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Paget's disease—A VDR coactivator disease?☆

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Abstract

Paget's disease is the most exaggerated example of bone remodeling with increased osteoclastic bone resorption followed by excessive bone formation. One of the earliest findings in our studies of Paget's disease is that pagetic osteoclast (OCL) precursors are hyper-responsive to 1,25-$(OH)_2D_3$ and form OCL at concentrations of 1,25-$(OH)_2D_3$ that are physiologic rather than pharmacologic. The increased responsivity to 1,25-$(OH)_2D_3$ is not due to increased levels of the Vitamin D receptor (VDR) or to increased infinity of 1,25-$(OH)_2D_3$ for VDR. We have recently shown using GST-VDR chimeric protein pull-down assays that TAF_{II}-17, a member of the TAF_{II}-D transcription complex, is increased in OCL precursors from patients with Paget's disease compared to normals. We further showed that TAF_{II}-17 can enhance VDR mediated gene transcription and allow formation of the transcription complex at very low levels of 1,25-$(OH)_2D_3$. In addition, coactivators of VDR including CPB300 and DRIP205 are also increased in OCL precursors from Paget's patients. These data suggest that the enhanced sensitivity of OCL precursors for 1,25-$(OH)_2D_3$ in Paget's disease results from increased expression of coactivators of VDR and suggest that part of the pathophysiology underlying OCL formation in Paget's disease may result from enhanced expression of VDR coactivators.

Keywords: Paget's disease; Vitamin D receptor; Osteoclasts; Coactivator

1. Introduction

Paget's disease is a chronic highly localized bone disorder that was originally described by Sir James Paget over 100-years-ago [1]. Bone lesions in Paget's disease are characterized by markedly increased osteoclast (OCL) formation and activity. Although large numbers of osteoblasts are also present near areas of resorbed bone, and bone matrix in Paget's disease is highly abnormal due to the rapid formation of bone that is of poor quality, it is abundantly clear that the OCL is the primary cell involved in the pathogenesis of Paget's disease. OCL are increased in number and in size in Paget's disease [2]. These cells contain up to 100 nuclei per OCL in contrast to normal OCL, which contain between 3 and 10 nuclei. A striking feature of OCL from Paget's patients is the characteristic nuclear inclusions, which consists of paracrystalline arrays that are similar to nucleocapsids of paramyxoviruses (Fig. 1) [3].

These nuclear inclusions are not present in other bone marrow cells or in nonpagetic bone in these patients. One report has suggested that there is budding-off of an infectious virus from pagetic OCL [4], suggesting a viral etiology for Paget's disease. However, the basis for the increased OCL formation in Paget's disease has not been clearly defined, and a strong genetic component is also involved [5].We have previously reported that pagetic OCL contain measles virus (MV) transcripts [6] and demonstrated that OCL formed of normal human OCL precursors transfected with the measles virus nucleocapsid gene (MVNP) have many of the features of pagetic OCL [7]. We have used this model to dissect the pathophysiology of Paget's disease and compared it to OCL formed in cultures of marrow from involved bones of patients with Paget's disease.

2. Paget's OCL precursors are hyper-responsive to 1,25-$(OH)_2D_3$

One of the earliest findings in our studies of OCL formation in Paget's disease is that OCL precursors from Paget's patients appear to be hyper-responsive to 1,25-$(OH)_2D_3$

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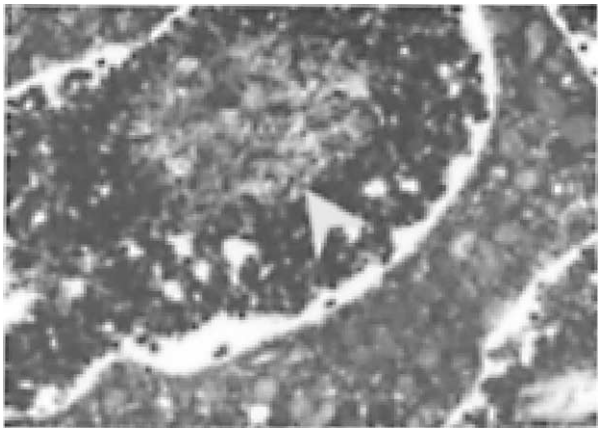

Fig. 1. Nuclear inclusions in OCL in Paget's disease.

