Pathogenesis of Paget's disease of bone

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Summary

Paget's disease (PD) is the most flagrant example of disturbed bone remodeling, with abnormalities in all phases of the bone remodeling process. Although the basis for the abnormal osteoclast (OCL) activity in PD is still unclear, several pathophysiologic features of PD and pagetic OCL have been demonstrated. These include: 1) pagetic OCL precursors and OCL express the measles virus nucleocapsid protein (MVNP) gene, suggesting a possible viral etiology for PD; 2) pagetic OCL express increased levels of the TAFII-17 gene; 3) pagetic OCL precursors are hyper-responsive to 1,25-(OH)2D3 and R-Na, and they can form OCL at very low concentrations of either ligand; 4) pagetic OCL has an increased rate of formation, increased nuclear number and bone resorbing capacity; and 5) a genetic component is involved in PD. Recently, mutations in the p62 gene (sequestasome-1) have been linked to PD in a third of patients with familial PD and about 10% of patients with sporadic PD. Currently, it is unknown if both the genetic and viral components are required for the development of PD. However, it is clear that MVNP gene expression in pagetic OCL precursors results in development of the pagetic phenotype in OCL. The genetic component appears to increase basal osteoclastogenesis but by itself may not be sufficient to induce PD.

KEY WORDS: osteoclast, Paget's disease, measles virus, sequestasome.

Introduction

Paget's disease (PD) of bone was first described in 1876 by James Paget as a disease that begins at middle-age or later and affects the long bones of the lower extremities, the pelvis and the skull. The bones enlarge and soften, and those bearing weight yield and become unnaturally curved or misshapen (1). This description remains accurate, although not all patients experience bone deformities. A recent study estimates that up to 70% of patients may be asymptomatic (2). Bone pain is the most common complication. Other symptoms may include deafness, nerve compression syndrome and pathological fractures (3). The most devastating complication of PD is osteochondroma, which is a very rare complication of the disease and occurs in less than 1% of patients. However, the vast majority of osteosarcomas in adults occur in patients with PD.

PD is particularly prevalent in populations of Northern European ancestry. Recently, the prevalence of the disease in the United States has been estimated to be approximately 1.3% in the population over the age of 55, which is a decrease from 5.4%, estimated in the 1970s (2). A similar decline in disease prevalence has also been reported in New Zealand (4). A current prevalence of 2% has been estimated in the United Kingdom (5). The prevalence of PD increases with age, such that the prevalence in the population over the age of 85 is almost 5 times that of the population under the age of 60. Differences in disease incidence is found at different geographic locations with a particular hot spot in the Lancashire region in the United Kingdom. PD is the second most common bone disease after the population over the age of 55 in the United States, the United Kingdom and in other Caucasian populations.

Bone histology and OCL phenotype in PD

Typical features of bones involved with PD include excessive osteoclast activity and a reflected in serum alkaline phosphatase, which is elevated in 85% of patients. Bisphosphonates, by inactivating OCL, in turn normalize the osteoblast activity, indicated by a reduction in serum alkaline phosphatase. X-rays and isotope bone scans are used to identify affected skeletal sites. PD can affect one or multiple bones, and is highly localized. The disease can spread within a particular site, but it rarely spreads to new sites. This feature distinguishes PD from three more progressive diseases with similar bone histology: Familial Expansile Osteolysis (FE0), Expansile Skeletal Hyperphosphatasia (ESH), and Juvenile Paget's Disease (JPD).

Bone histology and OCL phenotype in PD

Typical features of bones involved with PD include excessive numbers of OCL, which in turn are increased in size and nuclear number per OCL (Figure 1), large numbers of highly active osteoblasts, the presence of woven bone and often a highly cellular bone marrow. Pagetic OCL have been extensively investigated by TEM and immunohistochemistry (Figure 2) ever since they were first reported to contain intranuclear, or less frequently, intracytoplasmic inclusions. Intranuclear inclusions are found in almost all cases of PD (6) and the perceived similarity of these structures to paramyxovirus nucleocapsids (7) has led to the hypothesis that PD may be caused by a persistent infection by measles virus (MV) or another paramyxovirus (8, 9). This issue remains unresolved as evidence for or against the role of paramyxoviruses in the etiology of this disease has been presented over many years (10,11). The inability of various groups working in this field to confirm each other's findings has continued the discussion.

Reddy et al. reported the presence of measles virus nucleocapsid sequences in patients with PD, which can be amplified from bone marrow, blood cells, and early hematopoietic colony-forming cells in the United States (12). Recently, the
In support of the role for a virus in the etiology of PD, however, are studies indicating that measles virus infection of murine OCL, made susceptible by transfection with the human measles virus receptor (CD46), resulted in pagetic features in OCL, specifically enhanced nuclearity (16). The final analysis of a viral involvement in PD must await the elucidation of the undisputed genetic component in this disease (17). Only once the predisposing/causative genes have been identified will it be possible to assess whether viral infection is required for expression of the full phenotypic features of the disease.

**In vitro study of pagetic OCL**

Bone marrow culture techniques that allow the formation of OCLs in vitro have provided new insights into the potential pathogenesis of PD. Our laboratory was the first to examine OCL formation from bone marrow obtained from involved bones from patients with PD (18, 20). In vitro, increased numbers of OCLs are formed by pagetic precursors, and OCL are larger and contain more nuclei than in controls. We found that multinucleated cells that formed in marrow cultures from Paget’s patients shared many of the characteristics of pagetic OCLs. The OCLs were increased in number (10- to 100-fold) compared to normal marrow cultures, had increased numbers of nuclei per multinucleated cell (up to 100 nuclei per cell) compared to 3-10 nuclei in normal OCLs, and expressed high levels of tartrate-resistant acid phosphatase (TRAP). This appears to be caused by the activity of the culture media and not by viral infection (11).

