Cell. Mol. Life Sci. 63 (2006) 391–398
1420-682X/06/040391-8
DOI 10.1007/s00018-005-5473-9

Cellular and Molecular Life Sciences

Visions & Reflections

Etiologic factors in Paget's disease of bone

S. V. Reddy

Children's Research Institute, Medical University of South Carolina, 173 Ashley Avenue, Charleston, South Carolina 29425 (USA), Fax: +1 843 792 7466, e-mail: reddysv@musc.edu

Received 6 October 2005; received after revision 2 November 2005; accepted 24 November 2005
Online First 25 January 2006

Abstract. Paget's disease of bone is a chronic focal skeletal disorder characterized by increased bone resorption by the osteoclasts. Paramyxoviral gene products have been detected in pagetic osteoclasts. Paget's disease is an autosomal dominant trait with genetic heterogeneity. Several mutations in the ubiquitin-associated (UBA) domain of sequestosome 1 (SQSTM1/p62) have been identified in patients with Paget's disease. Similarly, mutations in the valosin-containing protein (VCP) gene have been shown to cause inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia. In addition, gene polymorphisms and enhanced levels of cytokine/growth factors associated with Paget's disease have been identified. However, the etiologic factors in Paget's disease remain elusive. A cause and effect relationship for the paramyxoviral infection and SQSTM1/p62 gene mutations responsible for pagetic osteoclast development and disease severity are unclear. This article will highlight the etiologic factors involved in the pathogenesis of Paget's disease.

Key words. Paget's disease; osteoclast; measles virus; sequestosome (p62); RANK ligand (RANKL).

Paget's disease of bone is a chronic focal skeletal disease that affects 2–3% of the population over the age of 60, with an increased incidence in Caucasians. The disease is associated with deformity and enlargement of single or multiple bones, among which the skull, clavicles, long bones and vertebral bodies are the most frequently involved [1]. Patients with Paget's disease are frequently asymptomatic, but approximately 10–15% have severe symptoms including bone pain, fractures, neurological complications due to spinal cord compression or nerve entrapment syndromes, deafness, and dental abnormalities. Paget's disease is a highly localized disease, and new lesions rarely develop during the course of the disease. It can be monostotic or polyostotic and the bone lesions continue to progress in size if untreated. Studies have also indicated that patients with Paget's disease have an increased incidence of osteosarcoma, approximately 1% of them developing osteosarcoma in an affected bone. Paget's disease has a very unusual geographic distribution, with an increased incidence in Caucasians of European descent, but it also occurs in African Americans. It is rare in those of Asian descent. Studies have also suggested high prevalence rates of radiographic Paget's disease in Britain, Australia, North America and western Europe. The incidence of Paget's disease appears to have been decreasing over the last several decades [2, 3], but the basis for this decrease is unknown.

Familial expansile osteolysis (FEO) is a rare disease related to Paget's disease, but occurs in patients at a much younger age and is a much more severe disease linked to activating mutations in the gene encoding the receptor activator of nuclear factor κB (RANK) on chromosome 18q [4].

Juvenile Paget's disease is characterized by widespread involvement of the skeleton, distinguishing it from Paget's disease of adults. It is caused by a homozygous deletion of the gene on chromosome 8q24.2 that encodes osteoprotegerin (OPG), a member of the tumor necrosis factor receptor family [5].

The primary pathologic abnormality in patients with Paget's disease is increased bone resorption, followed by abundant new bone formation that is disorganized and of

poor quality. Paget's disease has been described as a slow paramyxoviral infection process, suggesting a viral etiology for the disease. Familial incidence is common in Paget's disease and 40% of patients with the disease have an affected first-degree relative. Familial Paget's disease has an equal incidence in males and females. It is also evident that genetic factors play an important role in the familial and sporadic forms of Paget's disease of bone. Genetic linkage analysis has further indicated that Paget's disease is an autosomal dominant trait with genetic heterogeneity and incomplete penetrance. In this review, viral, genetic, and other etiologic factors that play an important role in the pathogenesis of Paget's disease of bone will be discussed.

Paramyxoviral etiology

A viral etiology has been proposed for Paget's disease due to an initial description of nucleocapsid-like structures in the nuclei and cytoplasm of pagetic osteoclasts by electron microscopy [6]. Immunocytochemical studies further confirmed that these nuclear inclusions cross-reacted with antibodies that recognized measles virus (MV) or respiratory syncytial virus (RSV) nucleocapsid antigens [7]. In situ hybridization techniques also identified the presence of MV messenger RNA sequences in up to 90% of osteoclasts and other mononuclear cells in pagetic bone specimens. Similarly, canine distemper virus (CDV) nucleocapsid antigens were also detected in osteoclasts from patients with Paget's disease [8]. These paramyxoviral-like nuclear inclusions are not unique to Paget's disease and were reported in patients with FEO and rarely in patients with osteopetrosis, pycnodysostosis, otosclerosis, and oxalosis [9]. This has raised the possibility that the virus may be a non-etiologic agent in a cell altered by a genetic defect. Alternatively, there may be sequence homologies between viral and cellular proteins. Since paramyxoviruses are RNA viruses, it is most unlikely that part of the viral genome is integrated into the genome of the affected population.

