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REVIEW

Emerging strategies and therapies for treatment of Paget's disease of bone

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Abstract: Paget's disease of bone (PDB) is a progressive monostotic or polyostotic metabolic bone disease characterized by focal abnormal bone remodeling, with increased bone resorption and excessive, disorganized, new bone formation. PDB rarely occurs before middle age, and it is the second most frequent metabolic bone disorder after osteoporosis, affecting up to 3% of adults over 55 years of age. One of the most striking and intriguing clinical features is the focal nature of the disorder, in that once the disease is established within a bone, there is only local spread within that bone and no systemic dissemination. Despite many years of intense research, the etiology of PDB has still to be conclusively determined. Based on a detailed review of genetic and viral factors incriminated in PDB, we propose a unifying hypothesis from which we can suggest emerging strategies and therapies. PDB results in weakened bone strength and abnormal bone architecture, leading to pain, deformity or, depending on the bone involved, fracture in the affected bone. The diagnostic assessment includes serum total alkaline phosphatase, total body bone scintigraphy, skull and enlarged view pelvis x-rays, and if needed, additional x-rays. The ideal therapeutic option would eliminate bone pain, normalize serum total alkaline phosphatase with prolonged remission, heal radiographic osteolytic lesions, restore normal lamellar bone, and prevent recurrence and complications. With the development of increasingly potent bisphosphonates, culminating in the introduction of a single intravenous infusion of zoledronic acid 5 mg, these goals of treatment are close to being achieved, together with long-term remission in almost all patients. Based on the recent pathophysiological findings, emerging strategies and therapies are reviewed: ie, pulse treatment with zoledronic acid; denosumab, a fully human monoclonal antibody directed against RANK ligand; tocilizumab, an interleukin-6 receptor inhibitor; odanacatib, a cathepsin K inhibitor; and proteasome and Dickkopf-1 inhibitors.

Keywords: Paget's disease of bone, bisphosphonates, sequestosome 1, p62, autophagy, pathogenesis, interleukin-6

Introduction

Paget's disease of bone (PDB) is a progressive monostotic or polyostotic metabolic bone disease characterized by focal abnormal bone remodeling, with increased bone resorption and excessive, disorganized new bone formation.[1] The disease is driven primarily by increased osteoclast activity, but intrinsic defects in other cell types in the bone microenvironment may contribute to disease onset and severity.[2] One of the most striking and intriguing clinical features is the focal nature of the disorder, in that once the disease is established within a bone, there is only local spread within that bone and no systemic dissemination.[3] Further supporting this focal nature, is the clinical observation of PDB transfer from one part of the skeleton to another as

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a result of autologous bone grafting after three years' latency.[4] While PDB is classically considered to be a focal disease, there is some evidence to suggest that patients have a mild generalized increase in bone turnover as measured by histomorphometry in nonpagetic sites.[5]

PDB affects both men and women, with a slight predominance in men.[1] PDB rarely occurs before middle age and its prevalence increases steadily with age. It is the second most frequent metabolic bone disorder after osteoporosis, affecting up to 3% of adults over 55 years of age,[6] with an unchanged prevalence (2.5%) over the last 1000 years,[7,8] although it appears to be declining over the last 50 years,[9] which is consistent with a major contribution of environmental triggers for PDB. PDB results in weakened bone strength and abnormal bone architecture, in which the collagen fibers assume a haphazard irregular mosaic pattern (woven bone) instead of the parallel symmetry observed in normal (lamellar) bone. PDB is often asymptomatic, but patients can present with pain, deformity or, depending on the bone involved, fracture in the affected bone.[10] Approximately 30%–50% of PDB patients experience disabilities due to bone pain, osteoarthritis secondary to deformities adjacent to weight-bearing joints, fractures, or nerve root compression.[11,12] Malignant transformation to osteosarcoma occurs in about 0.3% of patients.[11]

Despite many years of intense research, the etiology of PDB has still to be conclusively determined. A variety of evidence has implicated members of the Paramyxovirus family as causative agents;[13–18] UK researchers have previously shown molecular evidence of canine distemper virus in pagetic bone biopsies,[15–18] whereas groups in the US have predominantly identified measles virus.[19,20] Although controversial, these data may suggest a slow viral infection in pagetic bone.[21,22] Further supporting this viral hypothesis are the frequent associations between the development of PDB and contact with domesticated animals or residency in rural areas.[23–25] The high prevalence of PDB in Lancashire (England) and in New Zealand may be related to both environmental and genetic factors. The declining prevalence and severity of PDB in the British population also suggests that PDB is at least somewhat regulated by environmental factors,[26,27] although it may be partially due to the influx of migrants from low prevalence regions such as the Indian subcontinent and southeast Asia.[28] In contrast, the rural region of Campania (Italy) was recently reported to be a high prevalence area for PDB with an increased clinical severity.[29,30]

Diagnosis

PDB may present with obvious signs or symptoms or it may be an incidental finding during the investigation of other conditions.[10] In a recent study, PDB appears to be less severe, with 34% having a monostotic lesion, and an overall average of 5.5 lesions per patient.[31] The diagnosis of PDB is primarily radiological and confirmed with plain radiology of at least one skeletal site.[10] The radiological features include initial osteolytic changes (V-shaped lesions in long bones and osteoporosis circumscripta in the skull), followed by sclerotic changes, bone enlargement, and cortical thickening. Plain radiographs are also valuable in the diagnosis of secondary complications of PDB, eg, arthritis or fracture. Total body bone scintigraphy is more sensitive than x-rays and, it is recommended (where available) for patients with asymptomatic PDB and for patients with symptomatic PDB to assess the extent of skeletal involvement.[32] In contrast with focal assessment of disease by scintigraphy and radiography, biochemical markers of disease activity provide an integrated index, if not of the focal activity of the underlying disorder, then of its extent.[33,34] Measurement of serum total alkaline phosphatase is still the most frequently used and most useful biochemical marker for clinical management of PDB.[35] Serum bone alkaline phosphatase and procollagen type 1 N-terminal propeptide, as well as urinary N-terminal telopeptide of type 1 collagen and α-C-terminal telopeptide of type 1 collagen have been demonstrated to be similar[36] or slightly superior[37,38] to serum total alkaline phosphatase in assessing disease activity and response to therapy in small cohorts of patients. However, monostotic PDB may be associated with levels of serum total alkaline phosphatase within the reference range, introducing difficulties both in diagnosis and follow-up management of patients.[39] PDB patients with serum total alkaline phosphatase within the reference range may be discriminated from normal controls by an increased bone alkaline phosphatase isoform B2 measured by high-pressure liquid chromatography.[40] In contrast, serum osteocalcin is not a sensitive marker in PDB, being often in the normal range.[41,42]

