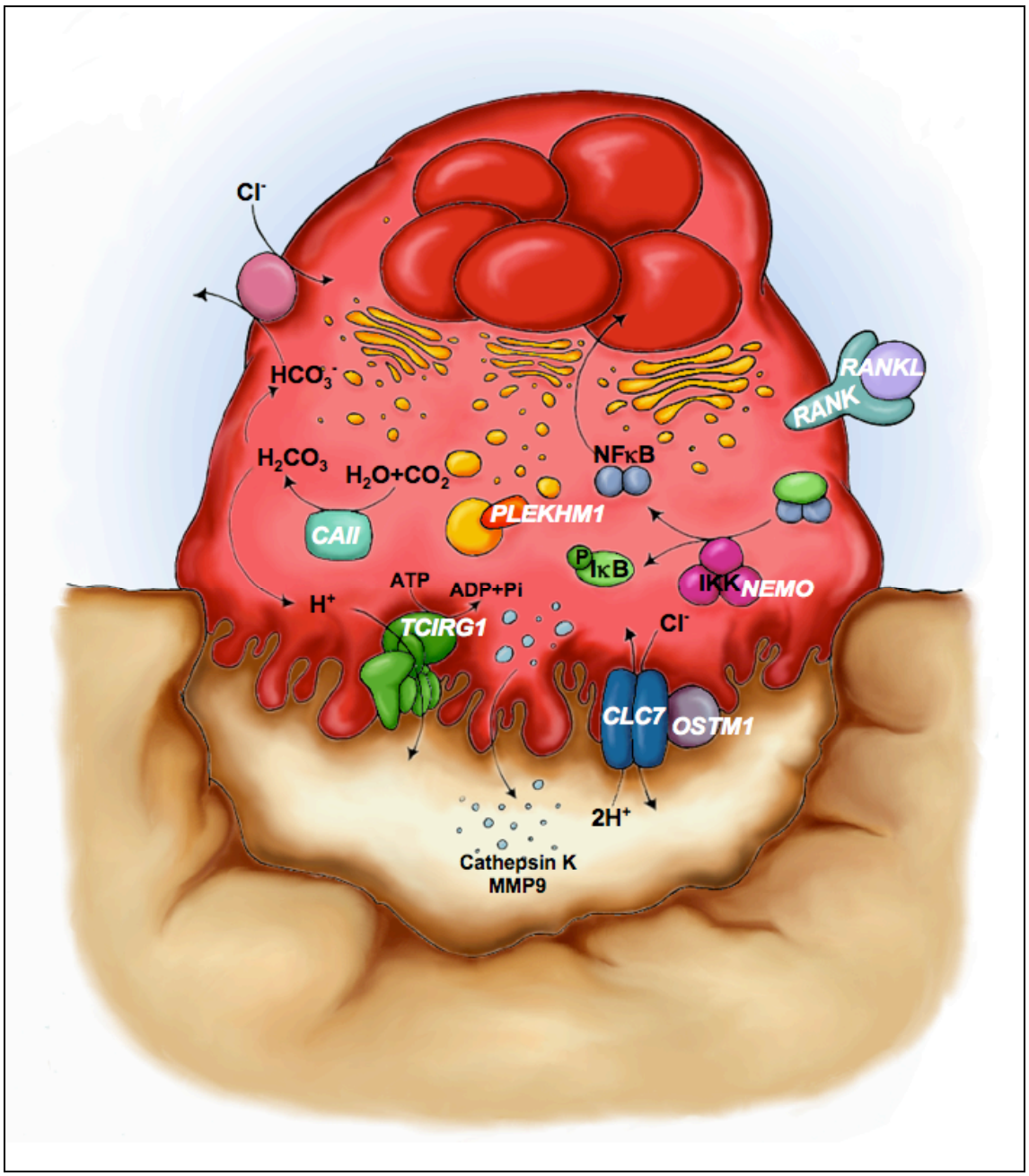

Fig. 2. The molecular mechanism of bone resorption and the genes (white) mutated in human osteopetrosis.

defect in six additional, unrelated families (28).

