Measles virus nucleocapsid protein modulates the Signal Regulatory Protein-β1 (SIRPβ1) to enhance osteoclast differentiation in Paget’s disease of bone☆

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1. Introduction

Paget’s disease of bone (PDB) is a chronic localized bone disorder in an elderly population. Environmental factors such as paramyxovirus are implicated in PDB and measles virus nucleocapsid protein (MVNP) has been shown to induce pagetic osteoclasts (OCLs). However, the molecular mechanisms underlying MVNP stimulation of OCL differentiation in the PDB are unclear. We therefore determined the MVNP regulated gene expression during OCL differentiation. Agilent microarray analysis of gene expression identified high levels of SIRPβ1 (353-fold) expression in MVNP transduced human bone marrow mononuclear cells stimulated with RANKL. Real-time PCR analysis further confirmed that MVNP alone upregulates SIRPβ1 mRNA expression in these cells. Also, bone marrow mononuclear cells derived from patients with PDB showed high levels of SIRPβ1 mRNA expression compared to normal subjects. We further show that MVNP increases SIRPβ1 interaction with DAP12 adaptor protein in the presence and absence of RANKL stimulation. shRNA knockdown of SIRPβ1 expression in normal human bone marrow monocytes decreased the levels of MVNP enhanced p-Syk and c-Fos expression. In addition, SIRPβ1 knockdown significantly decreased MVNP stimulated dendritic cell-specific transmembrane protein (DC-STAMP) and connective tissue growth factor (CTGF) mRNA expression during OCL differentiation. Furthermore, we demonstrated the contribution of SIRPβ1 in MVNP induced OCL formation and bone resorption. Thus, our results suggest that MVNP modulation of SIRPβ1 provides new insights into the molecular mechanisms which control high bone turnover in PDB.

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ABSTRACT

Paget’s disease of bone (PDB) is a chronic localized bone disorder in an elderly population. Environmental factors such as paramyxovirus are implicated in PDB and measles virus nucleocapsid protein (MVNP) has been shown to induce pagetic osteoclasts (OCLs). However, the molecular mechanisms underlying MVNP stimulation of OCL differentiation in the PDB are unclear. We therefore determined the MVNP regulated gene expression during OCL differentiation. Agilent microarray analysis of gene expression identified high levels of SIRPβ1 (353-fold) expression in MVNP transduced human bone marrow mononuclear cells stimulated with RANKL. Real-time PCR analysis further confirmed that MVNP alone upregulates SIRPβ1 mRNA expression in these cells. Also, bone marrow mononuclear cells derived from patients with PDB showed high levels of SIRPβ1 mRNA expression compared to normal subjects. We further show that MVNP increases SIRPβ1 interaction with DAP12 adaptor protein in the presence and absence of RANKL stimulation. shRNA knockdown of SIRPβ1 expression in normal human bone marrow monocytes decreased the levels of MVNP enhanced p-Syk and c-Fos expression. In addition, SIRPβ1 knockdown significantly decreased MVNP stimulated dendritic cell-specific transmembrane protein (DC-STAMP) and connective tissue growth factor (CTGF) mRNA expression during OCL differentiation. Furthermore, we demonstrated the contribution of SIRPβ1 in MVNP induced OCL formation and bone resorption. Thus, our results suggest that MVNP modulation of SIRPβ1 provides new insights into the molecular mechanisms which control high bone turnover in PDB.

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1. Introduction

Paget’s disease of bone (PDB) is a chronic focal skeletal disorder that affects 2 to 3% of the population over the age of 55 years. The primary pathologic abnormality in PDB resides in bone resorbing osteoclast (OCL) cells. Patients with PDB have an incidence of developing osteosarcoma in an affected bone (Hamdy, 1995). PDB is an autosomal dominant trait with genetic heterogeneity and incomplete penetrance (Leach et al., 2001). Mutations in gene encoding p62 (sequestosome 1/SQSTM1) have been identified in 5 to 10% of total patients with PDB (Daroszewska et al., 2011). Paramyxoviral-nuclear inclusions have been identified in 20–40% of OCL in these patients (Reddy, 2004; Mills and Singer, 1976). Immunohistochemical studies have confirmed the presence of measles virus (MV) and respiratory syncytial virus (RSV) nucleocapsids in pagetic OCLs (Mills et al., 1984; Reddy, 2016). Furthermore, measles virus nucleocapsid (MVNP) transcripts have been detected in >80% of bone marrow samples from patients with PDB (Friedrichs et al., 2002). Also, MVNP expression has been detected in pagetic osteosarcomas (Merchant et al., 2009). These findings implicated environmental factors such as MV play an important role in pathogenesis of PDB. Previously, canine distemper virus (CDV) nucleocapsid transcripts were also detected in pagetic bone samples (Mee et al., 1998). However, others have been unable to detect the expression of paramyxoviral transcripts in PDB (Helfrich et al., 2000; Matthews et al., 2008). Despite of viral etiology, no infectious virus is isolated from these patients.