In summary, the assessment of PDB includes serum total alkaline phosphatase, total body bone scintigraphy, skull and enlarged view pelvis x-rays (includes pelvis, 1/3 proximal femurs and L3 to L5 vertebrae), and if needed, additional x-rays. This clinical investigation is associated with very high diagnostic sensitivities for PDB, ie, 85%–91% for skull and enlarged view pelvis x-rays[43] and 97%–98% for bone scintigraphy.[44]

Pathogenesis

Genetic factors

Genetic factors play an important role in PDB.[45] One-third of patients with PDB have a familial form transmitted in an autosomal dominant pattern of inheritance with incomplete penetrance.[46–48] Genetic heterogeneity has been demonstrated in familial forms of PDB, which have been linked to several chromosomal regions.[49] A linkage between the *6p21.3* locus (*PDB1*) and PDB has been suggested, but not confirmed.[50] Four PDB families were linked to markers in the *18q22.1* locus (*PDB2*), a locus also involved in familial expansive osteolysis, a rare bone disease caused by a mutation in the tumor necrosis factor receptor superfamily, member 11a, NFKB activator (*RANK, TNFRSF11A*) gene.[51,52] However, *RANK* gene mutations are not a common cause of classical late-onset PDB,[53,54] although a genetic association to this gene was recently suggested in PDB patients.[55] The *5q35-qter* locus (*PDB3*) was identified by our research group in a genome-wide scan of three large French-Canadian families,[48] and replicated in British families.[56] Taking advantage of the influence of genetic drift and a strong founder effect of the French-Canadian population, we reported in this population the first and still most common germline mutation, *P392L*, within the *SQSTM1* gene,[57] and this was later confirmed in the British population.[58] The *5q31* locus (*PDB4*) was also linked to PDB in two French-Canadian families.[48] A genome-wide scan in 62 British families suggested the linkage of PDB with two other loci, *2q36* (*PDB5*) and *10p13* (*PDB6*).[56] Recently, data from this genome-wide scan were reanalyzed and confirmed a linkage to the *10p13* locus, but not to the *2q36* locus.[59] The *18q23* locus (*PDB7*) was suggested in an Australian family,[60] but this locus is more likely to contain a modifier gene rather than a causal gene because a *SQSTM1* mutation (*L394X*) was also found in this pedigree.[61] Although no linkage of the osteoprotegerin (*OPG, TNFRSF11B*) locus (*8q24*) was suggested with PDB, a British study reported a female sex-restricted association of this gene with PDB.[62,63] Mutations of the valosin-containing protein (*VCP*) gene, located at *9p13-p12*, were reported in rare families characterized by an autosomal dominant disorder associating PDB with frontotemporal dementia or inclusion body myopathy.[64] However, no mutations were found in pagetic patients in the absence of myopathy or dementia, suggesting that the *VCP* gene was not a common causal gene of PDB.[65] Finally, a recently published genome-wide association study in PDB patients, mostly of British descent, identified a significant association between PDB and six common variants, located at the *1p13* (*CSF1* gene) and *10p13* (*OPTN* gene) loci, and, as previously mentioned, at the *18q21* (*RANK* gene) locus.[55] These genetic associations have been strongly replicated in Belgian and Dutch populations, as well as the association of the dendritic cell-specific transmembrane protein (*DC-STAMP, TM7SF4*) gene, encoding for a protein involved in cell–cell fusion of osteoclasts.[66] Among the seven reported loci, the *5q35qter* (*PDB3*) locus is the only one for which a gene has been identified, namely the sequestosome 1 (*SQSTM1*) gene that encodes the SQSTM1/p62 protein.[57] More than 20 missense or truncating germline mutations of this gene have now been reported, although the *P392L* mutation is the most frequent.[67,68] In the French-Canadian population, the P392L recurrent mutation was involved in 46% of familial forms and 16% of unrelated cases of PDB.[57] Sequencing of the *SQSTM1* gene in unrelated French PDB patients allowed the identification of two novel mutations, *A381V* and *L413F*, and for the first time, the presence of double mutations of *SQSTM1* was reported in PDB.[69] In the American population, 10% of unrelated PDB patients living in the New York City area carried a *SQSTM1* mutation, most frequently the *P392L* mutation, but also the novel *S349T*, *A390V*, and *L417Q* mutations.[70] Almost all of the *SQSTM1* mutations are recurrent, and reported in different Caucasian populations on average in 40% of familial forms of PDB and 8% of unrelated patients.[61,67,69,71]

NF-κB signaling pathway

Interestingly, all of the reported *SQSTM1* germline mutations result in either missense or truncating mutations[67] enhancing the NF-κB signaling pathway. They are clustered either within or near the C-terminal region of the SQSTM1/p62 protein that embodies the ubiquitin-associated domain. This suggests that an alteration of ubiquitin-chain binding by SQSTM1/p62 is important in the development of PDB,[72,73] resulting in an aberrant RANK-NF-κB signaling pathway.[74] In osteoclasts, SQSTM1/p62 has been described as a scaffolding protein that interacts with TRAF6 following activation by the RANK ligand (Figure 1).[75] Activation of this complex results mainly in the activation of NF-κB and NFATc1 transcription factors. The overexpression of SQSTM1/p62 in osteoclasts from PDB patients induces major shifts in the pathways activated by the RANK ligand and upregulates osteoclast activity. The *P392L* mutation may contribute to the overactive state of osteoclasts in PDB,[76] and could potentially explain the generalized increase in bone turnover observed in nonpagetic bone sites.[5]

