Mini-review

Pathogenesis of Paget's disease of bone

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Summary

Paget's disease (PD) is the most flagrant example of disordered 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 TAF_{II}-17 gene; 3) pagetic OCL precursors are hyperresponsive to 1,25-$(OH)_2D_3$ and RANKL 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, sequestosome.

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 osteoclastoma, 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 in the population over the age of 55 in the United States, the United Kingdom and in other Caucasian populations.

PD is primarily a disease of osteoclasts (OCL) with abnormal function. This in turn leads to excessive osteoblast activity and is 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 (FEO), 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

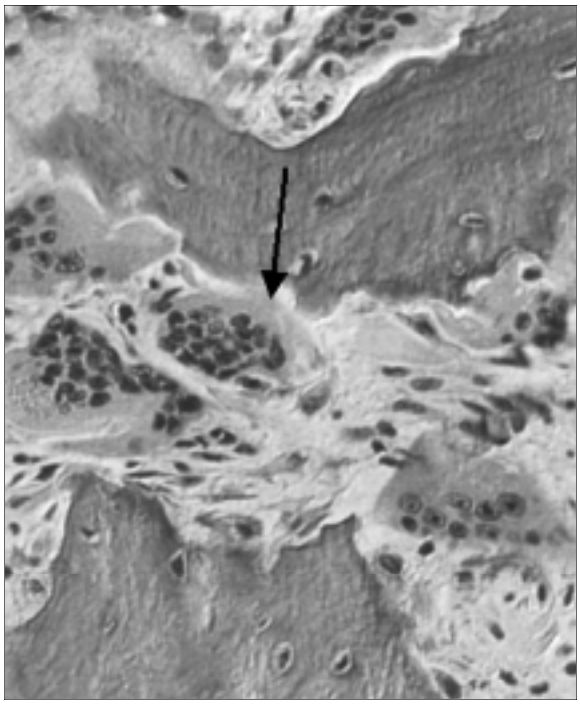

Figure 1 - Osteoclasts in Paget's disease. Pagetic osteoclasts are increased in both number and size and contain increased nuclei per multinucleated cells. In addition, these cells show an increased bone resorbing capacity.

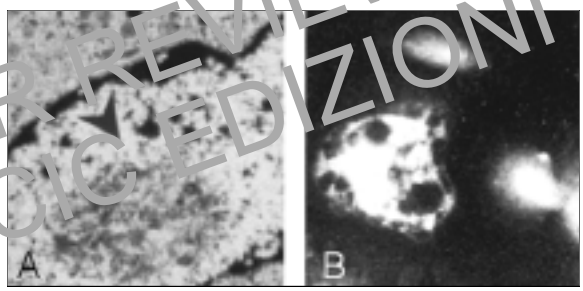

Figure 2 - Transmission Electron Microscopy (TEM) and immunocytochemistry of osteoclasts from patients with Paget's disease. A viral etiology has been suggested for Paget's disease. Paramyxoviral-like nuclear and cytoplasmic inclusions are present in osteoclasts from the majority of patients (A). Immunocytohistochemical studies have shown that pagetic osteoclasts express measles virus and respiratory syncytial virus nucleocapsid antigens (B).

amplification of a complete measles coding sequence has been reported (13). Contrary to these results, Mee et al. had reported the presence of canine distemper virus, but not measles virus (14). However, others were unable to detect any paramyxovirus in Paget's patients (15). No intact virus has ever been recovered from a patient with PD. Not only has the presence of viral nucleic acids in pagetic tissue been questioned, but the whole basis for considering paramyxovirus in the etiology of the disease (15), since the intranuclear inclusions in PD are ultrastructurally dissimilar to these seen in subacute sclerosing panencephalitis (SSPE), the only known persistent measles infection (11).

In support of the role for a virus in the etiology of PD, however, are our 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 as compared to 3-10 nuclei in normal OCL), and expressed high levels of tartrate-resistant acid phosphatase (TRAP). This appears to be caused by the fact that the precursors are hyper-responsive to $1,25\text{-}(OH)_2D_3$ and RANKL (18-21), rather than through an increase in the number of OCL precursors.

Immunocytochemical studies confirmed that MV and respiratory syncytial virus nucleocapsid antigens were expressed in OCLs formed *in vitro* in these cultures (22). OCL formation was induced by concentrations of $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ (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 *MVNP* gene, induce OCL precursors to form OCL that express a pagetic phenotype and are hyper-responsive to $1,25\text{-}(OH)_2D_3$ (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_{II}-17 (24), which is not expressed in normal OCL precursors, is overexpressed in pagetic OCL and may be playing a role in the hyper-responsivity to $1,25\text{-}(OH)_2D_3$ (25). The molecular basis for the increased affinity of $1,25\text{-}(OH)_2D_3$ for the VDR appears to result from enhanced expression of co-activators that interact with VDR, in particular TAF_{II}-17, a member of the $TF_{II}D$ transcription complex (24, 25). Menna et al. had also shown that OCL precursors from patients with PD are also hyper-responsive to RANK ligand (26).