Receptor activator of NF-κB ligand (RANKL) and RANK signaling is critical for OCL differentiation and function. RANKL interaction with RANK results in recruitment of TNF receptor-associated factor (TRAF) proteins and activation of NF-κB, c-Fos, c-Jun N-terminal kinase (JNK) during OCL differentiation (Boyle et al., 2003). Also, RANKL signaling induces nuclear factor of activated T cells, cytoplasmic 1 (NFATc1) which regulates OCL gene expression (Takayanagi, 2007). RANKL costimulatory signaling with immunoreceptor tyrosine-based activation motif
from Santa Cruz Biotechnology (Santa Cruz, CA). and peroxidase-conjugated secondary antibodies were purchased previously we demonstrated that MVNP expression in preosteoclast cells induces pagetic OCL differentiation (Kurihara et al., 2000). Previously, we have shown that MVNP stimulates the expression of ASK1, Rac1, c-Fos, p-JNK, and NFATc1 during OCL differentiation (Shanmugarajan et al., 2008). However, the molecular mechanisms that regulate pagetic OCL formation and bone resorption activity in the PDB are unclear.

Signal regulatory proteins (SIRPs) are transmembrane glycoproteins that are expressed in hematopoietic cells including macrophages, monocytes, granulocytes and dendritic cells (Kharkitonov et al., 1997). SIRPs are characterized by containing three Ig like domains with different transmembrane and cytoplasmic domains (Oldenberg et al., 2000). Signal regulatory proteins classified into SIRPα and SIRPβ based on cytoplasmic domains. SIRPα has been shown to inhibit OCL differentiation and bone resorption (van Beek et al., 2009). SIRPβ1 (CD172b) is a transmembrane protein, which contains three Ig like domains in the extracellular region and a short cytoplasmic domain (Seiffert et al., 2001). SIRPβ1 contains lysine residue within the hydrophilic transmembrane domain which interacts with DAP12. Previously, Zou et al. (Zou et al., 2010) have reported that DAP12 knock-out mice show defective in osteoclastogenesis and cytoskeleton organization.

SIRPα1 signaling has been shown to activate the MAP kinase pathway which regulates various cellular functions (Tomasetto et al., 2000; Dietrich et al., 2000). However, the MVNP regulation of SIRPβ1 and a functional role in OCL differentiation in the PDB is unknown. In this study, we showed that MVNP modulates SIRPβ1 which play an important role in the enhanced OCL differentiation and bone resorption activity associated with PDB.

2. Materials and methods
2.1. Reagents and antibodies

Cell culture and DNA transfection reagent, lipofectamine were purchased from Invitrogen Inc. (Carlsbad, CA). Normal primary human bone marrow mononuclear cells were purchased from ATCC (Manassas, VA) and peripheral blood monocytes (PBMC) were obtained from Stem Cell Technologies Inc., (Vancouver, BC). RANKL and M-CSF were obtained from R&D systems Inc. (Minneapolis, MN). Anti-c-Fos, anti-SIRPα1 and peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-DAP12, Syk and p-Syk antibodies were obtained from the Cell Signaling (Danvers, MA). SuperSignal enhanced chemiluminescence (ECL) reagent was purchased from Amersham (Bioscience, Piscataway, NJ), and nitrocellulose membranes were from Millipore (Bedford, MA).