and can form OCL-like cells in culture at concentrations of 1,25-$(OH)_2D_3$ that are at least 1–2 logs less than those required for normal marrow cultures [8]. Our laboratory reported, using highly purified populations of early OCL precursors from Paget's patients, that this high responsivity to 1,25-$(OH)_2D_3$ appears to be an intrinsic property of early OCL precursors, the granulocyte-macrophage colony-forming unit (CFU-GM)-derived cells [9]. However, the mechanism responsible for the enhanced 1,25-$(OH)_2D_3$ responsivity of these early precursors is still unknown, but could include for example, increased Vitamin D receptor (VDR) numbers, a mutated VDR, increased expression of a coactivator of VDR or decreased expression of a corepressor of VDR. Our group has previously reported that the enhanced sensitivity of pagetic OCL precursors was not due to increased VDR numbers [10]. It is unlikely that Paget's patients have a mutated VDR that has an intrinsically increased affinity for 1,25-$(OH)_2D_3$, since Paget's disease has been associated with at least four different chromosomal loci, none of which coincide with the chromosomal location of VDR, and all patients appear to have OCL precursors that are hyper-responsive to 1,25-$(OH)_2D_3$.

3. VDR mediated gene transcription and OCL formation

To determine the potential role of VDR mediated gene transcription in Paget's disease, we examined the capacity of bone marrow cells from VDR −/− mice to form OCL when transfected with MVNP gene or empty vector (EV). OCL formation was significantly decreased in bone marrow cultures from VDR −/− mice treated with RANK ligand (RANKL) and M-CSF, which induce normal OCL formation, compared to the marrow cultures from VDR +/+ mice. Furthermore, the OCL that formed in bone marrow cultures of VDR −/− mouse transfected with the measles virus nucleocapsid (MVNP) gene were small and did not express the characteristics of pagetic OCL. In contrast MVNP transfection of VDR +/+ mouse cells treated with RANKL/M-CSF formed large numbers of OCL that were hyper-responsive to 1,25-$(OH)_2D_3$ and had many of the features of pagetic OCL. As expected VDR −/− marrow cells did not form OCL in response to 1,25-$(OH)_2D_3$. These preliminary studies suggest that VDR mediated gene transcription is important for normal levels of OCL formation, and that VDR mediated gene transcription is required for expression of a pagetic phenotype in OCL.

4. Determination of the basis for increased 1,25-$(OH)_2D_3$ responsivity of OCL precursors from patients with Paget's disease

To confirm that the increased responsivity to 1,25-$(OH)_2D_3$ was mediated through VDR, our laboratory examined 24-hydroxylase (24-OHase) mRNA expression in response to 1,25-$(OH)_2D_3$ in bone marrow cells from involved bones of Paget's patients and MVNP transduced CFU-GM. As shown in Fig. 2, 24-OHase mRNA expression was increased in Paget's marrow cells and MVNP transduced normal CFU-GM derived cells at 1,25-$(OH)_2D_3$ concentrations that were 1–2 logs lower than required for normal CFU-GM to form OCL.

We then wanted to determine if the enhanced sensitivity of MVNP-transduced cells to 1,25-$(OH)_2D_3$ simply reflected an increased sensitivity of all steroid responsive genes to their ligand or was relatively specific for VDR. Therefore, a luciferase reporter vector containing a DR-3 (VDR) or DR-5 (retinoic acid receptor, RAR) response element was inserted into EV or MVNP transduced early OCL precursors, CFU-GM. The CFU-GM cells transduced with the MVNP cDNA were hyper-responsive to 1,25-$(OH)_2D_3$ and showed increased luciferase activity at concentrations of 1,25-$(OH)_2D_3$ that were 1 log less than

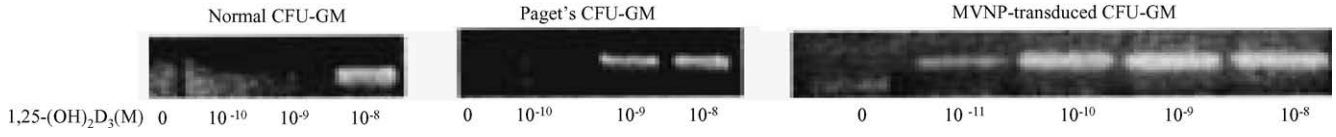

Fig. 2. 24-OHase mRNA expression in response to 1,25-$(OH)_2D_3$. Total RNA was extracted from each type of transduced cell treated with 10^{-11} to 10^{-8} M of 1,25-$(OH)_2D_3$ or media alone for 3 days and subjected to reverse transcription using random primers. The first-strand cDNAs were analyzed by PCR for 24-OHase mRNA expression with the specific primers. PCR was performed for 30 cycles. PCR products were separated by electrophoresis on 2% agarose gels and visualized by ethidium bromide staining with ultraviolet light illumination. Similar results were detected in two independent experiments.