We have previously identified the expression of MV nucleocapsid (MVNP) transcripts in freshly isolated bone marrow cells from patients with Paget's disease. These MVNP transcripts contain mutations which resulted in amino acid substitutions clustered at the C-terminal end [10]. The mutations occured at a 1% rate in the total MVNP gene isolated from a patient with Paget's disease. We further demonstrated that osteoclast precursors, the granulocyte macrophage colony-forming unit (CFU-GM), as well as mature osteoclasts from patients with Paget's disease, expressed MVNP transcripts. We also detected expression of MVNP transcripts in peripheral blood-derived monocytes from these patients, indicating that MV infection occurs in early osteoclast lineage cells [11]. MV infection has a similar incidence worldwide and occurs in very young patients, whereas Paget's disease is a disease of the elderly. These observations suggest that if paramyxoviruses have an etiologic role in Paget's disease, these viral infections must persist for long periods of time. Pluripotent hematopoietic stem cells, which can persist for long periods of time in a quiescent phase, may be the initial target for the paramyxoviral infection in patients with Paget's disease. We found that other hematopoietic lineages from patients with Paget's disease in addition to the osteoclast lineage, including the erythroid and the erythroid precursors, burst-forming unit-erythroid (BFU-E), and multipotent myeloid precursors (CFUGEMM) also express MVNP transcripts [11]. Thus, if the initial site of infection occurs in a small number of primitive pluripotent hematopoietic stem cells that predominantly remain in Go, this might explain the chronicity of the infection. Also, there may be a genetic predisposition for chronic paramyxoviral infections of hematopoietic precursors in patients with Paget's disease. However, a cause and effect relationship of paramyxoviruses in Paget's disease remains to be proven, as no infectious virus has been isolated from pagetic cells. Also, it is not clear how the focal lesions are initiated in Paget's disease. In contrast to these results, other workers have been unable to detect paramyxoviral nucleocapsid transcripts in samples obtained from patients with Paget's disease [12, 13].

The presence of paramyxoviral transcripts in osteoclasts and osteoclast precursors from patients with Paget's disease suggests a pathophysiologic role for the viral genes in the development of the pagetic lesions. In studies using normal osteoclast precursors (CFU-GM) transduced with retroviral vectors expressing the MVNP gene, the cells formed pagetic-like osteoclasts more rapidly, with an increased number of nuclei, hypersensitivity to 1,25-dihydroxyvitamin D3 (1,25-$[OH]_2D_3$), and an increased bone-resorbing capacity compared to normal osteoclasts. In contrast, normal osteoclast precursors transduced with the MV matrix gene did not express an abnormal phenotype [14]. Furthermore, infecting canine bone marrow cells with CDV results in the development of multinucleated cells that share some of the phenotypic characteristics of pagetic osteoclasts [15]. More recently, CDV was shown to be infectious to human osteoclast precursors and to enhance osteoclast differentiation and function. Previously, we have targeted CD46, the human MV receptor, to cells of the osteoclast lineage in transgenic mice and demonstrated that MV infection of osteoclast precursors from CD46 transgenic mice form osteoclasts, which express a pagetic phenotype *in vitro* [16]. However, TRAP-CD46 mice do not develop sustained MV infection, most likely reflecting the need for blocking interferon production for development of persistent MV infection in these mice. Transgenic mice targeted with

MVNP expression to cells of the osteoclast lineage in vivo results in a bone phenotype that is characteristic of Paget's disease and supports a pathophysiologic role for MVNP in Paget's disease. However, these studies do not exclude genetic factor(s) that may play an important role in disease severity and pathogenesis.

Taken together, these data suggest a potential pathophysiologic role for the paramyxoviral nucleocapsid gene that is expressed in patients with Paget's disease. Mouse models of MV infection were also developed in which CD46 is introduced into transgenic mice and bred to another transgenic mouse lacking the alpha-beta interferon receptor. Upon exposure to MV, these mice developed immune suppression similar to patients with acute MV infection. The mice lacking the alpha-beta interferon receptor demonstrated persistence of MV infection for at least 12 days [17]. Although several lines of evidence support a viral etiology for Paget's disease, it is still unclear how this is related to the late onset and focal nature of Paget's disease.

Genetic linkage of sequestosome 1/p62 and molecular signaling

A genome-wide search in familial Paget's disease of bone indicated genetic heterogeneity of the disease, with candidate loci on chromosomes 2q, 5q, 6p, 10p, and 18q [18, 19, 20]. Linkage studies, coupled with mutation screening, have excluded involvement of RANK and also osteoprotegerin in the majority of patients with Paget's disease of bone [21]. Genetic studies have demonstrated linkage in 7 of 7 patients with osteosarcoma to loss of heterozygosity in a region of 18q that is adjacent to or within a locus for Paget's disease on 18q [22].