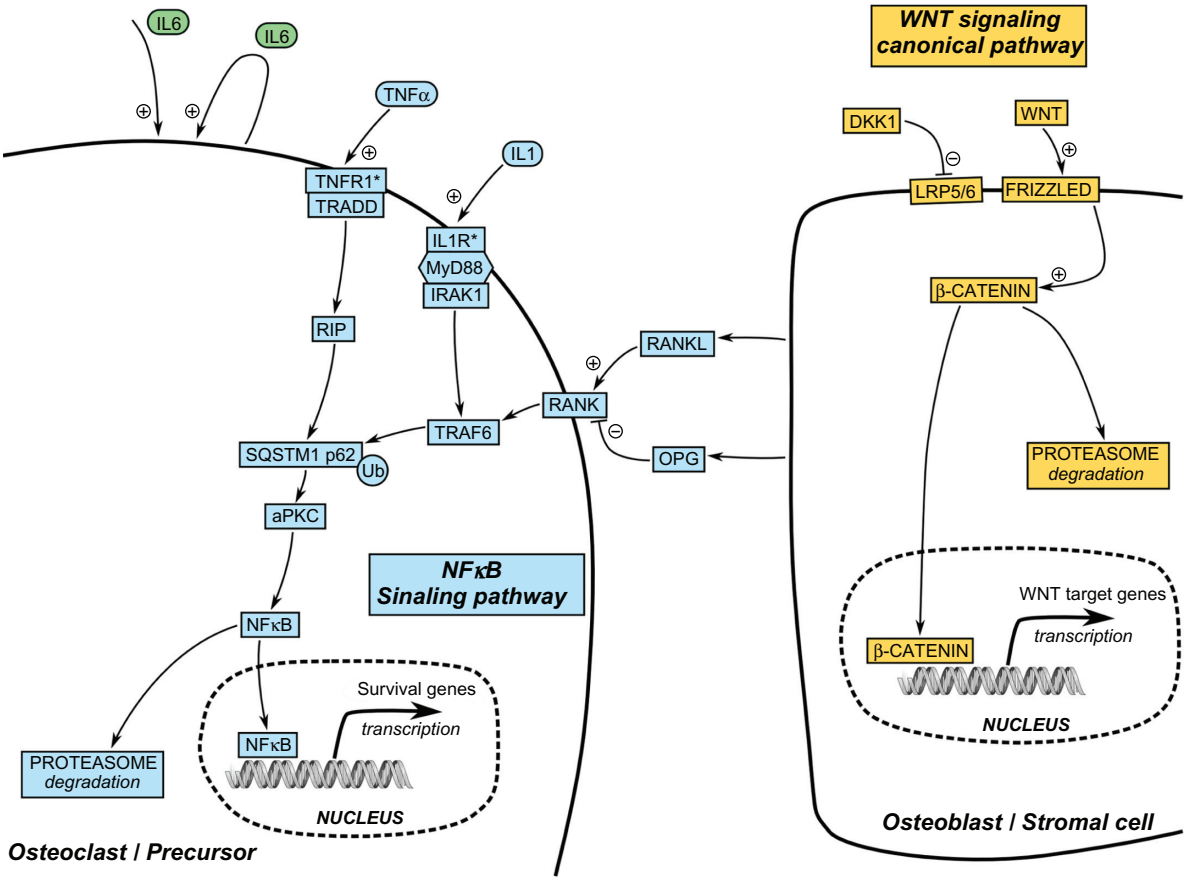

Figure 1 Most relevant pathways for the identification of potential novel therapeutic targets in Paget's disease of bone.

Ubiquitin-proteasome system, autophagy, and apoptosis

The ubiquitin-proteasome system is involved in the degradation of short-lived, damaged, or misfolded proteins. Target-to-be-degraded proteins are first tagged with ubiquitin then digested by the proteasome.[77,78] This system is important for protein degradation and controls various cell functions, including mitosis, signal transduction, gene transcription, immune response, and apoptosis.

Autophagy is another protein degradation system, and includes macroautophagy, microautophagy, and chaperon-mediated autophagy.[79,80] Macroautophagy (hereafter termed autophagy) involves the engulfing of a portion of cytoplasm by a double-membrane structure, the autophagosome. The autophagosome fuses with the lysosome, becoming the autolysosome, which undergoes autodigestion.[80,81] Autophagy maintains cellular homeostasis and participates in processes including differentiation, remodeling, growth control, cell defense, and adaptation to adverse environments,[82] and is involved in eliminating abnormal proteins.[83] Loss of autophagy in mice induces inclusion formation in neurons and hepatocytes.[84,85]

Ubiquitination, through binding of the ubiquitin-associated domain of the p62 protein (encoded by the SQSTM1 gene) to LC3 protein, mediates protein degradation by autophagy and also results in the delivery of p62 itself to autophagosomes for lysosomal degradation.[86–88] So far, only one PDB-associated germline missense mutation (D335E) has been shown to affect the LC3-binding region.[89] In PDB, autophagy appears to be defective, with impaired p62 clearance that leads to increased levels of p62 regardless of the SQSTM1 mutation status.[69,76] p62 not only functions as an adaptor protein that targets substrates to the autophagosome, but also as a scaffold protein interacting with TRAF6 and caspase 8, promoting polyubiquitination of TRAF6 and activation of NF-κB signaling,[90,91] as well as the aggregation of cullin-3 modified caspase 8, required for apoptosis, within p62-dependent foci[92] which leads to increases in osteoclast survival.[76] These increases in osteoclast survival can be induced by artificial overexpression of p62 and appear to be independent of SQSTM1 mutations because they are observed with wild-type and PDB-mutant p62.[76] Finally, it is interesting to note that osteoclasts from healthy carriers of germline SQSTM1/p62^{P392L} mutation show an intermediate

rate of apoptosis between affected individuals and healthy controls.[76] Exploring the precise nature of the potential link between autophagy and PDB has been judiciously proposed as a priority area because autophagy represents a cellular pathway that can be relatively easily manipulated in vivo by pharmacological agents.[93]

Viral factors

Canine distemper virus

Canine distemper virus can infect and replicate in human osteoclast precursors, raising possible zoonotic implications for canine distemper virus. Canine distemper virus transiently induces osteoclastogenesis in human osteoclast precursor cultures via NF-κB and SQSTM1/p62 activation.[94] A variety of other proteins have been shown to be upregulated in PDB, notably Bcl-2,[95] leading to an enhanced lifespan of pagetic osteoclasts. Hence, it is possible that the viral effects on ubiquitin and SQSTM1/p62 are only transient, but that the effects on other proteins, such as Bcl-2, lead to an enhanced lifespan of the enlarged osteoclast, with the subsequent recruitment of further precursor cells, thus increasing the size and bone resorbing capacity further.