The most frequent form of osteopetrosis is ADO. Only the *CLC7* gene, mutated in approximately 70% of ADO cases, is known to be involved in this extremely heterogeneous form of osteopetrosis (13;29). About 30% of ADO patients still lack a genetic diagnosis (13;21;30;31). *CLC7* ADO mutations are heterozygous dominant negative, mostly missense, and tend to affect the entire length of the gene, even if most of the mutations are found in the C-terminal CBS (Cystathionine Beta Synthase) domains of the protein, indicating this location as a “hot spot” (13). ADO has no obvious genotype-phenotype correlation and

its great phenotypic variability cannot be easily explained. In fact, there are cases in which the very same mutation leads to completely different clinical outcomes (13;29). This variability could be due to *CLC7* single nucleotide polymorphisms (SNPs) or other genetic determinants, such as "modifier" genes, that could affect the expression/function of the gene itself. Otherwise, the reason could lie in some instability of the mutant protein. Further investigation is still necessary to clarify this aspect of ADO.

As in ADO, in IRO a considerable clinical heterogeneity is observed and a clear genotype/phenotype correlation is not. Most IRO patients harbor mutations in the *CAII* gene that encodes carbonic anhydrase type II (10;32), and this form is clinically recognized due to renal tubular acidosis, cerebral calcifications and mental retardation. A novel gene recently associated with IRO is *PLEKHM1*, identified as the human homolog of the gene responsible for the *incisor absent* (*ia*) rat phenotype. Thus far *PLEKHM1* has been found to be mutated in one family carrying a highly truncated gene product (33). Moreover, a significant role of *PLEKHM1* in bone biology is also suggested by its involvement in the bone phenotype of a patient with generalized osteopenia but localized osteosclerosis harboring a heterozygous mutation (R714C) of the gene (34). The exact role of Plekhm1 in bone resorption is still unclear, but recent findings suggest it is involved with Rab7-regulated late endosomal trafficking, vesicular acidification and tartrate resistant acid phosphatase (TRACP) release in osteoclasts (33;34).

As previously described, there is a particularly rare and clinically complex form of osteopetrosis with X-linked inheritance (XLO). This form is due to mutations of the NFκB Essential Modulator (*NEMO*) gene that encodes a regulatory subunit of IKK (IkB Kinase). XLO patients harbor a mutation causing the replacement of the NEMO stop codon with tryptophan, leading to the addition of 27 irrelevant residues at the C-terminus of the protein. This mutation seems to strongly destabilize the NEMO protein, leading to almost undetectable levels of this molecule (14).

Bone Histology and Osteoclast Phenotype

The radiographic features of the osteopetroses can be diagnostic. Nevertheless, failure of osteoclasts to resorb bone provides a histological finding that is pathognomonic. As a result of the inability of the osteoclasts to resorb, osteopetrotic bone contains large areas of non-remodeled mineralized cartilage. These remnants of mineralized primary spongiosa persist as islands or bars of calcified cartilage within mature bone (35).

Human osteopetrosis may be associated with variable numbers of osteoclasts (36). Ultrastructural examination of the osteoclasts generally reveals a lack of ruffled borders and clear zones that characterize normal osteoclasts. However, some patients have osteoclasts with abundant ruffled border-clear zone complexes (36;37). In other cases, some resorption by a mechanism more akin to phagocytosis has been described (36).

Nuclear inclusions of uncertain etiology were found in the osteoclasts of three unrelated patients with benign osteopetrosis (38), but it is not clear if these structures are actually associated with the bone phenotype.

Moreover, there is heterogeneity regarding the number of osteoclasts found in bone biopsies of osteopetrosis patients. Adult osteopetrosis is usually characterized by numerous and large osteoclasts. However, in some cases osteoclasts can be few in number and without ruffled borders. In the infantile form these cells are usually abundant (osteoclast-rich) and are found at bone surfaces. However, in a small number of cases, osteoclasts are absent in bone biopsies (osteoclast-poor) (27;28;36) (Fig. 3). As described above, Sobacchi *et al*. (27) and Guerrini *et al*. (28) showed that mutations of the *TNFSF11* and *TNFRSF11A* genes were responsible for subsets of patients with osteoclast-poor forms. The

former paper points to osteoblasts as the cells causing osteoclast failure in this form of the disease.