that required for EV-transduced CFU-GM cells. Using a modified Hill equation to compare the dose response curves, we found that the concentration of 1,25-$(OH)_2D_3$ that induced a 50% of maximal response (C 50%) for the 1,25-$(OH)_2D_3$ dose response curve (0–10^{-8} M) for MVNP transduced cells, differed significantly from EV-transduced cells (0.47×10^{-9} M versus 2.0×10^{-9} M, $P < 0.05$). In contrast, transduction of the DR-5 reporter construct into CFU-GM cells transduced with the MVNP or EV construct demonstrated that although basal transcription was increased in CFU-GM cells transduced with the MVNP gene, the C50% of the retinoic acid dose-response curve (0–10^{-8} M retinoic acid) for MVNP-transduced cells did not differ significantly from that of the EV-transduced cells ($(0.7 \pm 1.25) \times 10^{-9}$ M versus $(1.5 \pm 0.72) \times 10^{-9}$ M, respectively).

5. A GST-VDR chimeric protein binds a 17-kDa protein—TAF_{II}-17

We then utilized a GST-VDR chimeric protein with lysates from MVNP transduced normal OCL precursors (CFU-GM derived cells) and marrow cells from involved bones from Paget's patients to try to isolate a potential coactivator of VDR. A 17-kDa peptide was detected that bound VDR but was not expressed in EV-transduced cells. Furthermore, NIH-3T3 cells transduced with the MVNP construct also expressed a similar 17-kDa VDR binding protein. Importantly, bone marrow cells derived from patients with Paget's disease also expressed this peptide. This peptide was not expressed in normal OCL precursors.

To establish the identity of the protein, microsequence analysis of the 17-kDa band was performed. The 17-kDa band in Paget's patients, MVNP transduced CFU-GM and NIH-3T3 cells was identical to TAF_{II}-17, which is a component of the TFIID transcription complex [11,12]. The sequence of the peptide is similar to that of histone H2B, and the peptide interacts with the TATA box binding protein and coactivators. It contains LXXLL motifs, which bind coactivators.

To determine if TAF_{II}-17 mRNA was expressed in cells that were hypersensitive to 1,25-$(OH)_2D_3$, RT-PCR for TAF_{II}-17 mRNA was performed. OCL precursors transduced with the MVNP gene expressed TAF_{II}-17 in the presence of 1,25-$(OH)_2D_3$, and marrow cells from involved sites of Paget's patients also expressed TAF_{II}-17 in the presence or absence of added 1,25-$(OH)_2D_3$. In contrast, very weak expression of TAF_{II}-17 was detected in normal cells.

To examine the interaction between VDR and TAF_{II}-17, NIH-3T3 cells were transfected with a TAF_{II}-17 construct (antisense or sense orientation) and DR-3 reporter constructs were inserted. NIH-3T3 cells transfected with TAF_{II}-17 demonstrated increased responsivity to 1,25-$(OH)_2D_3$ at concentrations of 1,25-$(OH)_2D_3$ that were 1 log less than that required for cells containing the antisense TAF_{II}-17 construct. In contrast, NIH-3T3 cells transfected with TAF_{II}-17 and a DR-5 reporter construct showed that the shape of the retinoic acid dose response curve was similar although basal transcription was increased. These data suggest that increased expression of TAF_{II}-17 allows VDR mediated gene expression to occur at lower concentration of 1,25-$(OH)_2D_3$.