In vitro studies have also shown that pagetic OCLs are hyper-responsive to 1,25-(OH)\(_2\)D\(_3\), induced by concentrations of 1,25-(OH)\(_2\)D\(_3\) that were 1 to 2 logs lower than that required for OCL formation in normal marrow cultures, suggesting that OCL precursors from Paget’s patients were hyper-responsive to 1,25-(OH)\(_2\)D\(_3\) (20). In support of this finding, Kurihara et al. have shown that transduction of normal OCL precursors, CFU-GM, with retroviral constructs containing the cDNA for the MVN gene, induce OCL precursors to form OCL that express a pagetic phenotype and may be playing a role in the hyper-responsivity to 1,25-(OH)\(_2\)D\(_3\) (23). They have further shown that using GST-VDR chimeric protein pull-down assays with lysates from MVNP transduced normal OCL precursors and marrow cells from involved bones from patients with PD, that a potential coactivator of VDR, TAF\(_{17}\), is overexpressed in pagetic OCL and may be playing a role in the hyper-responsivity to 1,25-(OH)\(_2\)D\(_3\) (24). The molecular basis for the increased affinity of 1,25-(OH)\(_2\)D\(_3\) for the VDR appears to result from enhanced expression of co-activators that interact with VDR, in particular TAF\(_{17}\), a member of the TF\(_D\) transcription complex (24, 25).

**VDR coactivator expression in PD**

As noted above, one of the earliest findings from in vitro studies of PD is that pagetic OCL precursors are hyper-responsive to 1,25-(OH)\(_2\)D\(_3\) and form OCL at concentrations of 1,25-(OH)\(_2\)D\(_3\) that are physiologic rather than pharmacologic. The increased responsivity to 1,25-(OH)\(_2\)D\(_3\) is not due to increased levels of the Vitamin D receptor (VDR) nor to increased affinity.
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Expression of the pagetic phenotype in OCL requires VDR dependent gene transcription

Transduction of normal CFU-GM with retroviral constructs containing the cDNA for the measles virus nucleocapsid (MVNP) gene can induce these cells to form OCL that express a pagetic phenotype and are hypertrophic. This result suggested that VDR plays a key role in the abnormal OCL activity in PD. Therefore, to test this hypothesis, OCL precursors from VDR−/− and VDR+/+ mice were transfected with the MVNP gene, and the characteristics of OCL formed examined. PCR analysis of MVNP transduced VDR−/− and VDR+/+ OCL precursors demonstrated that both cell types expressed high levels of TAF17. OCL formation was significantly decreased in bone marrow cultures from VDR−/− mice treated with RANKL compared to marrow cultures from VDR+/+ mice. Furthermore, the OCL that formed in marrow cultures of VDR−/− mouse transfected with the MVNP gene did not express a pagetic phenotype. In contrast, MVNP transduced VDR+/+ mouse cells treated with RANKL formed large numbers of abnormal OCL that expressed a pagetic phenotype. As expected, VDR−/− marrow cells failed to form OCL in response to 1,25-(OH)2D3. These data suggest that VDR mediates a VDR-dependent gene transcription that may be required for expression of a pagetic phenotype in OCL.

Genetics of PD

PD is a complex disorder where multiple genetic factors have been identified. A genetic component appears to be involved in PD, with at least six loci now linked to PD. The genetic basis for PD stems from the observation that familial clustering is common and that ethnic differences in prevalence persist after migration. Between 15-40% of patients have an affected first-degree relative, and first-degree relatives have a seven-fold increased risk of developing the disease compared to controls (28). Familial PD has an autosomal dominant mode of inheritance with high penetrance. Linkage analysis in families initially concentrated on HLA alleles and a susceptibility locus was described on chromosome 6p21 (29). However, further studies, including genome wide searches, have failed to confirm this region as important in PD (30,31). Following the cloning of RANK and the realization that RANK is critical for OCL differentiation (32) led to the examination of chromosome 18 in PD. RANK maps to critical region on chromosome 18q, previously linked to FEO, a more severe disease. Transgenic mice, harboring the various mutations in this heterogeneous disorder have been identified will it be possible to examine fully the etiology and biology of the disease. Transgenic mice, harboring the various mutations found in patients, may be useful as models, but may not develop the disease unless exposed to appropriate environmental triggers, for example, viral infection, ageing or fracture. Such studies will be complex, but may provide the only tool to help dissect their contribution to disease development. In addition, such studies will help to provide a better understanding of the unique role of the primary genes affected in the biology of the OCL.

Conclusion

PD appears to require both a genetic and environmental factor. VDR mediated gene transcription is required for expression of a pagetic phenotype in OCL, and the p62mut causes increased NF-κB signaling and OCL formation in response to RANKL and TNF-α. MVNP increases 1,25-(OH)2D3 responsivity and induces TAF17. These data suggest that p62mut increases basal OCL formation in PD patients but is not sufficient to induce the complete PD phenotype in OCL precursors without an environmental component that affects VDR mediated gene transcription.
References


44. Neale SD, Smith R, Wasse JA, Athanasou NA. Osteoclast differentiation from circulating mononuclear precursors in Paget’s disease
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