Measles virus nucleocapsid protein

Osteoclasts in PDB are increased in number and size and express a "pagetic phenotype" that distinguishes them from normal osteoclasts. They contain up to 100 nuclei/osteoclast compared with 3–10 nuclei in normal osteoclasts, their precursors are hyperresponsive to the RANK ligand, tumor necrosis factor alpha, and $1,25(OH)_2$ vitamin D_3,[96–98] and form osteoclast at physiologic concentrations of $1,25(OH)_2$ vitamin D_3 (10^{-11} M) rather than the pharmacologic $1,25(OH)_2$ vitamin D_3 concentrations (10^{-8} M) required for normal osteoclast formation. The $1,25(OH)_2$ vitamin D_3 hyperresponsivity results from elevated levels of a novel vitamin D receptor coactivator, TAF12 (formerly TAF_{II}-17) in osteoclasts.[97] Furthermore, osteoclasts in PDB secrete high levels of interleukin (IL)-6, which are detectable in marrow plasma and peripheral blood from patients with Paget's disease.[99]

Both measles virus nucleocapsid (MVNP) and the SQSTM1/p62^{P392L} mutation have been implicated in the pathogenesis of PDB, but their relative contributions are not yet clearly defined. We recently reported that osteoclast from approximately 70% of PDB patients express MVNP, and that normal osteoclast precursors expressing MVNP formed osteoclasts that express the "pagetic phenotype".[100,101] Furthermore, 30% of transgenic mice with targeted expression of MVNP to osteoclasts developed osteoclast and bone lesions characteristic of PDB.[102]

At least 21 genetic mutations of the SQSTM1/p62 gene are linked to PDB, with p62^{P392L} mutation being the most frequent.[57,67,103] However, the role of p62^{P392L} in PDB is unclear because normal osteoclast precursors expressing p62^{P392L} are hyperresponsive to the RANK ligand but not to $1,25(OH)_2D_3$, do not express high levels of IL-6 or TAF12, or form bone lesions or osteoclasts characteristic of PDB.[104,105]

Therefore, to assess the roles of MVNP and p62^{P392L} in PDB, marrow from clinically involved and uninvolved bones of PDB patients with p62^{P392L} and normals was tested for MVNP, and the effects of antisense MVNP on the osteoclast formed were determined.[101] To delineate the mechanism(s) responsible for the abnormal osteoclast activity and bone formation seen with coexpression of MVNP and p62^{P392L}, p62^{P394L} knockin mice (the mouse equivalent of p62^{P392L}) were bred to transgenic mice expressing MVNP in osteoclasts producing the p62^{P394L} knockin/MVNP mice. These mice developed more pagetic osteoclast and pagetic bone lesions than transgenic mice expressing MVNP in osteoclasts.[101] The p62^{P392L} gene increased RANK ligand sensitivity of osteoclast precursors while MVNP was responsible for osteoclast hypermultinucleation, increased TAF-12 expression, and IL-6 production through enhanced p38MAPK signaling induced by $1,25(OH)_2D_3$.[101] Furthermore, when transgenic mice expressing MVNP in osteoclasts were bred to IL-6 knockout mice, pagetic osteoclast formation no longer occurred.[101]

In conclusion, studies in mice have demonstrated that the p62^{P392L} mutation leads to some of the phenotypic characteristics of PDB, but this single mutation is seemingly unable to result in the whole PDB phenotype. This mutation may predispose to PDB by increasing the sensitivity of osteoclastic precursors to osteoclastogenic cytokines[104] and/or the osteoclastogenic potential of the bone microenvironment,[105] probably in association with other biological mechanisms, such as the presence of MVNP, which is responsible for osteoclast hypermultinucleation, increased TAF-12 expression, and IL-6 production.[101,106]

SQSTM1/p62, selective autophagy, and measles virus persistence

A unifying hypothesis for SQSTM1/p62, selective autophagy, and measles virus persistence is shown in Figure 2. It has been recently suggested that successful clearance of viral proteins through p62-mediated selective autophagy may