2.2. Retroviral expression of MVNP

We have previously developed a retroviral plasmid construct, pLTRXAN#1 that transcribes MVNP mRNA expression under the control of a 5′LTR viral promoter. The recombinant retroviral plasmid construct was transiently transfected into the PT67 packaging cell line using lipofectamine. The stable cell lines were established by selecting for resistance to neomycin (600 μg/ml). Similarly, a control retrovirus producer cell line was established by transfecting cells with the pLXS empty vector (EV). Normal human bone marrow derived mononuclear cells and PBMC were transduced with EV or MVNP retroviral supernatants (20%) from the producer cell lines in the presence of polybrene (4 μg/ml) for 24 h at 37 °C in a 5% CO₂ incubator as described earlier (Kurihara et al., 2000).

2.3. Lentiviral expression of SIRPβ1 shRNA

A human GIPZ lentiviral SIRPβ1 shRNAmir (Thermo Scientific Open Biosystem, Huntsville, AL) recombinant plasmid construct was transfected into the 293T amphotropic packaging cell line using lipofectamine. Stable clonal cell line producing SIRPβ1 shRNAmir recombinant lentivirus at high titer (1 × 10⁹ virus particles/ml) was established by selecting for resistance to puromycin (1.5 μg/ml). Similarly, a control lentivirus producer cell line was established by transfecting the cells with the GIPZ lentiviral empty vector (EV). The producer cell line was maintained in DMEM containing 10% FBS, 100 U/ml each of streptomycin and penicillin, 4 mM L-glutamine and high glucose (4.5 g/L). Lentiviral transduction into the human bone marrow derived mononuclear cells and PBMC were performed as described above.

2.4. Microarray analysis

Human bone marrow derived mononuclear cells were transduced with EV, MVNP and stimulated with M-CSF (10 ng/ml) and RANKL (100 ng/ml) for 48 h. Total RNA was isolated using RNAzol reagent (Biocex Labs, Houston, TX) and subjected to Agilent whole genome 4 × 44 K array analysis of ~26,000 genes. Agilent feature extraction and GeneSpring GX v7.3.1 software packages (Genus Biosystem, Inc. Northbrook, IL) were employed to analyze the data. Gene Ontology Tree Machine (GOTM) is a web-based platform used for interpreting gene expression profile of microarray data (Zhang et al., 2004).

2.5. Western blot analysis

Normal bone marrow derived mononuclear cells were transduced with MVNP in the presence and absence of SIRPβ1 shRNA and stimulated with M-CSF (10 ng/ml) and RANKL (100 ng/ml) for indicated time point. Total cell lysates were prepared in a lysis buffer (20 mM Tris-HCL, pH 7.4, 1% Triton X-100, 1 mM EDTA, 1.5 mM MgCl₂; 10% glycerol, 150 mM NaCl, 0.1 mM Na₃VO₄) containing 1 × protease inhibitor cocktail. The protein content of the samples was measured using the BCA protein assay reagent (Pierce, Rockford, IL). Protein (30 μg) samples were then subjected to SDS–PAGE (4–15% Tris–HCl gradient gels) and blot transferred onto a PVDF membrane, immunoblotted with anti-c-Fos, anti-Syk, anti-phospho-Syk (p-Syk) and anti-i-β-actin antibodies. The bands were detected using the ECL and the intensity was quantified by densitometry using the NIH ImageJ Program.

2.6. Co-Immunoprecipitation assay

Human bone marrow mononuclear cells were transduced with EV or MVNP in the presence and absence of M-CSF (10 ng/ml) and RANKL (100 ng/ml) for 48 h. Total cell lysates collected in a lysis buffer (50 mM HEPES (pH 7.5), 250 mM NaCl, 10 μM NaF, 0.2 mM EDTA and 0.5% Nonidet P-40) and were subjected to immunoprecipitation using anti-DAP12 antibody or control IgG overnight at 4 °C. The immune complexes were captured upon incubation with 100 μl of protein A agarose beads (Sigma, St. Louis, MO) for 2 h at 4 °C and subjected to western blot analysis using anti-SIRPβ1 antibody as described earlier (Sundaram et al., 2011).