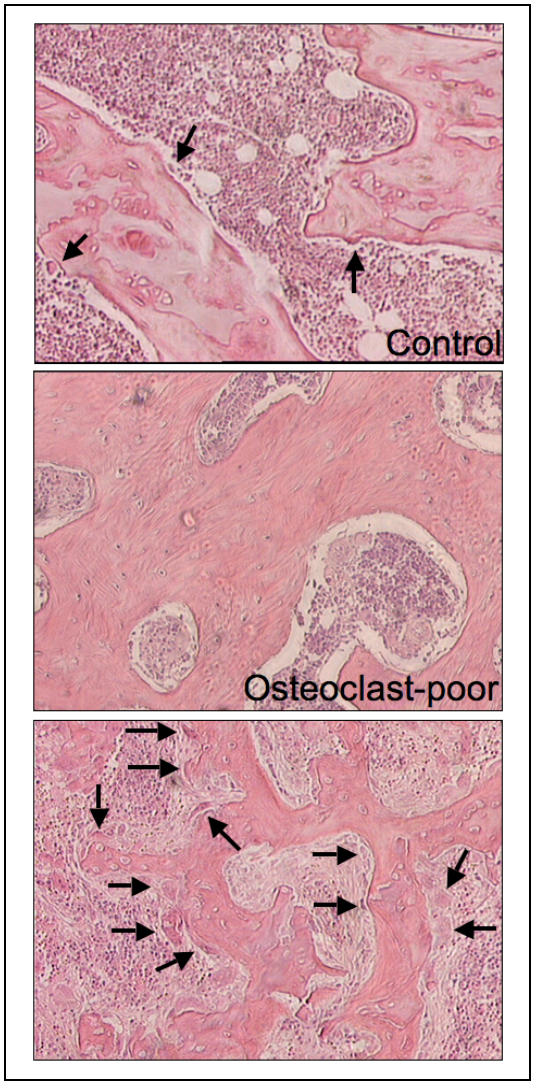

Fig. 3. Iliac crest biopsies from a control, an ARO patient with osteoclast-poor osteopetrosis, and an ARO patient with osteoclast-rich osteopetrosis. Ematoxylin/eosin staining. Arrows indicate osteoclasts. Original magnification, 10X.

The primary involvement of osteoblasts in the increased bone mass observed in osteopetrosis is controversial. Osteopetrosis is regarded as an osteoclast disease although two cases have been reported in which increased primary osteoblast activity was suggested (39). It is interesting, however, that bone biopsies of osteopetrotic patients often show active osteoblasts and increased amounts of osteoid (40). In fact, some extent of osteosclerosis, namely increased osteoblast activity and bone formation, has been claimed to occur in this disease, likely secondary to osteoclast dysfunction. This aspect could be explained if osteoclast-osteoblast coupling is taken into account (41). Recent reports have pointed to some factors, other than matrix-stored components (including TGFβ, BMPs and PDGF), as mediators of osteoclast-osteoblast interplay. These factors include the osteoclast membrane protein ephrin B2 (42), soluble sphingosine 1-phosphate (S1P) (43), hepatocyte growth factor (HGF) (44) and TRAcP enzyme (34). The ephrinB2 ligand is expressed by osteoclasts and binds to the EphB tyrosine kinase receptor expressed by osteoblasts. Interaction between ephrin- and Eph-expressing cells results in bidirectional signal transduction. Reverse signaling through the ephrinB2 ligand into osteoclasts suppresses osteoclast differentiation by blocking expression of the transcription factor c-Fos, while forward signaling through the EphB4 receptor into mesenchymal precursors promotes osteoblast differentiation (42). S1P is produced by sphingosine kinase (Sphk) in osteoclasts and the secreted protein interacts with the S1P receptor expressed by osteoblasts, enhancing RANKL expression as well as migration and survival of osteoblasts (43). HGF, secreted by osteoclasts, binds to HGF receptors expressed by both osteoblasts and osteoclasts. HGF treatment increases DNA synthesis and proliferation of both cell types (44). We have recently demonstrated that TRAcP stimulates bone formation (34). Moreover, transgenic mice overexpressing TRAcP show decreased trabecular bone volume but increased bone formation. Sheu *et al*. (45) used a phage display to screen an osteoblast expression library for sequences binding to TRAcP in search of osteoblastic proteins that would account for why osteoblastic bone formation occurs in resorption lacunae, and identified the candidate protein, TGF-β receptor-interacting protein (TRIP-1).