Recently, the sequestosome 1 gene encoding the protein p62 (SQSTM1/p62) mapped within the critical region on chromosome 5q35-qter identified a proline-leucine amino acid change at codon 392 (P392L) in French-Canadian patients with Paget's disease of bone [23]. The frequency of the mutation was 16% and 46% for sporadic and familial cases tested, respectively. Further studies also identified different mutations affecting the highly conserved ubiquitin-associated (UBA) domain of the SQSTM1/p62 protein in patients with familial and sporadic Paget's disease [24–26]. In addition to the P392L mutation, two novel mutations (M404V and G425R) were also identified in exon 8 of the SQSTM1 gene in Italian sporadic patients; however, no significant differences in the clinical history was observed in these patients [27]. Studies with patients with familial disease in The Netherlands further identified three new mutations, S399P, M404T, and G425R, which correlated with serum alkaline phosphatase activity similar to patients with P392L mutations [28]. Insertion mutations introducing a stop codon or abolishing the splice donor site at the start of the intron 7 region of SQSTM1 were also identified in UK-derived familial and sporadic Paget's disease cases [29].

Structural analysis studies have classified p62 mutations that retain or abolish the ability of the isolated UBA domain to bind to K48-linked polyubiquitin [30]. Cavey et al. [31] have recently studied the effects of various p62 mutants associated with Paget's disease on the *in vitro* ubiquitin-binding properties of p62 protein. These studies indicated that several SQSTM1 mutations associated with Paget's disease impair p62 binding to ubiquitinylated targets at physiological temperature, suggesting that p62 mutations predispose to Paget's disease through a common mechanism that depends on loss of ubiquitin binding by p62. Using an *in vitro* expression cloning approach, 11 proteins that interact with the p62 UBA domain that are associated with neurodegenerative disorders have been identified [32]. These studies have shown that the heat shock protein-70 (HSP70) interacts with the p62 UBA domain. HSPs are well known to play a role in protein binding, assembly, intracellular transport and degradation within cells. Development of methods such as the yeast two-hybrid screening of an osteoclast cDNA library using the p62 UBA domain as bait should identify further genes that play an important role in pagetic osteoclast development.

The atypical protein kinase C (aPKC) interaction with SQSTM1/p62 has been implicated in signaling cascades that control NF-κB activation (fig. 1). It is evident that p62 provides a scaffold linking the aPKCs to the tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) receptor signaling complexes through its interaction with RIP and TRAF-6, respectively [33]. Thus, SQSTM1/p62 mediates IL-1 and TNF-α cytokine signaling to activate NF-κB. TRAF-6, plays an essential role in receptor activator of NF-κB ligand (RANKL) signaling during osteoclastogenesis. Recently, RANKL stimulation has been shown to result in upregulation of p62 expression in osteoclast precursor cells, and the genetic inactivation of p62 in mice impaired PTHrP-induced osteoclastogenesis *in vivo*. However, p62 null mice have a grossly normal skeletal phenotype and no alterations were found in the trabecular size and number of osteoclasts compared to wild type mice. In vitro studies demonstrated that p62 deficiency leads to inhibition of IKK activation and NF-κB nuclear translocation during osteoclastogenesis [34]. These studies also demonstrated that RANKL stimulation induces formation of a ternary complex involving TRAF-6, p62 and aPKC during osteoclastogenesis. Recent evidence indicates that TNF-α stimulation of osteoclast precursors in the presence of cofactors such as transforming growth factor-beta (TGF-β) results in osteoclastogenesis independent of the RANKL-RANK-TRAF-6 axis [35]. Also, TRAF-2 has been shown to be essential for TNF-α signaling to induce osteoclastogenesis [36]. It is unclear if p62

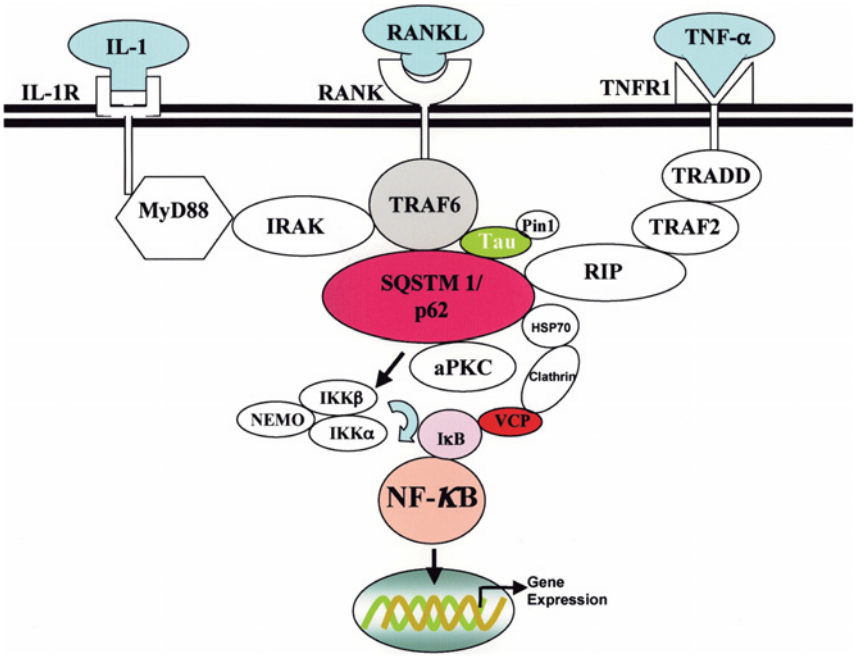

Figure 1. Sequestosome 1 (SQSTM1/p62) protein-protein interactions and associated cellular signaling cascades. RANKL-RANK signaling induces p62 to form a ternary complex with TRAF-6 and aPKCs during osteoclast differentiation. Several mutations in the UBA domain of SQSTM1/p62 have been identified in patients with Paget's disease. Similarly, mutations in VCP, a multiubiquitin chain-targeting factor required in ubiquitin-proteasome degradation have been shown to cause inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia.