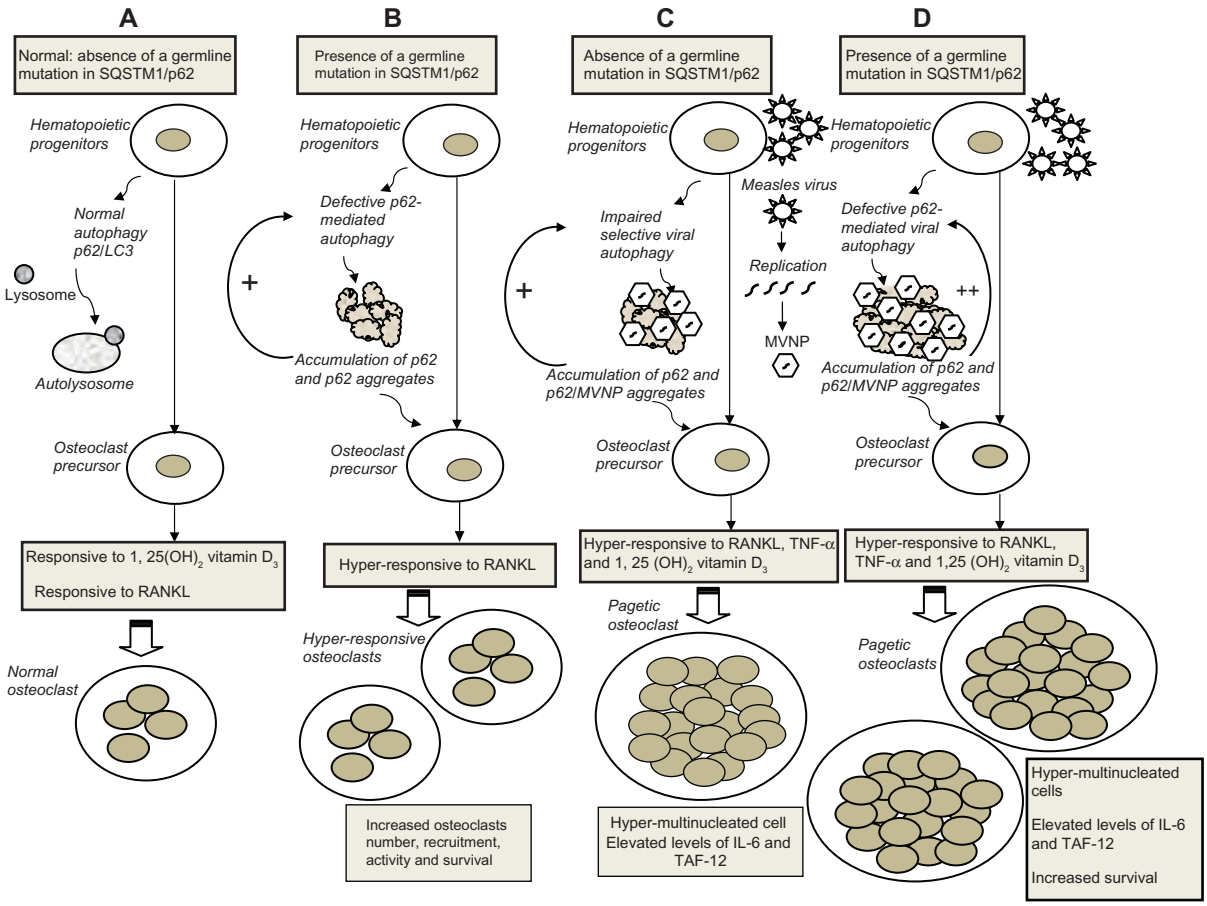

Figure 2 Pathogenesis of Paget's disease of bone: Viral and genetic interactions, unifying hypothesis. Schematic models of cytoplasmic autophagy in **A**) normal hematopoietic progenitors with adequate clearance of the autolysosome by the proteasome, **B**) hematopoietic progenitors carrying a germline SQSTM1/p62 mutation leading to defective p62-mediated autophagy, accumulation of p62, further amplifying the process, and p62 aggregates, **C**) hematopoietic progenitors with persistent measles virus infection and replication leading to impaired autophagy with accumulation of MVNP/p62 aggregates, **D**) persistent measles virus infection of hematopoietic progenitors carrying a germline SQSTM1/p62 mutation further amplifies the genetically-induced defective p62-mediated autophagy. **B**–**D**) These abnormalities in the autophagy process are perpetuated in cells differentiated from the hematopoietic cells with specific functional consequences on mature osteoclasts (see text).

represent an integral component of the normal host antiviral defense response.[79] Virus-induced autophagy usually requires viral replication[80] and is then followed by viral persistence. The measles virus is a monotypic virus existing as a single serotype and is among the most infectious viruses.[89] Measles virus infections predominantly occur in children, and infection or vaccination with any one strain appears to provide life-long protection from the disease.[89] It would be difficult to accept life-long persistence of measles virus RNA or protein in the absence of viral replication and low level gene expression.[86] Indeed, intracellular nonreplicating MVNP are inactivated within three days.[88] Osteoclasts have a short lifespan (2–3 weeks) and are not self-renewing cells, but are rather formed by fusion of postmitotic precursors of the monocyte-macrophage lineage.[90] Immature multipotential hematopoietic progenitors that give rise to granulocytes, erythrocytes, macrophages, and platelets, also express MVNP transcripts, while nonhematopoietic stromal cells do not.[87] These cells could easily be the reservoir for measles virus persistence in PDB, although direct evidence is lacking. Toll-like receptors 2 and 4 have been shown to increase susceptibility to measles virus infection,[91] suggesting a predisposing role of innate immunity.

We can then speculate that measles virus persistence could explain the latency between measles virus infection in childhood and PDB development in middle age.[86] Measles virus persistence would also explain the presence of MVNP in lifelong immature multipotential hematopoietic progenitors,[87] later differentiating into osteoclasts, and would be responsible for osteoclast hypermultinucleation, increased TAF-12 expression, $1,25(OH)_2$ vitamin D_3 responsivity, and IL-6 production.[101,106] Defective p62-mediated selective autophagy of MVNP, by germline SQSTM1/p62 mutation or other causes, would lead to accumulation of p62 itself as well as MVNP-p62 aggregates in osteoclasts and antigen-presenting cells, reducing their clearance by the proteasome.[79]

Mutant p62^{P392L},[76] or any other SQSTM1/p62 mutations associated with PDB and overexpression of native p62, are increasing osteoclast responsiveness to RANK ligand[76,104,105] and osteoclast survival,[76] which translates clinically into a more severe phenotypic expression of the disease.[67]

Treatment

Indications for therapy

Guidelines on clinical management of PDB have been published by expert committees from various countries.[10,32,107–109] Pain in pagetic bone is the only symptom of PDB for which there is firm evidence that therapy confers a clinical benefit,[110] and is, therefore, a definite indication for antipagetic therapy. It is important to distinguish bone pain that occurs as a result of pagetic activity (ie, pain in pagetic bone) from pain in a bone and/or joint deformity that occurs as a consequence of the disease (ie, osteoarthritic pain). The former is usually present at rest, whereas the latter occurs during mobilization of the joint and can, therefore, respond to analgesics, but not to antipagetic drugs.

Pharmacological treatment to prevent future complications, such as osteoarthritis, fracture, hearing loss, or other neurological complications, is more controversial.[111] In a 12-year study of 41 patients with PDB, osteoarthritic complications occurred in 62% of patients, in whom serum total alkaline phosphatase levels were halved after therapy compared with 33% of those who had normal serum total alkaline phosphatase following treatment.[112] Therefore, a reasonable strategy is to treat pain even when its cause is unclear, because it can often be difficult to distinguish between PDB pain and osteoarthritic pain.[10] Both symptomatic and asymptomatic patients with metabolically active PDB requiring therapy include those with involvement of long bones at risk of future bowing deformities, those with extensive skull involvement at risk for future hearing loss, those with pagetic changes in one or more vertebrae with the risk of various neurological complications, and those with PDB in bones adjacent to major joints with the risk of secondary arthritis.[32,107] Because current therapies improve radiographic osteolytic lesions[113] and allow normal lamellar bone deposition,[114] it is likely that associated complications could be prevented if treatment is administered at an early stage.[111,112,115] A recent three-year prospective study known as PRISM (Paget's Disease: a Randomized Trial of Intensive Versus Symptomatic Management)[116] in 1324 patients with long established PDB, concluded that intensive bisphosphonate therapy has no beneficial effect on quality of life, bone pain, or clinical complications (fracture and osteoarthritis) compared with symptomatic management. The negative findings from the PRISM trial could be explained by numerous limitations in the trial design.[115,117] Unevenly potent treatment was given late in the disease process, the primary endpoint was inadequate (ideally pain or alternatively fracture in pagetic bone should have been used rather than clinical fracture at any site), and the sample size was too small and the observational period too short to impact on the clinical management of PDB. For preventing complications associated with PDB, initiating pharmacological therapy at the right time (at an early disease stage) is clearly more important than using a highly potent bisphosphonate.