Osteopetrosis probably offered the very first evidence of a potential paracrine osteoclast-

osteoblast cross-talk independent of bone resorbing activity (13;41;46). In fact, patients with osteoclast-rich osteopetrosis have high numbers of osteoclasts and, in the face of severely decreased resorption, normal or even increased bone formation. Interestingly, we have found that in bone biopsies, the number of non-resorbing osteoclasts correlates with the number and activity of osteoblasts and that serum bone alkaline phosphatase levels are 6.5-fold greater than normal values (13;46). In contrast, osteoclast-poor osteopetrotic patients show significantly fewer osteoblasts and normal values of serum bone alkaline phosphatase activity (13). Together with earlier reports suggesting that hyperactive osteoblasts could be observed in human and animal forms of osteopetrosis, these observations support the hypothesis that osteoblasts themselves could contribute to the increased bone mass characterizing this disease. This suggests that factors produced by osteoclasts, and not only their resorptive activity, are important for supporting bone formation. This hypothesis was recently confirmed by Karsdal *et al*. (47) who collected conditioned media from human osteoclasts cultured on either bone or plastic, and tested their effects on bone nodule formation by osteoblasts. Both types of conditioned media were shown to dose-dependently induce bone nodule formation, whereas non-conditioned medium had no effect (47).

Treatment

Because etiology, pathogenesis, pattern of inheritance, and prognosis can differ for the various forms of osteopetrosis, a correct diagnosis is critical before therapy is administered. A precise diagnosis among the various forms of osteopetrosis may require investigation of the family and careful evaluation of the patient's disease severity and progression. Management of patients requires a comprehensive approach to characterize clinical problems, including hematologic and metabolic abnormalities, fractures, deformities, back pain, bone pain, osteomyelitis, and neurologic sequel. Medical treatment of osteopetrosis is based on efforts to provide an alternative source of osteoclasts by HSCT or to stimulate patients' osteoclasts.

Currently, the only curative treatment for ARO is HSCT (5), while there is virtually no cure for ADO. Unfortunately, HSCT is associated with high mortality when HLA-identical donors are not available. A retrospective analysis was made of 122 children who had received an allogeneic HSCT for ARO. The probabilities of 5-year disease-free survival were 73% for recipients of a genotype HLA-identical HSCT, 43% for recipients of a phenotype HLA-identical or one HLA-antigen mismatch graft from a related donor, 40% for recipients of a graft from a matched unrelated donor, and 24% for patients who received a graft from an HLA-haplotype-mismatch related donor.

Causes of death after HSCT were graft failure and early transplant-related complications. After successful HSCT, the majority of children had no further deterioration of vision and preservation of vision was better in children transplanted before the age of three months, suggesting that HSCT should be offered as early as possible (5). Unfortunately, in some cases disease progression continues. In fact, osteoclast appearance after HSCT is a slow process. Our unpublished study shows that such an outcome could be improved if readily available committed osteoclast precursors were injected. We optimized the method to obtain cells suitable for therapy and tested engraftment and phenotype improvement in animal models. We observed decreased bone volume and longer survival in treated animals, providing first-hand information on the feasibility of a support osteoclast precursor therapy in osteopetrosis. However, it has to be taken into account that patients with retinal atrophy and neurodegeneration due to *CLC7* or *OSTM1* mutations do not benefit from HSCT. In fact, transplanted HSCs can replace the hematopoietic tissue but have no effect on the neural system.

