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PERSPECTIVES

The Role of Genes in the Pathogenesis of Paget's Disease of Bone

Pui Yan Jenny Chung and Wim Van Hul

Department of Medical Genetics, University of Antwerp, Antwerp, Belgium

Abstract

Paget's disease of bone (PDB) is the second most common metabolic bone disease in the Western world after osteoporosis. Although not always recognized in the early years, genetic factors do contribute to the pathogenesis of PDB in at least a subset of patients. This review summarizes how the current understanding of the role of genes in this condition was obtained, discusses what can be learned from that process, and outlines remaining questions. The first insights were gained by studying monogenic conditions that to some extent resemble the pathogenic mechanisms of increased bone turnover seen in PDB patients. With the involvement, in these conditions, of the genes encoding the OPG and RANK proteins, the importance of the NFκB pathway was solidified. This was also underscored by genetic association studies indicating an effect of genetic variance within *TNFRSF11B* (OPG) and *TNFRSF11A* (RANK) on the susceptibility for PDB. Genetic linkage studies in multicase PDB families suggested up to 7 loci but, most likely, most of these will be false positive gene localizations. At present, only one gene, *SQSTM1* encoding p62, has been identified by this approach. Together with the *VCP* gene encoding the valosine-containing protein and being identified as causative for a syndromal form of PDB, the evidence seems to support a pathogenic mechanism caused by increased NFκB signaling. However, more recent functional studies have suggested a more complex situation. The role of both p62 and VCP in the processes of protein degradation, and especially autophagy, indicates that disturbance of these might contribute to the pathogenic mechanisms in PDB. This hypothesis might also be linked with the observation of cellular inclusion bodies in some PDB patients and the hypothesis that these might be due to paramyxovirus infection. Much progress has been made in unraveling the genetic factors involved in PDB but questions such as the tissue-specific and focal aspect of the disease, as well as the observation of a declining prevalence, remain open, making further research of interest. Ongoing genome-wide association studies will definitely contribute to the further unraveling of the pathogenesis of PDB. *IBMS BoneKEy*. 2010 March;7(3):124-133.

Keywords: Paget's disease of bone; SQSTM1; OPG; RANK; VCP

Introduction

Paget's disease of bone (PDB) is a focal disease characterized by increased bone turnover, sometimes resulting in deformities or fractures of the affected bone(s). As the initial lesions are osteolytic due to increased bone resorption, PDB was generally viewed as an osteoclastic disease. However, more recent data indicate that osteoblasts from PDB patients also show abnormalities and it cannot be excluded that this might also contribute to the pathogenesis of the disease (1;2). Regarding the primary cause of the disease, it is generally accepted that genetic factors are contributing but belief also remains in a putative role of slow virus infection with paramyxovirus (3-5).

In his initial description of what he called "osteitis deformans, a form of chronic inflammation of bones," Sir James Paget reported, "I have tried in vain to trace any hereditary tendency to the disease. I have not found it twice in the same family." This observation is not completely unexpected taking into account some of the main characteristics of the disease. The very late onset of the disease (mostly over 55 years of age), in combination with the fact that up to 95% of patients are clinically asymptomatic, makes it very hard to find

multigenerational or even multicase PDB families (6-8). However, mainly based on large epidemiological studies, evidence later became available for the role of genetic factors in at least a subset of cases. A seven-fold increased risk for first degree relatives of PDB patients was calculated and some large multigenerational families were described with a clear autosomal dominant mode of inheritance (9). Furthermore, some studies illustrated that the prevalence differences between some populations do persist after emigration, supporting the role of genetic rather than environmental factors (10). However, even today the field still lacks a precise estimate of the quantitative role of genetic factors for PDB, generally expressed as heritability and calculated by concordance and discordance rates in mono- and dizygotic twins. Nevertheless, the fact that PDB presents both as a condition segregating in families and as a condition without evidence for familial segregation means that genetic studies were performed considering it to be a monogenic disease, while others studies have approached it as a complex, multifactorial disease.

PDB as a Monogenic Condition

Over the last decade, genetic studies of PDB have made use of the restricted number of multicase families as these can provide interesting tools for linkage studies and positional cloning of PDB-causing genes. Up to 7 chromosomal loci were described but, unfortunately, from only one of these has a disease-causing gene been identified (11-16). The underlying explanation for this lack of success might be that the majority of these loci are false positives due to characteristics of PDB (such as a high age of onset and high prevalence of asymptomatic cases) that complicate linkage studies. PDB3 on chromosome 5q35 is thus far the only region where the second step of positional cloning was made by the identification of the gene encoding sequestosome1 (SQSTM1) or p62 protein (15;17). This turned out to be an important gene accounting for about 30-50% of the familial cases and, in 5-10% of cases without a proven familial history, the gene was mutated as well (17-31). Currently more than 20 different mutations have been described in this gene but in more than 70% of the cases with a *SQSTM1* mutation, the mutation was identical, an amino acid substitution at position 392 (P392L) located within the ubiquitin-binding domain of SQSTM1. Interestingly, analysis of polymorphisms flanking this mutation identified an identical genetic background for this chromosomal region in almost all cases from different Western European populations and was even found in about 50% of the P392L mutations in the French Canadian population (17;24;25;27). These findings imply the presence of a founder mutation, and that one ancestral mutation originating many centuries ago is causative for a large number of current “familial” PDB patients.