Contraindications to therapy

Elderly asymptomatic patients whose life span would likely limit the chance of future complications[111] and those with metabolically inactive pagetic lesions (no radiographic osteolytic lesions nor increased uptake on bone scintigraphy) are not candidates for pharmacological therapy. Patients with vitamin D deficiency should be repleted before therapy is initiated to prevent severe hypocalcemia.[118]

Goals of therapy

Physicians treating PDB should aim for a complete remission, as defined by normalization of serum total alkaline phosphatase and even a nadir value in the lower half of the reference range.[119] The ideal therapeutic option would eliminate bone pain, normalize serum total alkaline phosphatase with prolonged remission, heal radiographic osteolytic lesions, restore normal lamellar bone, and prevent recurrence and complications.[32]

Current pharmacological therapies

The first effective therapy was salmon calcitonin, available in the 1970s as daily subcutaneous formulations, followed by human calcitonin. Calcitonin acts directly on calcitonin receptors expressed on osteoclasts.[120] Because calcitonin was associated with partial response, acquired resistance, and a short-lived action, it is not used clinically any more.

Bisphosphonates are a class of drugs related to the naturally occurring mineralization inhibitor, inorganic pyrophosphate.[121] In biological systems, they are able to bind to the surface of hydroxyapatite crystals within bone, especially on those surfaces undergoing active osteoclastic resorption. Bisphosphonates work according to one of two main mechanisms of action, depending on the chemical nature of the side chain attached to the basic bisphosphonate core. The relatively weak, non-nitrogen, simple bisphosphonates

(ie, etidronate, clodronate, and tiludronate) inhibit bone resorption by generating a toxic analog of adenosine triphosphate, which then targets the mitochondria.[122] For the more potent, nitrogen-containing bisphosphonates (ie, alendronate, ibandronate, pamidronate, risedronate, and zoledronic acid), the direct intracellular target in osteoclasts is the farnesyl pyrophosphate synthase enzyme in the mevalonate pathway.[122] Its inhibition suppresses a process called protein prenylation, which is essential for the basic cellular processes required for osteoclastic bone resorption and cell survival.

Nitrogen-containing bisphosphonates are the treatment of choice for PDB. The advent of ever more powerful bisphosphonates has led to an aim for a more stringent definition of biochemical response to therapy, ie, a reduction of serum total alkaline phosphatase into the normal range and even a nadir value in the lower half of the reference range.[119]

Comparative trials have been published evaluating the relative efficacy of the bisphosphonates in the treatment of PDB. These trials typically use extent of suppression of serum total alkaline phosphatase and duration of remission as evidence of superior treatment. Table 1 provides a summary of the clinical trials assessing the efficacy of bisphosphonates in PDB, as measured by the proportion of patients achieving normalization of serum total alkaline phosphatase.[42,123–128] This table reports the results from independent studies without any attempt to compare efficacy between therapies that have not been compared in a head-to-head trial. Although of somewhat differing protocols, these trials demonstrate that alendronate and risedronate are superior to etidronate. In a small comparative study of previously untreated patients, oral alendronate (40 mg/day in three-month blocks) and intravenous pamidronate (60 mg every three months) were given until normalization of serum total alkaline phosphatase, which was observed at one year in 86% and 56% of patients, respectively.[129] In previously treated patients, alendronate resulted in normalization of serum total alkaline phosphatase in 79% compared with 14% for pamidronate.[129] At one year, nonresponders to pamidronate were crossed over to alendronate treatment, and 71% achieved normalization of serum total alkaline phosphatase.[129] In another comparative trial, normalization of serum total alkaline phosphatase was achieved at six months in 93% of patients treated with intravenous zoledronic acid 4 mg and in 35% of patients treated with intravenous pamidronate 60 mg every three months.[130] At six months, nonresponders to pamidronate were treated with intravenous zoledronic acid 4 mg or intravenous neridronate 100 mg, and normalization of serum total alkaline phosphatase was achieved in more than 90%.[130] A once-weekly alendronate 280 mg oral buffered solution was recently compared with an alendronate 40 mg/day tablet. While both were similarly effective (percentage of patients with serum total alkaline phosphatase normalization not provided), the 40 mg daily tablet was better tolerated.[131] Recent comparison of intravenous zoledronic acid 5 mg and oral risedronate 30 mg daily for 60 days in 357 patients after six months showed normalization of serum total alkaline phosphatase in 89% of zoledronic acid-treated patients and 58% of risedronate-treated patients.[128]

In the zoledronic acid group, mean scores for each of the eight components of the SF-36 trended upward at both three and six months, suggesting improvements in quality of life, whereas the responses were more mixed in the risedronate group.[128] Patients in remission at six months were followed for duration of response and, after two years,[132] zoledronic acid 5 mg extended remission in 98% of patients with one single dose, compared with 57% with risedronate, and at 5–6 years these figures were 87% and 38%, respectively.[133] Acquired resistance has been commonly observed with etidronate and pamidronate, but not with alendronate, risedronate, or zoledronic acid.[134] Upper gastrointestinal intolerance and abdominal pain have been reported as the most frequent adverse events associated with oral bisphosphonates.[135] Postinfusion syndrome (a flu-like illness) occurs in about 15% of patients treated with intravenous bisphosphonates (pamidronate, ibandronate, and zoledronic acid), and this almost exclusively at the first infusion.[119,128] Oral bisphosphonates should not be used in patients with esophageal stricture or dysmotility. All bisphosphonates should be avoided in patients with renal insufficiency and severe vitamin D deficiency. Osteonecrosis of the jaw and subtrochanteric fractures are very rare events and their pathophysiology remains unclear.[135] However, overall, only a very small proportion of patients treated with

Table 1 Summary of clinical trials assessing bisphosphonate efficacy in Paget's disease of bone as measured by the proportion of patients with normalization of serum total alkaline phosphatase

Drug name	Regimen	Duration	% n sTALP
Etidronate[123]	400 mg/day, oral	6 months	15
*Clodronate[124]	1600 mg/day, oral	6 months	60
*Tiludronate[125]	400 mg/day, oral	3 months	39
*Pamidronate[126]	60 mg/day, IV	3 days	53
*Alendronate[127]	40 mg/day, oral	6 months	63
Risedronate[123]	30 mg/day, oral	2 months	73
*Ibandronate[42]	6 mg/day, IV	2 days	70
Zoledronic acid[128]	5 mg, IV	One dose	89

Note: *Small sample size.
Abbreviations: sTALP, serum total alkaline phosphatase; IV, intravenously.

bisphosphonates experience adverse events and the overall benefits have consistently outweighed their potential risks.