UBA mutants affect the status of NF-κB activation and result in a pagetic phenotype of the osteoclast. However, it is reasonable to assess ligand specificity and alternative signaling mechanisms or alternative protein-protein interactions in pagetic-like osteoclast development. For example, it has been shown that inositol 5′ phosphatase-deficient mice are severely osteoporotic with an increased number of osteoclast precursors and hyperactive osteoclasts. In addition, serum levels of IL-6 are markedly increased in these mice as in Paget's disease [37].

p62 was shown to bind ubiquitin non-covalently and sequester into cytoplasmic aggregates in some systems. p62 has been proposed to function as a polyubiquitin shuttling factor for proteasomal degradation through its interaction with the proteasome. Therefore, it is reasonable to speculate that the occurrence of mutations in the UBA domain of p62 in patients with Paget's disease results in cellular accumulation of insoluble polyubiquinated protein aggregates due to failure of proteasomal degradation. For example, accumulation of hyperphosphorylated Tau, the microtubule-associated protein in the brain of patients with Alzheimer's disease, contributes to neurodegeneration. Recently Tau has been identified as a K63-polyubiquitinated substrate of TRAF6 that interacts with the UBA domain of p62 and is targeted for proteasomal degradation [38]. Furthermore, recent evidence also suggests that ubiquitin-proteasome regulatory mechanisms play an important role in osteoblast differentiation [39]. Smad ubiquitin regulatory factor-1 (Smurf1) ubiquitin ligase deficiency results in an age-dependent increase in bone mass due to accumulation of phosphorylated MEKK2 and activation of the JNK signaling cascade in osteoblast cells [40]. Although patients with Paget's disease demonstrate high levels of alkaline phosphatase activity, the molecular defect or alteration in osteoblast cells and the role that the p62 UBA mutant may play in osteoblast activity in these patients is not clear. Osteoblasts are also increased in lesions in patients with Paget's disease, and they appear to be morphologically normal.

Normal human osteoclast precursors transduced with a P392L mutant p62 retroviral expression vector displayed enhanced sensitivity to RANKL and increased osteoclast formation. However, the osteclast precursors demonstrated no pagetic characteristics such as hypersensitivity to 1,25-$(OH)_2D_3$ and increased number of nuclei in the osteoclasts formed *in vivo*. Furthermore, transgenic mice with the P392L mutant p62 gene targeted to cells in the osteoclast lineage using the tartrate-resistant acid phosphatase (TRAP) promoter demonstrated increased osteoclast numbers and were osteopenic but did not develop the increased osteoblast activity that is characteristic of pagetic lesions. These studies suggested that the P392L mutation in p62 enhances osteoclast formation, possibly through increased RANK signaling. Therefore, the precise role that SQSTM1/p62 and signaling mechanisms

may play in pagetic-like osteoclast development and pathogenesis of disease remain to be elucidated.

Inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia (IBMPFD) were recently reported to be caused by mutant valosin-containing protein (VCP) that maps to chromosome 9p21.1-p12 [41]. VCP, a member of the AAA-ATPase superfamily, is a multiubiquitin chain targeting factor for proteasome degradation. VCP is known to function in cell cycle control, membrane fusion, and the ubiquitin-proteasome degradation pathway. It has also been shown that VCP may provide a physical and functional link between IKB-α and the 26S proteasome and play an important role in the proteasome degradation of IKB-α [42, 43]. Formation of protein aggregates and or accumulation of cellular signaling molecules may occur upon failure of proteasomal degradation due to mutations in the N-terminal ubiquitin-binding domain of VCP or UBA domain of p62. This ubiquitous mechanism does not explain specific molecules associated with the pathogenesis of Paget's disease or dominant negative effects on gene expression which regulate osteoclastogenesis and bone resorption activity. However, identification of molecules which interact with p62/VCP provide further insights into ligand specificity for altered signaling cascades responsible for pagetic-like osteoclast development. Recent evidence suggests that a fraction of IKB-α physically associates with nuclear corepressors and histone acetylases. It has further been shown that recruitment of IKKs to the nucleus in response to TNF-α may induce chromatin-associated IKB-α release and gene activation [44]. However, genetic linkage analysis indicated that mutations in the p62 gene may not completely account for the pathogenesis of Paget's disease. The severity of disease in family members carrying the same mutation can vary widely, and up to 20% of individuals who harbor p62 mutations and are older than 55 years do not have PD. Therefore, it is reasonable to hypothesize that other gene loci may be involved in the genetic predisposition and osteoclast abnormalities associated with Paget's disease of bone.