For patients not considered for transplantation, other therapeutic strategies, such as stimulating osteoclasts with calcium

restriction, calcitriol, steroids, parathyroid hormone and interferon γ, were used to reverse the phenotype or slow down the progression of the disease. Unfortunately, minimal success has been obtained to date. Some satisfactory outcomes have been reported with a calcium-deficient diet alone. However, supplementation of dietary calcium may be necessary for symptomatic hypocalcemia in severely affected infants or children (11). Large oral doses of calcitriol, together with limited dietary calcium intake to prevent hypercalciuria/hypercalcemia, have been reported to occasionally improve infantile osteopetrosis (11). Calcitriol seems to be helpful because it may stimulate dormant osteoclasts, but some patients appear to became resistant to this treatment (11;48). Long-term infusion of parathyroid hormone was helpful for one infant, perhaps because it stimulated calcitriol synthesis. The observation that the generation of superoxide by peripheral-blood leucocytes is defective in patients with osteopetrosis has led to recombinant human interferon γ-1b therapy with clinical, laboratory, and histopathological evidence of a successful response. In fact, all the patients treated with interferon γ-1b for 18 months showed sustained biochemical evidence of increased bone resorption. Bone biopsies revealed decreases in the area of trabecular bone (11). High-dose glucocorticoid treatment stabilizes pediatric patients with pancytopenia and hepatomegaly (11;49). Some patients treated with prednisone and a low calcium/high phosphate diet exhibited formation of bone marrow cavity and radiological improvement of the skull bones, with a corresponding decrease of exophthalmus and better visual function (49).

Conclusions and Challenges for the Future

Osteopetrosis has been a very important contributor to a better understanding of bone biology and physiology, allowing for the identification of many pathways involved in osteoclast differentiation and function. It also provided the first clues regarding a direct osteoclast-osteoblast cross-talk independent of bone resorbing activity and the involvement of osteoblasts in its pathogenesis. Our challenge for the future, in addition to the recognition of the as yet unknown genetic defects, will be the identification of effective therapeutic approaches based on cell and pharmacological therapies. RANKL-dependent ARO could potentially be treated with soluble RANKL or by transplantation of bone marrow mesenchymal stem cells. Moreover, more effort to find effective therapeutics for ADO is needed since only palliative intervention is available now. The striking advances made in the field in the last few years make us confident that new hope and better therapies will be offered in the future to patients with osteopetrosis and their families.

Acknowledgments

We are indebted to Dr. Rita Di Massimo for the editing of this manuscript. We also gratefully acknowledge the generous support provided by Telethon (grants #E.0831 and #GGP06019) to AT.

Conflict of Interest: None reported.

Peer Review: This article has been reviewed by Miep H. Helfrich.

References

1. Whyte MP. Osteopetrosis. In: Royce PM, Steinman B, eds. *Connective Tissue and Its Heritable Disorders: Medical, Genetic, and Molecular Aspects*. New York: Wiley-Liss; 2002:753-70.

2. Albers-Schönberg HE. Röntgenbilder einer seltenen Knockenerkrankung. *Munch Med Wochenschr.* 1904;51:365-8.

3. Balemans W, Van Wesenbeeck L, Van Hul W. A clinical and molecular overview of the human osteopetroses. *Calcif Tissue Int*. 2005 Nov;77(5):263-74.

4. Loría-Cortés R, Quesada-Calvo E, Cordero-Chavarri C. Osteopetrosis in

children: a report of 26 cases. *J Pediatr.* 1977 Jul;91(1):43-7.

5. Driessen GJ, Gerritsen EJ, Fischer A, Fasth A, Hop WC, Veys P, Porta F, Cant A, Steward CG, Vossen JM, Uckan D, Friedrich W. Long-term outcome of haematopoietic stem cell transplantation in autosomal recessive osteopetrosis: an EBMT report. *Bone Marrow Transplant*. 2003 Oct;32(7):657-63.

6. Del Fattore A, Cappariello A, Teti A. Genetics, pathogenesis and complications of osteopetrosis. *Bone*. 2008 Jan;42(1):19-29.

7. Kornak U, Kasper D, Bösl MR, Kaiser E, Schweizer M, Schulz A, Friedrich W, Delling G, Jentsch TJ. Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. *Cell*. 2001 Jan 26;104(2):205-15.

8. Kasper D, Planells-Cases R, Fuhrmann JC, Scheel O, Zeitz O, Ruether K, Schmitt A, Poët M, Steinfeld R, Schweizer M, Kornak U, Jentsch TJ. Loss of the chloride channel ClC-7 leads to lysosomal storage disease and neurodegeneration. *EMBO J*. 2005 Mar 9;24(5):1079-91.