Monitoring and retreatment

Serum total alkaline phosphatase is the most commonly used method of monitoring disease activity.[10] It should be measured every three months for the first six months after therapy and every six months thereafter.[10] Pretherapeutic serum total alkaline phosphatase is often within the normal range in monostotic disease, and it cannot be used for monitoring.[136] Bone scintigraphy (normal uptake) or plain radiographs (filling of osteolytic lesions[113]) performed six months after treatment would constitute the ideal monitoring. Retreatment is usually indicated when there are persistent symptoms of PDB or biochemical relapse.[10] Although there is no clinical trial evidence to guide clinicians, it is generally accepted that an increase of 25% above nadir indicates significant relapse requiring retreatment.[10]

Mechanisms of action of bisphosphonates in PDB

Recent in vitro studies suggest that pulse treatment with zoledronic acid, achieving micromolar concentrations (rather than the nanomolar concentrations usually observed in clinical use) similar to what is observed with a single intravenous infusion of 5 mg, causes inhibition of proliferation and induction of apoptosis in human mesenchymal stem cells and enhances differentiation through the osteoblastic lineage.[137] Emerging preclinical and clinical evidence suggests that, in addition to their selected inhibition of osteoclastic activity, bisphosphonates exert anticancer activity by interacting with monocytes, macrophages, and tumor cells, and by stimulating the expansion of γδ T cells, a subset of human T cells with antitumor activity.[138] Focal high bone turnover lesions like PDB or bone metastases do enrich bisphosphonates in the surrounding bone. Only under those circumstances may it be envisaged that bisphosphonate concentrations in the microenvironment exceed micromolar concentrations for a longer period of time and thus propagate apoptosis of pluripotential hematopoietic progenitors, leading to the long-term remissions observed in PDB after a single intravenous zoledronic acid infusion.[133]

Potential therapeutic targets

Although bisphosphonates are currently the treatment of choice for the management of PDB, uncertainty about the long-term health consequences of these drugs may now lead to consideration of potential alternative therapies, particularly targeted therapies already designed and used, or about to be used, in clinical practice for the management of other bone disorders.

RANK ligand inhibition

RANK ligand inhibition by the use of a fully human monoclonal antibody (denosumab) induces, in clinical trials, a profound but reversible inhibition of bone resorption. This targeted therapy may be considered for the treatment of OPG/RANK/RANK ligand pathway-mediated diseases, mainly postmenopausal osteoporosis, bone erosion in inflammatory arthritis, and cancer-induced bone disease.[139] In PDB, the OPG/RANK/RANK ligand system is usually normal, although enhanced RANK ligand expression and responsivity in bone marrow cells have been reported.[98] Moreover, the pathophysiology of several PDB-related diseases involves the OPG/RANK/RANK ligand system, such as mutation in the signal peptide region of the *RANK* gene in familial expansile osteolysis and a mutation in the *OPG* gene in juvenile Paget's disease.[140]

Interleukin-6 inhibition

Almost 20 years ago, osteotropic factors, such as $1,25(OH)_2$ vitamin D_3, parathyroid hormone, and IL-1, were shown to stimulate osteoblast release of IL-6 which, at low concentrations (<10 ng/mL), stimulates osteoclast formation from precursors, and at higher concentrations, stimulates mature osteoclasts to resorb bone.[141] IL-6 plays a central role in the development of the abnormal phenotype of osteoclast in PDB, mainly in the multinucleation and hypersensitivity to $1,25(OH)_2$ vitamin D3.[90] IL-6 was found to be overexpressed in pagetic osteoblasts, and may be involved in both stimulation of osteoclast proliferation and inhibition of osteoblast growth.[2] However, a recent study did not find any association of common polymorphisms in IL-6, IL-8, and tumor necrosis factor alpha genes with PDB.[142] Tocilizumab, an IL-6 receptor inhibitor, has recently been approved for the treatment of rheumatoid arthritis.[143] Although IL-6 plays a key role in causing joint damage in rheumatoid arthritis through possible indirect effects on osteoclastogenesis and bone resorption,[144] no clinical trials have been initiated to date in metabolic bone disorders associated with high levels of IL-6.

Dickkopf-1 inhibition

Dickkopf-1 is a natural secreted antagonist of the Wnt/β-catenin signaling interacting with the LRP5/6 coreceptor (Figure 1). Surprisingly, Dickkopf-1 RNA and protein levels are increased in pagetic osteoblast and stromal cells,[2]

giving new insights into the role of the osteoblast in PDB. A later independent study reported increased circulating Dickkopf-1 levels in serum from PDB patients,[145] and suggested Dickkopf-1 as a potential therapeutic target in PDB.[146] Indeed, high levels of Dickkopf-1 have also been reported in multiple myeloma, osteosarcoma, and rheumatoid arthritis, and Dickkopf-1 targeted therapy gave preliminary promising results in multiple myeloma and rheumatoid arthritis.[146]

Strategies for novel therapeutic target identification

Relevant strategies for the identification of novel therapeutic targets in PDB may rely mostly on the investigation of novel targets developed for the management of other bone disorders and of the results from genetic studies.

Investigation of novels targets developed in other bone disorders

Several metabolic disorders share common pathophysiological features with PDB, such as multiple myeloma, osteoporosis, rheumatoid arthritis-induced bone erosions, and bone metastases of cancer with high affinity for bone, such as prostate and breast cancers. In both PDB and bone metastases, increased osteoclast formation and the increased osteoclastogenic nature of the bone microenvironment are mediated by common factors, namely IL-6 and RANK ligand.[147] Available data suggest that, in the case of PDB, there is increased RANK ligand and IL-6 production, and IL-6 enhances responsivity of the osteoclast precursors to RANK ligand, contributing to the elevated numbers of osteoclasts. In patients with multiple myeloma, 95%–100% of whom develop osteolytic bone lesions, both IL-6 and RANK ligand levels are increased.[147] We will mainly focus the remaining discussion on therapeutic targets for multiple myeloma and osteoporosis

Bone destruction in multiple myeloma is associated with increased osteoclast formation and activity like in PDB, but with decreased or absent osteoblast differentiation and activity.[148] The impairment of osteoblast activity in multiple myeloma results primarily from blockade of osteogenic differentiation of mesenchymal progenitors into mature osteoblasts. Multiple myeloma patients have low to normal levels of bone formation markers, such as alkaline phosphatase and osteocalcin, in the setting of increased bone resorption. In contrast, multiple myeloma patients without bone lesions display balanced bone remodeling with increased osteoclastogenesis and normal or increased bone formation rates. Both soluble factors and cell-to-cell contact between multiple myeloma cells and osteoblast progenitors are responsible for the suppression of osteoblast differentiation in multiple myeloma. Current approaches for the development of target-specific treatment in multiple myeloma concern mainly second-generation proteasome inhibitors, new immunomodulating drugs or thalidomide derivatives, histone deacetylase, and heat shock protein 90 inhibitors.[149–151]