Gene polymorphisms and mRNA expression in Paget's disease

Several studies have examined the linkage of HLA begenes cause of their highly polymorphic nature, and significant associations were observed between class II antigens and Paget's disease [45]. Similarly, studies also indicated that the TNFRSF11B gene encoding OPG with lysine at the codon 3 position predisposes to the development of sporadic and familial forms of Paget's disease that is not caused by SQSTM1 mutations [46]. Recent studies also identified significant variations in genotype frequency of polymorphisms in estrogen receptor-alpha and calcium-sensing receptor genes in patients with Paget's disease compared to normal subjects, which may contribute genetic susceptibility to Paget's disease [47]. Allelic association determines the risk of disease severity and susceptibility but does not explain the focal nature of the disease. Inhibition of apoptosis has been hypothesized to lead to an increased osteoclast lifespan resulting in an increase in the size and number of osteoclasts responsible for enhanced bone resorption activity in patients with Paget's disease. In support of this, in situ hybridization studies have identified increased levels of Bcl2 mRNA expression in pagetic osteoclasts. Further studies indicated that the polymorphic mutations present in the Bcl2 gene promoter region are responsible for elevated Bcl2 expresssion in patients with paget's disease [48]. In situ hybridization studies have also identified increased levels of IL-6 and c-fos proto-oncogene mRNA expression in pagetic osteoclasts. IL-6 receptor and NF-IL-6 mRNA levels were also increased in osteoclasts from bone samples from patients with Paget's disease compared to those with osteoarthritis [49]. It is essential to delineate whether enhanced gene expression or half-life of mRNA and recruitment of coactivators for gene transcription play a pivotal role in pagetic osteoclast development. This is evident from the studies which indicated that pagetic osteoclast precursors are hypersensitive to 1,25-$(OH)_2D_3$ compared to normals. The increased sensitivity of osteoclast precursors from Paget's patients to 1,25-$(OH)_2D_3$ is mediated through the vitamin D3 receptor (VDR); however, this is not due to increased numbers of VDR in pagetic osteoclast precursors compared to normals, but appears to be due to enhanced affinity of the VDR in pagetic cells for its ligand compared to normals [50]. In support of a viral etiology, MVNP gene expression in osteoclast precursors was recently demonstrated to result in increased levels of TAF_{II}-17 transcription factor gene expression. The high levels of TAF_{II}-17 permit formation of a VDR transcription complex at low levels of receptor occupancy by 1,25-$(OH)_2D_3$ [51]. These results support the hypothesis that part of the pathophysiology underlying the increased osteoclast activity in Paget's disease is due to increased levels of VDR coactivators that enhance VDR-mediated gene transcription at low levels of 1,25-$(OH)_2D_3$. Enhanced levels of general transcription factors such as TAF_{II}-17 may not explain the cellular specificity and chronic pathogenesis. However, this does not exclude the possibility of their involvement in pagetic osteoclast development at the involved sites.

Systemic factors

Bones not clinically involved with Paget's disease appear to show increased bone remodeling. This increased bone remodeling in unaffected bones has been ascribed to sec-

ondary hyperparathyroidism rather than to subclinical involvement of the bones with Paget's disease. However, less than 20% of patients with Paget's disease have elevated parathyroid hormone (PTH) levels [2]. Enhanced levels of IL-6, RANKL, M-CSF and endothelin-1 have been associated with Paget's disease. These systemic factors are implicated in the pathogenesis and as an indicator of disease activity [52, 53]. We have recently detected elevated levels of high molecular-weight serum kininogen in patients with Paget's disease [unpublished data]. Because Paget's lesions are focal, pagetic cells may be more sensitive to the elevated systemic factors. The increased levels of IL-6 in the peripheral blood of patients with Paget's disease may in part explain the increased bone remodeling seen in bones not clinically involved with Paget's disease. We need, therefore, to define a pathologic role of systemic factors that are upregulated in patients with Paget's disease. Identification of such factors will also provide further insights into the localized nature and progression of pagetic lesions and disease activity. Osteoclasts from patients with Paget's disease also appear to produce increased levels of IL-6 and express higher levels of IL-6 receptors than normal osteoclasts. IL-6, which is a stimulator of human osteoclast formation, may act as an autocrine/paracrine factor to enhance osteoclast formation in patients with Paget's disease and increase the osteoclast precursor pool. The number of early osteoclast precursors, CFU-GM, was increased significantly in marrow aspirates from patients with Paget's disease compared to normals [54]. The osteoclast precursors from patients with Paget's disease also appear to be hyperresponsive to RANKL and marrow stromal cells from pagetic lesions have increased RANKL expression [55, 56]. RANKL is a critical osteoclast differentiation factor that is expressed on marrow stromal and osteoblast cells in response to several osteotropic factors. The increased sensitivity of osteoclast precursors from Paget's patients to RANKL appears to be due to interactions of these precursors with IL-6. Therefore, it has been hypothesized that Pagetic osteoclasts expressing the MVNP gene produce high levels of cytokines that increase the osteoclast precursor pool. Chronic exposure to cytokines produced by the pagetic osteoclasts results in constitutive overexpression of RANKL in stromal/osteoblast cells further enhancing the abnormal osteoclast development and localized nature in pagetic bone lesions in patients with Paget's disease [57]. Although osteoclasts are thought to be the primary cells affected in Paget's disease, osteogenic cells may be either indirectly or directly affected by the elevated systemic factors or intrinsic genetic defect. Immature osteoblasts are the major responders to RANKL-inducing cytokines and studies have also suggested that expression of RANKL decreases with osteoblast maturation [58]. Therefore, the increased numbers of highly active osteoblasts rapidly form large amounts of woven bone in patients with Paget's disease.