9. Maranda B, Chabot G, Décarie JC, Pata M, Azeddine B, Moreau A, Vacher J. Clinical and cellular manifestations of OSTM1-related infantile osteopetrosis. *J Bone Miner R*es. 2008 Feb;23(2):296-300.

10. Sly WS, Hewett-Emmett D, Whyte MP, Yu YS, Tashian RE. Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. *Proc Natl Acad Sci U S A*. 1983 May;80(9):2752-6.

11. Askmyr MK, Fasth A, Richter J. Towards a better understanding and new therapeutic of osteopetrosis. *Br J Haematol*. 2008 Mar;140(6):597-609.

12. Bollerslev J, Andersen PE Jr. Radiological, biochemical and hereditary evidence of two types of autosomal dominant osteopetrosis. *Bone*. 1988;9(1):7-13.

13. Del Fattore A, Peruzzi B, Rucci N, Recchia I, Cappariello A, Longo M, Fortunati D, Ballanti P, Iacobini M, Luciani M, Devito R, Pinto R, Caniglia M, Lanino E, Messina C, Cesaro S, Letizia C, Bianchini G, Fryssira H, Grabowski P, Shaw N, Bishop N, Hughes D, Kapur RP, Datta HK, Taranta A, Fornari R, Migliaccio S, Teti A. Clinical, genetic, and cellular analysis of 49 osteopetrotic patients: implications for diagnosis and treatment. *J Med Genet*. 2006 Apr;43(4):315-25.

14. Smahi A, Courtois G, Rabia SH, Döffinger R, Bodemer C, Munnich A, Casanova JL, Israël A. The NF-kappaB signalling pathway in human diseases: from incontinentia pigmenti to ectodermal dysplasias and immune-deficiency syndromes. *Hum Mol Genet*. 2002 Oct 1;11(20):2371-5.

15. Frattini A, Orchard PJ, Sobacchi C, Giliani S, Abinun M, Mattsson JP, Keeling DJ, Andersson AK, Wallbrandt P, Zecca L, Notarangelo LD, Vezzoni P, Villa A. Defects in TCIRG1 subunit of the vacuolar proton pump are responsible for a subset of human autosomal recessive osteopetrosis. *Nat Genet*. 2000 Jul;25(3):343-6.

16. Kornak U, Schulz A, Friedrich W, Uhlhaas S, Kremens B, Voit T, Hasan C, Bode U, Jentsch TJ, Kubish C. Mutations in the a3 subunit of the vacuolar H+-ATPase cause infantile malignant osteopetrosis. *Hum Mol Genet*. 2000 Aug 12;9(13):2059-63.

17. Nishi T, Forgac M. The vacuolar (H+)-ATPase--nature's most versatile proton pumps. *Nat Rev Mol Cell Biol.* 2002 Feb;3(2):94-103.

18. Teitelbaum SL, Ross FP. Genetic regulation of osteoclast development

and function. *Nat Rev Genet*. 2003 Aug;4(8):638-49.

19. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature*. 2003 May 15;423(6937):337-42.

20. Bothwick KJ, Kandemir N, Topaloglu R, Kornak U, Bakkaloglu A, Yordam N, Ozen S, Mocan H, Shah GN, Sly WS, Karet FE. A phenocopy of CAII deficiency: a novel genetic explanation for inherited infantile osteopetrosis with distal renal tubular acidosis. *J Med Genet*. 2003 Feb;40(2):115-21.

21. Frattini A, Pangrazio A, Susani L, Sobacchi C, Mirolo M, Abinum M, Andolina M, Flanagan A, Horwitz EM, Mihci E, Notarangelo LD, Ramenghi U, Teti A, Van Hove J, Vujic D, Young T, Albertini A, Orchard PJ, Vezzoni P, Villa A. Chloride channel ClCN7 mutations are responsible for severe recessive, dominant, and intermediate osteopetrosis. *J Bone Miner Res*. 2003 Oct;18(10):1740-7.

22. Graves AR, Curran PK, Smith CL, Mindell JA. The Cl-/H+ antiporter ClC-7 is the primary chloride permeation pathway in lysosomes. *Nature*. 2008 Jun 5;453(7196):788-92.