Another gene involved in typical PDB was identified by studying IBMPFD, a syndrome where PDB is associated with inclusion body myopathy and frontotemporal dementia and is caused by mutations in the valosin-containing protein (*VCP*) gene (32-35). Finally, relevant insight into the pathogenesis of PDB was obtained by studying monogenic conditions with clinical and radiological similarities to PDB. Familial expansile osteolysis (FEO), expansile skeletal hyperphosphatasia, and early-onset PDB on one hand and juvenile PDB (juvenile hyperphosphatasia) on the other hand are all characterized by increased bone turnover resulting in medullary expansion, deformities and fractures. The first group of conditions results from duplications in the signal peptide of the *TNFRSF11A* gene encoding RANK (36-43), while the latter is due to loss of functional OPG protein encoded by *TNFRSF11B* (44-48).

PDB as a Complex, Multifactorial Disease

Since PDB presents in most cases without a familial background, association studies have been performed on such non-familial cases in order to reveal genetic variants that influence the susceptibility to develop PDB. No evidence was found for such an effect of genetic variance within the *SQSTM1* gene

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(49). However, we and others have found evidence for the presence of such a variant in the *TNFRSF11B* gene encoding OPG (50-52), in different populations. More recently, we were able to find a similar effect in the *TNFRSF11A* gene encoding RANK in a Belgian cohort of PDB patients (53). Furthermore, we were able to replicate these findings both in a British and Dutch cohort (53). The real functional variant causing the increased susceptibility for PDB has not been identified for either gene. Apparently, a somewhat modulated balance between the two receptors for RANKL protein can, in combination with local factors, increase the risk for a focal PDB lesion. Therefore the variant could be within the promoter region slightly influencing the expression of either gene or could be within the coding sequence influencing the functioning of one of both proteins. Intriguingly, the association in both genes was almost exclusively found in the female patient cohort (52;53). This suggests that the regulation of the NFκB pathway by the RANKL-RANK-OPG balance is for some reason more critical in females than in males or that in males other pathways are relatively more important mimicking the effect of differences in NFκB signaling.

Lessons Learned About Pathogenesis

Genetic studies thus far have indicated the involvement of genes encoding RANK, OPG, VCP and SQSTM1 in Pagetoid diseases, providing strong arguments for the role of deregulated NFκB signaling. Providing further support for this idea is that this pathway is a key player in osteoclastogenesis that is to a large degree regulated by RANK and OPG. Moreover, both for SQSTM1 and VCP, a role in intracellular NFκB signaling has been found; the former is a scaffolding protein facilitating NFκB signaling (54-56) and the latter participates in proteasomal degradation of IκB, a downstream mediator of NFκB signaling (Fig. 1).

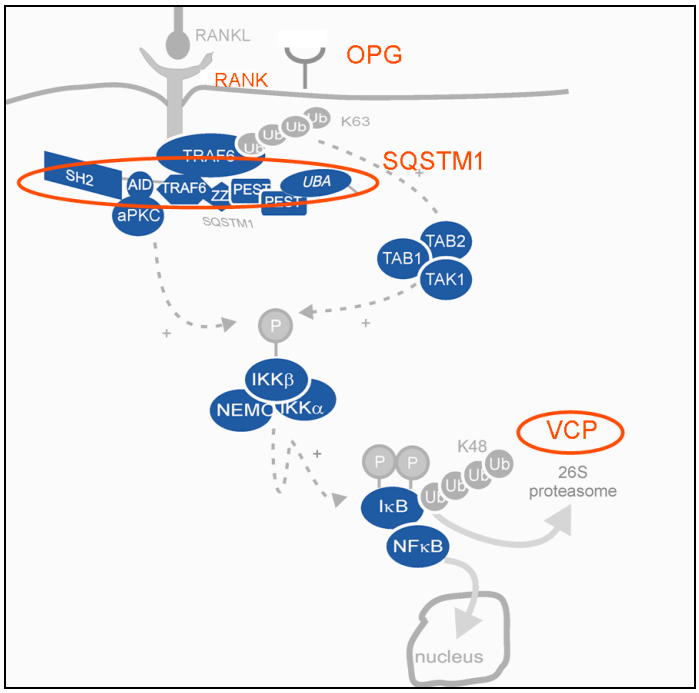

Fig. 1. A model of RANKL-mediated NFκB signaling, with genes involved in Pagetoid diseases shown in red. RANKL, if not bound to its decoy receptor OPG, can bind its receptor RANK, thus causing intracellular recruitment of TRAF6, SQSTM1 and aPKC. The latter can phosphorylate the IKK complex leading to phosphorylation of IκB, which will be degraded by proteasomal degradation with the help of VCP. Consequently NFκB is released and can be translocated to the nucleus to induce the expression of target genes.