Conclusions and perspectives

In recent years significant progress has been made with respect to etiologic factors associated with Paget's disease of bone, an autosomal dominate trait with genetic heterogeneity. Although recurrent mutations in the UBA domain of sequestosome 1 (SQSTM1/p62) in patients with Paget's disease have been identified and implicated as a common cause of familial and sporadic Paget's disease, it is still unclear if mutant p62 is sufficient to cause Paget's disease, and what its precise role is in osteoclast abnormalities. Future perspectives are to identify novel cellular protein interactions with the ubiquitin-binding domain of VCP or the UBA domain of p62 and to develop animal models to further delineate the role of SQSTM1/p62-associated signaling cascades in pagetic osteoclast development. Lack of skeletal abnormalities in p62-deficient mice further suggests a potential role for genes present in other candidate loci that have been linked with Paget's disease. Alternatively, a genetic defect may favor environmental factors such that MV infection plays a potential role in the pathogenesis of the disease. However, the molecular basis for the abnormalities associated with osteoclasts, the role of paramyxoviral infection, and the persistence of the virus in patients with Paget's disease are unclear. It will be important to determine a cause and effect relationship for the persistence of paramyxoviral infection and genetic predisposition in patients with Paget's disease. Essential also, will be to define the role of elevated systemic factors and underlying molecular mechanisms in the initiation and progression of focal lesions in patients with Paget's disease.

Acknowledgements. This work is supported by the National Institutes of Health Grants DE 12603, AR 049363 and a DOD Medical Research Award.

1 Paget J. (1877) On a form of chronic inflammation of bones (osteitis deformans). Med. Chir. Tr. **60:** 37–63
2 Siris E. S. (1999) Paget's disease of bone. In: Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, 4th edn., pp. 415–425, Favus M. J. (ed.), Lippincott Williams & Wilkins, Philadelphia
3 Doyle T., Gunn J., Anderson G., Gill M. and Cundy T. (2002) Paget's disease in New Zealand: evidence for declining prevalence. Bone **31:** 616–619
4 Hughes A. E., Ralston S. H., Marken J., Bell C., MacPherson H., Wallace R. G. et al. (2000) Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis. Nat. Genet. **24:** 45–48
5 Whyte M. P., Obrecht S. E., Finnegan P. M., Jones J. L., Podgornik M. N., McAlister W. H. et al. (2002) Osteoprotegerin deficiency and juvenile Paget's disease. N. Eng. J. Med. **347:** 175–184