23. Chalhoub N, Benachenhou N, Rajapurohitam V, Pata M, Ferron M, Frattini A, Villa A, Vacher J. Grey-lethal mutation induces severe malignant autosomal recessive osteopetrosis in mouse and human. *Nat Med*. 2003 Apr;9(4):399-406.

24. Pangrazio A, Poliani PL, Megarbane A, Lefranc G, Lanino E, Di Rocco M, Rucci F, Lucchini F, Ravanini M, Facchetti F, Abinun M, Vezzoni P, Villa A, Frattini A. Mutations in OSTM1 (grey lethal) define a particularly severe form of autosomal recessive osteopetrosis with neural involvement. *J Bone Miner Res*. 2006 Jul;21(7):1098-105.

25. Lange PF, Wartosch L, Jentsch TJ, Fuhrmann JC. ClC-7 requires Ostm1 as a beta-subunit to support bone resorption and lysosomal function. *Nature*. 2006 Mar 9;440(7081):220-3.

26. Feigin ME, Malbon CC. OSTM1 regulates beta-catenin/Lef1 interaction and is required for Wnt/beta-catenin signalling. *Cell Signal*. 2008 May;20(5):949-57.

27. Sobacchi C, Frattini A, Guerrini MM, Abinun M, Pangrazio A, Susani L, Bredius R, Mancini G, Cant A, Bishop N, Grabowski P, Del Fattore A, Messina C, Errigo G, Coxon FP, Scott DI, Teti A, Rogers MJ, Vezzoni P, Villa A, Helfrich MH. Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL. *Nat Genet*. 2007 Aug;39(8):960-2.

28. Guerrini MM, Sobacchi C, Cassani B, Abinun M, Kilic SS, Pangrazio A, Moratto D, Mazzolari E, Clayton-Smith J, Orchard P, Coxon FP, Helfrich MH, Crockett JC, Mellis D, Vellodi A, Tezcan I, Notarangelo LD, Rogers MJ, Vezzoni P, Villa A, Frattini A. Human osteoclast-poor osteopetrosis with hypogammaglobulinemia due to TNFRSF11A (RANK) mutations. *Am J Hum Genet*. 2008 Jul;83(1):64-76.

29. Letizia C, Taranta A, Migliaccio S, Caliumi C, Diacinti D, Delfini E, D'Erasmo E, Iacobini M, Roggini M, Albagha OM, Ralston SH, Teti A. Type II benign osteopetrosis (Albers-Schönberg disease) caused by a novel mutation in CLCN7 presenting with unusual clinical manifestations. *Calcif Tissue Int*. 2004 Jan;74(1):42-6.

30. Waguespack SG, Hui SL, Dimeglio LA, Econs MJ. Autosomal dominant osteopetrosis: clinical severity and natural history of 94 subjects with a chloride channel 7 gene mutation. *J Clin Endocrinol Metab*. 2007 Mar;92(3):771-8.

31. Kornak U, Ostertag A, Branger S, Benichou O, de Vernejoul MC. Polymorphisms in the CLCN7 gene

modulate bone density in postmenopausal women and in patients with autosomal dominant osteopetrosis type II. *J Clin Endocrinol Metab*. 2006 Mar;91(3):995-1000.

32. Bolt RJ, Wennink JM, Verbeke JI, Shah GN, Sly WS, Bökenkamp A. Carbonic anhydrase type II deficiency. *Am J Kidney Dis*. 2005 Nov;46(5):A50, e71-3.

33. Van Wesenbeeck L, Odgren PR, Coxon FP, Frattini A, Moens P, Perdu B, MacKay CA, Van Hul E, Timmermans JP, Vanhoenacker F, Jacobs R, Peruzzi B, Teti A, Helfrich MH, Rogers MJ, Villa A, Van Hul W. Involvement of PLEKHM1 in osteoclastic vesicular transport and osteopetrosis in incisors absent rats and humans. *J Clin Invest*. 2007 Apr;117(4):919-30.