As stated above, TAF_{II}-17 is upregulated in cells that contain the MVNP gene including OCL precursors from patients with Paget's disease. This increased responsivity to 1,25-$(OH)_2D_3$ appears to be relatively specific, since there is no increased responsivity to other steroid hormones such as retinoic acid or thyroid hormone in cells transfected with either the MVNP gene or the TAF_{II}-17 gene. The increased responsivity to 1,25-$(OH)_2D_3$ appears to result from increased expression of a VDR coactivator possibility TAF_{II}-17. Increased concentration of TAF_{II}-17 allow TAF_{II}-17 to associate with VDR at low concentrations of 1,25-$(OH)_2D_3$, permitting VDR mediated gene transcription to occur. This does not occur in EV transfected cells.

6. Measles virus (MV) infection of OCL precursors induces TAF_{II}-17

To further determine if MV infection increased the responsivity of OCL precursors to 1,25-$(OH)_2D_3$ and resulted in upregulation of TAF_{II}-17, a cellular MV receptor, human CD46, was targeted to cells in the OCL lineage in transgenic mice using the mouse tartrate-resistance acid phosphatase (TRAP) gene promoter. Targeting hCD46 to murine cells allows MV infection. The MV infected cells were hyper-responsive to 1,25-$(OH)_2D_3$ and expressed TAF_{II}-17. OCL formed by MV infected marrow cells from TRAP-CD46 mice shared characteristics of pagetic OCLs [13].

7. TAF_{II}-17 anti-sense oligonucleotide (AS-ODN) reduces OCL formation but does not change the 1,25-$(OH)_2D_3$ hyper-responsivity of pagetic OCL precursors

The effects of the TAF_{II}-17 AS-ODN on OCL formation by Paget's OCL precursors and MVNP-transduced CFU-GM were then investigated. Treatment of Paget's OCL precursors with TAF_{II}-17-antisense ODN markedly decreased the protein expression levels of TAF_{II}-17 compared with untreated OCL precursors. In contrast, the mismatched TAF_{II}-17 anti-sense oligonucleotide treatment did not alter the protein levels of TAF_{II}-17. When Paget's patient derived or MVNP transduced CFU-GM were cultured for 14 days in the presence or absence of 3 μM TAF_{II}-17 AS-ODN or MS-ODN and treated with 10^{-11} to 10^{-7} M 1,25-$(OH)_2D_3$, OCL formation was inhibited from $37 \pm 6\%$ (Paget's pa-

tients) to 64 ± 4% (MVNP transduced cells). Interestingly, treatment with the TAF_{II}-17 AS-ODN did not change the hyper-responsivity of the OCL precursors to 1,25-$(OH)_2D_3$. In contrast, 3 μM of TAF_{II}-17 AS-ODN did not block OCL formation in cultures of normal or EV-transduced CFU-GM. Importantly, TAF_{II}-17 AS-ODN did not affect OCL formation induced by IL-6 in Paget's patients and normal donors. The data suggest that other cofactors in addition to TAF_{II}-17 are mediating the hyper-responsivity of OCL precursor to 1,25-$(OH)_2D_3$.

8. Additional VDR coactivators are also increased in MVNP transduced OCL precursors

To determine if known coactivators of VDR, such as DRIP205, SRC1, or CBP300 were also increased in MVNP transduced cells or pagetic cells, RT-PCR analysis and GST-VDR pull down experiments were performed with lysates from MVNP or EV transfected CFU-GM to detect high molecular weight bands. Expression of both TAF_{II}-17 and DRIP205 mRNA and protein were increased in MVNP transduced cells treated with 10^{-8} M 1,25-$(OH)_2D_3$. These data suggest that another coactivator(s) is also increased in pagetic cells. Furthermore, the data suggest that high levels of TAF_{II}-17 allow formation of the VDR transcription complex at low concentrations of 1,25-$(OH)_2D_3$. Other coactivators such as DRIP205, and CBP300 are also increased in cells expressing the MVNP gene, and may be responsible for the hyper-responsivity of these cells to 1,25-$(OH)_2D_3$.

9. Serum concentrations of 1,25-$(OH)_2D_3$ in Paget's patients

To determine if the increased responsivity of pagetic OCL precursors to 1,25-$(OH)_2D_3$ altered Vitamin D metabolism, the profile of Vitamin D metabolites, calcium (Ca), and inorganic phosphate (Pi) in the sera of 9 patients with Paget's disease and 10 age-matched normal healthy volunteers was determined. The concentrations of Vitamin D metabolites, 25-OH-D_3, 24,25-$(OH)_2D_3$ and 1,25-$(OH)_2D_3$, Ca and Pi in sera of patients with Paget's disease were almost identical to those of age-matched normal healthy volunteers. No abnormality in Vitamin D metabolism was detected in patients with Paget's disease.