6 Mills B. G. and Singer F. R. (1976) Nuclear inclusions in Paget's disease of bone. Science **194:** 201–202
7 Basle M. F., Fournier J. G., Rozenblatt S., Rebel A. and Bouteille M. (1986) Measles virus RNA detected in Paget's disease bone tissue by in situ hybridization. J. Gen. Virol. **67:** 907–913
8 Gordon M. T., Mee A. P., Anderson D. C. and Sharpe P. T. (1992) Canine distemper virus transcripts sequenced from pagetic bone. Bone Miner. **19:** 159–174
9 Singer F. R. (1999) Update on the viral etiology of Paget's disease of bone. J. Bone Miner. Res. **14 (suppl 2):** 29–33
10 Reddy S. V., Singer F. R. and Roodman G. D. (1995) Bone marrow mononuclear cells from patients with Paget's disease contain measles virus nucleocapsid messenger ribonucleic acid that has mutations in a specific region of the sequence. J. Clin. Endocrinol. Metab. **80:** 2108–2111
11 Reddy S. V., Kurihara N., Menaa C. and Roodman G. D. (2001) Paget's disease of bone: a disease of the osteoclast. Rev. Endocr. Metab. Disord. **2:** 195–201
12 Helfrich M. H., Hobson R. P., Grabowski P. S., Zurbriggen A., Cosby S. L., Dickson G. R. et al. (2000) A negative search for a paramyxoviral etiology of Paget's disease of bone: molecular, immunological, and ultrastructural studies in UK patients. J. Bone Miner. Res. **15:** 2315–2329
13 Ooi C. G., Walsh C. A., Gallagher J. A. and Fraser W. D. (2000) Absence of measles virus and canine distemper virus transcripts in long-term bone marrow cultures from patients with Paget's disease of bone. Bone **27:** 417–421
14 Kurihara N., Reddy S. V., Menaa C., Anderson D. and Roodman G. D. (2000) Osteoclasts expressing the measles virus nucleocapsid gene display a pagetic phenotype. J. Clin. Invest. **105:** 607–614
15 Gordon M. T., Mee A. P., Anderson D. C. and Sharpe P. T. (1992) Canine distemper virus transcripts sequenced from pagetic bone. Bone Miner. **19:** 159–174
16 Reddy S. V., Kurihara N., Menaa C., Landucci G., Forthal D., Koop B. A. et al. (2001) Osteoclasts formed by measles virus-infected osteoclast precursors from hCD46 transgenic mice express characteristics of pagetic osteoclasts. Endocrinology **142:** 2898–2905
17 Peng K. W., Frenzke M., Myers R., Soeffker D., Harvey M., Greiner S. et al. (2003) Biodistribution of oncolytic measles virus after intraperitoneal administration into Ifnar-CD46Ge transgenic mice. Hum. Gene Ther. **14:** 1565–1577
18 Hocking L. J., Herbert C. A., Nicholls R. K., Williams F., Bennett S. T., Cundy T. et al. (2001) Genomewide search in familial Paget disease of bone shows evidence of genetic heterogeneity with candidate loci on chromosomes 2q36, 10p13, and 5q35. Am. J. Hum. Genet. **69:** 1055–1061
19 Leach R. J., Singer F. R. and Roodman G. D. (2001) The genetics of Paget's disease of the bone. J. Clin. Endocrinol. Metab. **86:** 24–28
20 Good D. A., Busfield F., Fletcher B. H., Duffy D. L., Kesting J. B., Andersen J. et al. (2002) Linkage of Paget disease of bone to a novel region on human chromosome 18q23. Am. J. Hum. Genet. **70:** 517–525
21 Sparks A. B., Peterson S. N., Bell C., Loftus B. J., Hocking L., Cahill D. P. et al. (2001) Mutation screening of the TNFRSF11A gene encoding receptor activator of NF kappa B (RANK) in familial and sporadic Paget's disease of bone and osteosarcoma. Calcif. Tissue Int. **68:** 151–155
22 Hansen M. F., Nellissery M. J. and Bhatia P. (1999) Common mechanisms of osteosarcoma and Paget's disease. J. Bone Miner. Res. **14 (suppl 2):** 39–44
23 Laurin N., Brown J. P., Morissette J. and Raymond V. (2002) Recurrent mutation of the gene encoding sequestosome 1 (SQSTM1/p62) in Paget disease of bone. Am. J. Hum. Genet. **70:** 1582–1588
24 Johnson-Pais T. L., Wisdom J. H., Weldon K. S., Cody J. D., Hansen M. F., Singer F. R. et al. (2003) Three novel mutations in SQSTM1 identified in familial Paget's disease of bone. J. Bone. Miner. Res. **18:** 1748–1753
25 Hocking L. J., Lucas G. J., Daroszewska A., Cundy T., Nicholson G. C., Donath J. et al. . (2004) Novel UBA domain mutations of SQSTM1 in Paget's disease of bone: genotype phenotype correlation, functional analysis, and structural consequences. J. Bone Miner. Res. **19:** 1122–1127
26 Good D. A., Busfield F., Fletcher B. H., Lovelock P. K., Duffy D. L., Kesting J. B. et al. (2004) Identification of SQSTM1 mutations in familial Paget's disease in Australian pedigrees. Bone **35:** 277–282
27 Falchetti A., Di Stefano M., Marini F., Del Monte F., Mavilia C., Strigoli D. et al. (2004) Two novel mutations at exon 8 of the Sequestosome 1 (SQSTM1) gene in an Italian series of patients affected by Paget's disease of bone (PDB). J. Bone Miner. Res. **19:** 1013–1017
28 Eekhoff E. W., Karperien M., Houtsma D., Zwinderman A. H., Dragoiescu C., Kneppers A. L. et al. (2004) Familial Paget's disease in The Netherlands: occurrence, identification of new mutations in the sequestosome 1 gene, and their clinical associations. Arthritis Rheum. **50:** 1650–1654
29 Hocking L. J., Lucas G. J., Daroszewska A., Mangion J., Olavesen M., Cundy T. et al. (2002) Domain-specific mutations in sequestosome 1 (SQSTM1) cause familial and sporadic Paget's disease. Hum. Mol. Genet. **11:** 2735–2739
30 Layfield R., Ciani B., Ralston S. H., Hocking L. J., Sheppard P. W., Searle M. S. et al. (2004) Structural and functional studies of mutations affecting the UBA domain of SQSTM1 (p62) which cause Paget's disease of bone. Biochem. Soc. Trans. **32:** 728–730
31 Cavey J. R., Ralston S. H., Hocking L. J., Sheppard P. W., Ciani B., Searle M. S. et al. (2005) Loss of ubiquitin-binding associated with Paget's disease of bone p62 (SQSTM1) mutations. J. Bone. Miner. Res. **20:** 619–624
32 Pridgeon J. W., Geetha T. and Wooten M. W. (2003) A method to identify p62's UBA domain interacting proteins. Biol. Proced. Online **5:** 228–237
33 Moscat J. and Diaz-Meco M. T. (2000) The atypical protein kinase Cs: functional specificity mediated by specific protein adapters. EMBO Rep. **1:** 399–403
34 Duran A., Serrano M., Leitges M., Flores J. M., Picard S., Brown J. P. et al. (2004) The atypical PKC-interacting protein p62 is an important mediator of RANK-activated osteoclastogenesis. Dev. Cell **6:** 303–309
35 Kim N., Kadono Y., Takami M., Lee J., Lee S. H., Okada F. et al. (2005) Osteoclast differentiation independent of the TRANCE-RANK-TRAF6 axis. J. Exp. Med. **202:** 589–595
36 Kanazawa K. and Kudo A. (2005) TRAF2 is essential for TNF-alpha-induced osteoclastogenesis. J. Bone. Miner. Res. **20:** 840–847
37 Takeshita S., Namba N., Zhao J. J., Jiang Y., Genant H. K., Silva M. J. et al. (2002) SHIP-deficient mice are severely osteoporotic due to increased numbers of hyper resorptive osteoclasts. Nat. Med. **8:** 943–949
38 Babu J. R., Geetha T. and Wooten M. W. (2005) Sequestosome 1/p62 shuttles polyubiquitinated tau for proteasomal degradation. J. Neurochem. **94:** 192–203
39 Zhao M., Qiao M., Harris S. E., Oyajobi B. O., Mundy G. R. and Chen D. (2004) Smurf1 inhibits osteoblast differentiation and bone formation *in vitro* and *in vivo*. J. Biol. Chem. **279:** 12854–12859
40 Yamashita M., Ying S. X., Zhang G. M., Li C., Cheng S. Y., Deng C. X. et al. (2005) Ubiquitin ligase Smurf1 controls osteoblast activity and bone homeostasis by targeting MEKK2 for degradation. Cell **121:** 101–113
41 Watts G. D., Wymer J., Kovach M. J., Mehta S. G., Mumm S., Darvish D. et al. (2004) Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat. Genet. **36:** 377–381