Other potential targets are represented by inhibitors of Akt and of PI3K/Akt signaling (rapamycin inhibitors), Bcl2 inhibitors and other promoters of apoptotic pathways, MAPK and telomerase inhibitors, to name a few.[151] Antibodies have also been designed in multiple myeloma to inhibit IL-6, CD56 (neuronal cell adhesion molecule), CD138 (syndecan-1, a receptor for endothelial growth factor ligands) and Cs1, a cell surface glycoprotein.[151] In addition to bisphosphonates, novel therapies are considered for the treatment of bone disease in multiple myeloma, such as denosumab, which specifically inhibits RANK ligand-RANK interaction, bortezomib which is a proteasome antagonist inducing myeloma cell apoptosis, and immunomodulating drugs, which inhibit osteoclast activity by decreasing the expression of cathepsin K.[152] Other inhibitors targeting natural antagonists of Wnt signaling, such as Dickkopf-1 and secreted frizzled-related proteins, have been targeted, as well as inhibitors of IL-3 and Il-7.[152]

Osteoporosis is characterized by a generalized increase in bone resorption, whereas PDB has both focal excesses of bone resorption and many unaffected bones that preserve normal bone remodeling. Both antiresorptive and anabolic agents have being designed as potential novel therapies in osteoporosis. In addition to denosumab, another antiresorptive agent called odanacatib, which is an inhibitor of cathepsin K, is currently being investigated in osteoporosis, as well as glucagon-like peptide 2, an intestinal hormone which may act as an antiresorptive agent with no reduction in bone formation.[153] Novel anabolic agents targeting the Wnt signaling pathway designed for future osteoporosis management should be considered with caution, and may probably be contraindicated in PDB, considering the increased risk of osteosarcoma in this disorder.

Results of genetic studies

Gene expression profiling in RNA extracted from various cell types in pagetic patients has revealed that a huge number of genes may be significantly upregulated or downregulated in PDB, providing novel insights for potential future targeted therapies (Table 2).[2,154,155] Considering difficulties of performing large-scale proteomic studies in bone cells,

Table 2 Genes which showed statistically significant differential gene expression in various cell types from patients affected by Paget's disease of bone

Gene symbol	Encoded protein	Human cell type	Reference
Downregulated genes			
ACP5	Acid phosphatase 5, tartrate resistant	Osteoclast*	154
CASP3	Caspase 3, apoptosis-related cysteine peptidase	Osteoclast*	154
CTSK	Cathepsin K	Osteoclast*	154
FLJ23191	Chromosome 4 open reading frame 31	Osteoblast	2
GCA	Grancalcin, EF-hand calcium binding protein	Osteoblast	2
GLRB	Glycine receptor, beta	Osteoblast	2
MAFB	V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)	Osteoblast	2
MAPT	Microtubule-associated protein tau	Osteoclast*	154
SATB2	SATB homeobox 2	Osteoblast	2
TNFα	Tumor necrosis factor	Monocytes Lymphocytes	155
TNFRSF10A	Tumor necrosis factor receptor superfamily, member 10a	Osteoclast*	154
TNFRSF11A	Tumor necrosis factor receptor superfamily, member 11a, NFKB activator	Osteoclast*	154
Upregulated genes			
EPB41L4B	Erythrocyte membrane protein band 4.1 like 4B	Osteoblast	2
GULP1	GULP, engulfment adaptor PTB domain containing 1	Osteoblast	2
IFNα	Interferon, alpha 1	Monocytes Lymphocytes	155
IFNβ	Interferon, beta 1, fibroblast	Monocytes Lymphocytes	155
IFNγ	Interferon, gamma	Monocytes Lymphocytes	155
IFNγR1	Interferon gamma receptor 1	Monocytes Lymphocytes	155
IFNγR2	Interferon gamma receptor 2 (interferon gamma transducer 1)	Monocytes Lymphocytes	155
KRT18	Keratin 18	Osteoblast	2
P38 β2 MAPK	Mitogen-activated protein kinase 14	Monocytes	155
RAI3	G protein-coupled receptor, family C, group 5, member A	Osteoblast	2
RBPMS	RNA binding protein with multiple splicing	Osteoblast	2
STAT1	Signal transducer and activator of transcription 1, 91 kDa	Monocytes	155
STAT2	Signal transducer and activator of transcription 2, 113 kDa	Lymphocytes	155
TNXB	Tenascin XB	Osteoblast	2

Note: *Peripheral blood monocytes differentiated in vitro into mature osteoclasts.

genome-wide analyses, such as the genome-wide association study recently published in PDB,[55] or genome-wide investigations of copy number alterations or epigenetic modifications, may be considered as innovative and promising ways to identify novel targets or novel pathways for potential future therapies in PDB. Indeed, the recently published genome-wide association study reported a strong genetic association with three common polymorphisms located upstream of the CSF1 gene.[55] CSF1 gene encodes macrophage colony-stimulating factor, which is a key cytokine secreted by bone marrow stromal cells and osteoblasts, which induces the expression of RANK in osteoclast precursors, further inducing osteoclast differentiation and osteoclast activity and survival regulation.[156] Serum levels of macrophage colony-stimulating factor were reported to be significantly increased in PDB patients who were not currently treated, suggesting that serum measurement of macrophage colony-stimulating factor may be an indicator of disease activity.[157] Although CS1 antibody, antisense oligonucleotide, and CSF1 small interfering RNA strategies have demonstrated tumor suppression capabilities in several disease (excluding PDB) and model systems,[158,159] it is not yet clear enough how specific is their intervention on osteoclast formation, in bone disorders such as PDB, because other cell lineages derived from hematopoetic precursors use similar signaling pathways.[160]

Conclusion

In conclusion, nitrogen-containing bisphosphonates are currently the treatment of choice for PDB, particularly with the last generation and more powerful bisphosphonates, which have led us to aim for a more stringent definition of biochemical response to therapy. Major advances in the understanding of PDB pathophysiology in recent years could give rise to novel alternative treatment, such as targeted therapies, as a medium-term perspective for the management of PDB and other bone metabolic disorders.

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