The concentrations of 1,25-$(OH)_2D_3$ in sera of patients with Paget's disease were 41.0 ± 9.1 pg/ml serum (10^{-10} M), similar to that of age-matched normal healthy volunteers. These data suggest that there are adequate levels of 1,25-$(OH)_2D_3$ in Paget's patients, and that these levels may be sufficient to induce OCL formation in the pagetic lesions.

10. Conclusion

The results presented here suggest that pagetic OCL precursors are hyper-responsive to 1,25-$(OH)_2D_3$ and can form OCL at physiologic concentrations (10^{-11} M) 1,25-$(OH)_2D_3$. The potential mechanism responsible for the increased 1,25-$(OH)_2D_3$ responsivity is shown in Fig. 3. In this model, increased levels of TAF_{II}-17 and other coac-

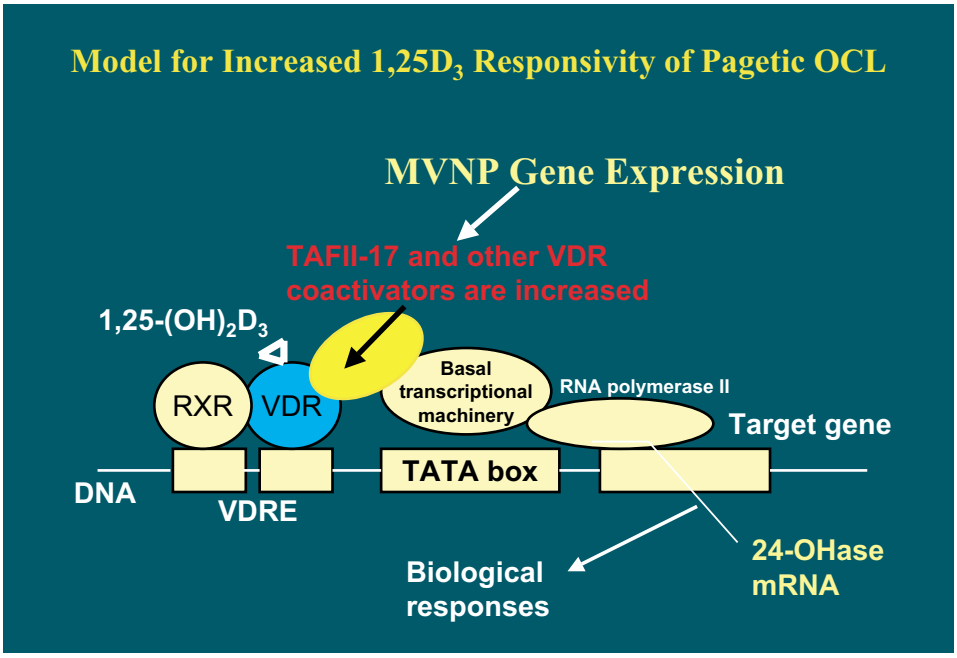

Fig. 3. Increased levels of TAF_{II}-17 and other coactivators such as DRIP205 and CBP300 are induced by expression of the MVNP gene in pagetic OCL precursors. The high levels of TAF_{II}-17 permit formation of VDR transcription complexes at low levels of receptor occupancy by 1,25-$(OH)_2D_3$, which is then enhanced by increased levels of DRIP205 and CBP300.

tivators such as DRIP205 and CBP300 are induced by expression of the MVNP gene in pagetic OCL precursors. The high levels of TAF_{II}-17 permit formation of VDR transcription complexes at low levels of receptor occupancy by 1,25-$(OH)_2D_3$. VDR mediated transcription is then enhanced by increased levels of DRIP205 and CBP300. These results support the hypothesis that part of the pathophysiology underlying the increased OCL activity in Paget's disease is due to increased levels of VDR coactivators that enhance VDR-mediated transcription at low levels of 1,25-$(OH)_2D_3$. However, we cannot rule out that corepressors of VDR transcription may also be decreased in pagetic cells since we have not studied these systematically. The data presented here suggest that Paget's disease may be a VDR coactivator disease, and that VDR antagonists may be able to inhibit pagetic OCL formation while not affecting normal OCL formation in patients with Paget's disease.

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