42 Dai R. M., Chen E., Longo D. L., Gorbea C. M. and Li C. C. (1998) Involvement of valosin-containing protein, an ATPase co-purified with IkappaBalpha and 26 S proteasome, in ubiquitin-proteasome-mediated degradation of IκBα. J. Biol. Chem. **273:** 3562–3573
43 Daroszewska A. and Ralston S. H. (2005) Genetics of Paget's disease of bone. Clin. Sci. (Lond.) **109:** 257–263
44 Aguilera C., Hoya-Arias R., Haegeman G., Espinosa L. and Bigas A. (2004) Recruitment of IkappaBalpha to the hes1 promoter is associated with transcriptional repression. Proc. Natl. Acad. Sci. USA **101:** 16537–16542
45 Leach R. J., Singer F. R. and Roodman G. D. (2001) The genetics of Paget's disease of the bone. J. Clin. Endocrinol. Metab. **86:** 24–28
46 Daroszewska A., Hocking L. J., McGuigan F. E., Langdahl B., Stone M. D., Cundy T. et al. (2004) Susceptibility to Paget's disease of bone is influenced by a common polymorphic variant of osteoprotegerin. J. Bone. Miner. Res. **19:** 1506–1511
47 Donath J., Speer G., Poor G., Gergely P. Jr, Tabak A. and Lakatos P. (2004) Vitamin D receptor, oestrogen receptor-alpha and calcium-sensing receptor genotypes, bone mineral density and biochemical markers in Paget's disease of bone. Rheumatology (Oxford) **43:** 692–695
48 Brandwood C. P., Hoyland J. A., Hillarby M. C., Berry J. L., Davies M., Selby P. L. et al. (2003) Apoptotic gene expression in Paget's disease: a possible role for Bcl-2. J. Pathol **201:** 504–512
49 Hoyland J. A., Freemont A. J. and Sharpe P. T. (1994) Interleukin-6, IL-6 receptor, and IL-6 nuclear factor gene expression in Paget's disease. J. Bone Miner. Res. **9:** 75–80
50 Menaa C., Barsony J., Reddy S. V., Cornish J., Cundy T. and Roodman G. D. (2000) 1,25-Dihydroxyvitamin D3 hypersensitivity of osteoclast precursors from patients with Paget's disease. J. Bone Miner. Res. **15:** 228–236
51 Kurihara N., Reddy S. V., Araki N., Ishizuka S., Ozono K., Cornish J. et al. (2004) Role of TAFII-17, a VDR binding protein, in the increased osteoclast formation in Paget's disease. J. Bone Miner. Res. **19:** 1154–1164
52 Tarquini R., Perfetto F. and Tarquini B. (1998) Endothelin-1 and Paget's bone disease: is there a link? Calcif. Tissue Int. **63:** 118–120
53 Neale S. D., Schulze E., Smith R. and Athanasou N. A. (2002) The influence of serum cytokines and growth factors on osteoclast formation in Paget's disease. QJM **95:** 233–240
54 Roodman G. D., Kurihara N., Ohsaki Y., Kukita A., Hosking D., Demulder A. et al. (1992) Interleukin-**6:** a potential autocrine/paracrine factor in Paget's disease of bone. J. Clin. Invest. **89:** 46–52
55 Neale S. D., Smith R., Wass J. A. and Athanasou N. A. (2000) Osteoclast differentiation from circulating mononuclear precursors in Paget's disease is hypersensitive to 1,25-dihydroxyvitamin D(3) and RANKL. Bone **27:** 409–416
56 Menaa C., Reddy S. V., Kurihara N., Maeda H., Anderson D., Cundy T. et al. (2000) Enhanced RANK ligand expression and responsivity of bone marrow cells in Paget's disease of bone. J. Clin. Invest. **105:** 1833–1838
57 Roodman G. D, and Windle J. J. (2005) Paget's disease of bone. J. Clin. Invest. **115:** 200–208
58 Gori F., Hofbauer L. C., Dunstan C. R., Spelsberg T. C., Khosla S. and Riggs B. L. (2000) The expression of osteoprotegerin and RANK ligand and the support of osteoclast formation by stromal-osteoblast lineage cells is developmentally regulated. Endocrinology **141:** 4768–4776