34. Del Fattore A, Fornari R, Van Wesenbeeck L, de Freitas F, Timmermans JP, Peruzzi B, Cappariello A, Rucci N, Spera G, Helfrich MH, Van Hul W, Migliaccio S, Teti A. A new heterozygous mutation (R714C) of the osteopetrosis gene, pleckstrin homolog domain containing family M (with run domain) member 1 (PLEKHM1), impairs vesicular acidification and increases TRACP secretion in osteoclasts. *J Bone Miner Res*. 2008 Mar;23(3):380-91.

35. Revell PA. *Pathology of Bone*. Berlin: Springer-Verlag; 1986.

36. Helfrich MH, Aronson DC, Everts V, Mieremet RH, Gerritsen EJ, Eckhardt PG, Groot CG, Scherft JP. Morphologic features of bone in human osteopetrosis. *Bone*. 1991;12(6):411-9.

37. Shapiro F, Key LL, Anast C. Variable osteoclast appearance in human infantile osteopetrosis. *Calcif Tissue Int*. 1988 Aug;43(2):67-76.

38. Mills BG, Yabe H, Singer FR. Osteoclasts in human osteopetrosis contain viral-nucleocapsid-like nuclear inclusions. *J Bone Miner Res*. 1988 Feb;3(1):101-6.

39. Lajeunesse D, Busque L, Ménard P, Brunette MG, Bonny Y. Demonstration of an osteoblast defect in two cases of human malignant osteopetrosis. Correction of the phenotype after bone marrow transplant. *J Clin Invest*. 1996 Oct 15;98(8):1835-42.

40. Bollerslev J, Steiniche T, Melsen F, Mosekilde L. Structural and histomorphometric studies of iliac crest trabecular and cortical bone in autosomal dominant osteopetrosis: a study of two radiological types. *Bone*. 1989;10(1):19-24.

41. Matsuo K, Irie N. Osteoclast-osteoblast communication. *Arch Biochem Biophys.* 2008 May 15;473(2):201-9.

42. Zhao C, Irie N, Takada Y, Shimoda K, Miyamoto T, Nishiwaki T, Suda T, Matsuo K. Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab*. 2006 Aug;4(2):111-21.

43. Ryu J, Kim HJ, Chang EJ, Huang H, Banno Y, Kim HH. Sphingosine 1-phosphate as a regulator of osteoclast differentiation and osteoclast-osteoblast coupling. *EMBO J*. 2006 Dec 13;25(24):5840-51.

44. Grano M, Galimi F, Zambonin G, Colucci S, Cottone E, Zallone AZ, Comoglio PM. Hepatocyte growth factor is a coupling factor for osteoclasts and osteoblasts in vitro. *Proc Natl Acad Sci U S A*. 1996 Jul 23;93(15):7644-8.

45. Sheu TJ, Schwarz EM, O'Keefe RJ, Rosier RN, Puzas JE. Use of a phage display technique to identify potential osteoblast binding sites within osteoclast lacunae. *J Bone Miner Res*. 2002 May;17(5):915-22.

46. Taranta A, Migliaccio S, Recchia I, Caniglia M, Luciani M, De Rossi G, Dionisi-Vici C, Pinto RM, Francalanci P, Boldrini R, Lanino E, Dini G, Morreale G, Ralston SH, Villa A, Vezzoni P, Del Principe D, Cassini F, Palombo G, Teti A. Genotype-phenotype relationship in

human ATP6i-dependent autosomal recessive osteopetrosis. *Am J Pathol*. 2003 Jan;162(1):57-68.

47. Karsdal MA, Neutzsky-Wulff AV, Dziegiel MH, Christiansen C, Henriksen K. Osteoclasts secrete non-bone derived signals that induce bone formation. *Biochem Biophys Res Commun*. 2008 Feb 8;366(2):483-8.

48. Key LL Jr, Rodriguiz RM, Willi SM, Wright NM, Hatcher HC, Eyre DR, Cure JK, Griffin PP, Ries WL. Long-term treatment of osteopetrosis with recombinant human interferon gamma. *N Engl J Med*. 1995 Jun 15;332(24):1594-9.

49. Iacobini M, Migliaccio S, Roggini M, Taranta A, Werner B, Panero A, Teti A. Apparent cure of a newborn with malignant osteopetrosis using prednisone therapy. *J Bone Miner Res*. 2001 Dec;16(12):2356-60.