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 $1,25\text{-}(OH)_2D_3$ and form OCL at concentrations of $1,25\text{-}(OH)_2D_3$ that are physiologic rather than pharmacologic. The increased responsivity to $1,25\text{-}(OH)_2D_3$ is not due to increased levels of the Vitamin D receptor (VDR) nor to increased affinity

of 1,25-$(OH)_2D_3$ for VDR. Kurihara et al. had shown by using GST-VDR chimeric protein pull-down assays that TAF_{II}-17, a member of the $TF_{II}D$ transcription complex, is increased in OCL precursors from patients with PD compared to normals. Kurihara et al. further showed that TAF_{II}-17 could enhance VDR mediated gene transcription and allow formation of the transcription complex at very low levels of 1,25-$(OH)_2D_3$. In addition, other coactivators of VDR, including CPB/p300 and DRIP205, are also increased in OCL precursors from Paget's patients (27). These data suggest that the enhanced sensitivity of OCL precursors to 1,25-$(OH)_2D_3$ in PD results from increased expression of coactivators of VDR.

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 hyper-responsive to 1,25-$(OH)_2D_3$. 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 TAF_{II}-17. 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 transfected VDR+/+ mouse cells treated with RANKL formed large numbers of abnormal OCL that expressed a pagetic phenotype. As expected, VDR-/- marrow cells did not form OCL in response to 1,25-$(OH)_2D_3$. These data suggest that VDR mediated gene transcription may be required for expression of a pagetic phenotype in OCL.

Genetics of PD

PD is a complex disease 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 6q21 (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 serve disease with clinical and histological similarities to PD. Linkage studies also explored the involvement of 18q21-22 and mutations in RANK in PD. Linkage to 18q21-22 was found in a number of families (33,34), however, only one Japanese family with severe, early onset PD was found to have a mutation in RANK (35). It is now well-established that PD is rarely linked to 18q21-22 (36) and mutations in RANK and OPG genes do not give rise to adult PD (37), but rather, to more severe, early onset disorders.

Having found a link of PD to chromosome 5q in a number of French-Canadian families, Laurin et al. (38) recently described a non-conservative heterozygous point mutation in a novel gene, sequestosome 1 (*SQSTM1*) in 11% of familial and 16% of sporadic PD (38). Mutations in p62 are the most frequently reported genetic mutations and affects about 26% of patients with familial PD and 9% of sporadic cases (39). More recently, the identification of new mutations in *SQSTM1* have been reported in the Netherlands (40) and Belgian patients with PD (41).

In the familial PD, the mutations co-segregated with the disease but were seen in small numbers of unaffected family members but not in a large number of non-pagetic controls. These data indicated that these mutations may not by themselves be sufficient to cause PD. *SQSTM1* codes for p62, a widely expressed ubiquitin-binding protein (41), which acts as a scaffold protein in the NF-κB signaling pathway, facilitating TNF-α, CD40 and IL-1 through its interactions with the atypical protein kinases, ζPKC or λPKC (42-44).

The mutations found in PD so far are all predicted to affect the ubiquitin-binding domain of the protein and to abolish or reduce ubiquitin binding. The P392L mutation is the most frequent mutation reported. The biological consequences of p62 remain to be substantiated in transgenic mice and knockout mice (45) and further *in vitro* experiments. Whereas ubiquitinated proteins, destined for degradation in the proteasome, generally bind ubiquitin in a covalent manner on single residues, p62 appears to use a large domain to bind ubiquitin in a non covalent manner. Given this difference, it is unclear whether ubiquitin binding by p62 leads to its own degradation, or whether instead it has a role to play in regulating ubiquitination of other signaling molecules by controlling intracellular availability of ubiquitin. Further cell biological experiments are required to elucidate the role of p62 and its specific importance in OCL formation. Recently, we have targeted p62 (P392L) to the OCL lineage in transgenic mice. These mice show increased OCL formation and activity but do not develop PD by age 4 months.

The genetics of PD is under intense investigation and the other genes mutated in PD will, no doubt, soon be found in the remaining susceptibility loci. Only once all the genes involved 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 $p62_{MUT}$ causes increased NF-κB signaling and OCL formation in response to RANKL and TNF-α. MVNP increases 1,25-$(OH)_2D_3$ responsivity and induces TAF_{II}-17. These data suggest that $p62_{MUT}$ 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.

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