However, further functional studies also looking at the effect of mutations on NFκB signaling have made clear that more than just increased NFκB signaling may be involved. These other aspects have been reviewed by our group and others in recent publications (57-59). In the last few years, evidence has been obtained for involvement of both the SQSTM1 and VCP proteins in the process of autophagy (60;61). This mechanism enables the cell to recycle material by enclosing it in intracellular organelles for degradation. It is important for cells to protect themselves against apoptosis under conditions such as starvation. If this mechanism is disturbed it can result in intracellular accumulation of inclusion bodies as seen in PDB (58). Both proteins have a ubiquitin-binding domain and are found in intracellular protein aggregates where they are assumed to play a role in the degradation process of these aggregates. Interestingly, the different mutations found in PDB patients are clustered, affecting precisely the ubiquitin-binding domain of both proteins. The involvement of this important cellular mechanism could also explain long-lasting questions related to PDB. First, it could explain the presence of intracellular inclusion bodies as seen in osteoclasts of some PDB patients as well as in patients with other osteoclast-related diseases such as FEO, osteopetrosis and otosclerosis. Second, it could explain the late age of onset of the disease as the mechanism of autophagy is known to become less efficient at later ages (62). Furthermore, it could explain why increased loading of this protein degradation system, for example by environmental factors possibly including paramyxovirus infections, could result in the abnormal osteoclasts seen in individuals who, for genetic reasons, have a somewhat impaired protein degradation machinery.

Missing Links and Future Perspectives

The unraveling of the genetic factors contributing to the pathogenesis of PDB has definitely not come to an end, as illustrated, for instance, by the fact that several proven familial cases do not have a mutation in any of the currently-identified genes. An obvious way to proceed is to aim for gene identification within the chromosomal regions that were delineated by linkage studies. As mentioned, only one gene was identified but 7 loci were suggested. However, it is clear that the majority of these localizations will, in the end, turn out to be false positives for different reasons. Based on the available data, it is our feeling that, at a maximum, gene identifications can be hoped only for PDB4 (chromosome 5q31) (15) and PDB6 (chromosome 10p13) (63). Further insights can be generated by association studies. Thus far all reported studies have used a candidate gene approach analyzing the role of selected genes. However, much can be expected from hypothesis-free genome-wide association studies. This approach allows the identification of genes with, at present, no suggested role in the pathogenesis of PDB. These studies are ongoing and preliminary results (64) indicate that they definitely will lead somewhere.

There are several remaining questions. First, there is the fact that a constitutional mutation such as a *SQSTM1* mutation leads to a bone-specific phenotype despite the broad expression pattern of the *SQSTM1* gene. The explanation for this could be within the local environment needed for bone remodeling or possibly within the specific nature of the osteoclast as a very large and multinuclear cell type, as well as within the important role of the protein degradation and autophagy processes in these cells. Alternatively, it cannot be excluded that an interaction between SQSTM1 and a more cell- specific protein is essential for its functioning. A further remarkable observation is the focal aspect of the disease that affects only one or a few bones. For several other diseases this observation has been explained by the presence of a second somatic hit within the affected tissue. A recent study found evidence for a somatic *SQSTM1* mutation in two out of five PDB patients studied (65) but this finding still needs replication in other studies. Similarly, evidence has been obtained that somatic mutation can explain why a very small percentage of Pagetic lesions can develop into true malignancies,

in most cases osteosarcomas. Currently both *SQSTM1* somatic mutations (65) as well as somatic deletions of the chromosome 18 region close to the gene encoding RANK have been reported (66;67). Another intriguing observation is the decreasing prevalence and severity of the disease seen in several populations (68). Definitely this cannot be explained by changing genetic factors as the allelic frequency of genetic variants can, at a maximum, change only very slowly over many generations. Certainly, this observation provides further support for the role of environmental factors as triggers but the precise nature of these factors is still unclear.

Conclusion

Genetic research over the past several years has resulted undoubtedly in further insights into the pathogenesis of PDB but many questions remain unanswered. Both functional studies on genes already identified, as well as the hunt for unrevealed disease-causing genes, will be instrumental for obtaining a fuller understanding. The different transgenic animal models that are being developed in an attempt to mimic the disease will be important tools for such studies since the role of environmental factors in the pathogenesis of the disease can be tested.

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