2.7. Real-time RT-PCR analysis of mRNA expression

Bone marrow mononuclear cells were derived from patients with PDB and normal. All human samples were obtained following the IRB approved protocol at the Medical University of South Carolina. Briefly, 15 ml of bone marrow sample was mixed equal volume of α-MEM and layered over 15 ml of Ficoll-Paque (Sigma, MO) and centrifuged (1500 × g, 30 min) at room temperature. The cell layer on top of the Ficoll-Paque was collected and washed with α-MEM. The resulting mononuclear cells were used for further studies. Cells were transduced
with EV or MVNP with and without SIRPβ1 shRNA and stimulated with M-CSF (10 ng/ml) and RANKL (100 ng/ml) for 48 h. Total RNA was isolated from these cells using RNAzol. The reverse transcription reaction was performed using a iScript cDNA synthesis kit (Bio-Rad, Hercules, CA) at 25 °C for 5 min, 42 °C for 30 min followed by 85 °C for 5 min. The quantitative real-time PCR was performed using IQ™ SYBR Green Supermix in an iCycler and the primers specific for SIRPβ1 sense 5′-GCA GGC TTC AAA TAC TGC TC-3′ and antisense 5′-GAC AGA GAC ACC AAC CAC CA-3′; DC-STAMP sense 5′-TCC TCA TCT TGG GAC GAC T-3′ and antisense 5′-TGG AAA ATG TTA GGA AAA GCT C-3′; CTGF sense 5′-CAG CAG AAA GGT TAG TAT CAT A-3′ and antisense 5′-CCT AGC TGT CAC TGG GGC TA-3′; and GAPDH sense 5′-CCT ACC CCC AAT GTA TCC GTT GTG-3′ and antisense 5′-GGA ATG GGA GTT GCT GTT GAA-3′. Thermal cycling parameters were 94 °C for 3 min, followed by 40 cycles of amplifications at 94 °C for 30 s, 60 °C for 1 min, 72 °C for 1 min, and 72 °C for 5 min as the final elongation step as described earlier (Sundaram et al., 2015). The mRNA expression was normalized with respect to GAPDH amplification.

2.8. Osteoclast differentiation and bone resorption activity assay

Normal human Peripheral blood mononuclear cells (PBMC) were isolated as described earlier (Shanmugarajan et al., 2008). PBMC (6 × 10⁵) was plated in 96-well plates per well in 0.2 ml alpha-MEM medium and supplemented with hRANKL (100 ng/ml), hM-CSF (10 ng/ml) and 1 μM dexamethasone. At the end of the culture period (10 days) the cells were fixed with 2% glutaraldehyde in PBS and stained for tartrate resistant acid phosphate (TRAP) activity using a histochemical kit (Sigma, St Louis, MO). TRAP positive multinucleated cells (MNC) containing three or more nuclei were scored as OCL under a microscope. Bone resorption activity of the OCLs was assayed by culturing cells for 10 days on sterile dentine slices as described (Shanmugarajan et al., 2008). The cells on dentine slices were removed using 1 M NaOH and stained with 0.1% toluidine blue. The bone resorption area was quantified using computerized image analysis (Adobe Photoshop and Scion Microlmaging version 4.2). The percentage of the resorbed area was calculated relative to the total dentine disc area.

2.9. Statistical analysis

Data obtained from three independent experiments is presented as mean ± SD and were compared by Student’s t-test. Values were considered significant at p < 0.05.

3. Results

3.1. MVNP upregulation of SIRPβ1 expression

MVNP has been shown to play an important role in pathogenesis of PDB (Kurihara et al., 2006). However, the molecular mechanism underlying MVNP induction of pagetic OCLs is unclear. Therefore, to determine the MVNP modulated gene expression profile during OCL differentiation, we transduced retroviral expression of MVNP in normal human bone marrow derived mononuclear cells and stimulated with M-CSF (10 ng/ml) and RANKL (100 ng/ml) for 48 h. Total RNA isolated from these cells was subjected to Agilent microarray analysis as described in Methods. Gene cluster analysis and scatter plot of MVNP regulated gene expression profiling demonstrated 8.4% of genes were upregulated (>4-fold) in MVNP transduced cells compared to EV (Fig. 1). The data were deposited in the Gene Expression Omnibus (GEO) database repository at NCBI (Accession # GSE29106). We thus identified MVNP transduced cells show a very high level of SIRPβ1 (353-fold) expression in these cells. To further validate the MVNP regulated SIRPβ1 expression, total RNA isolated from normal human bone marrow derived mononuclear cells transduced with MVNP or EV was stimulated with M-CSF and RANKL for 48 h was subjected to real-time-RT-PCR analysis. As shown in Fig. 2A, MVNP increased (35-fold) the SIRPβ1 mRNA expression compared to EV transduced cells. We also confirmed SIRPβ1 mRNA expression was elevated (130 fold) in bone marrow mononuclear cells obtained from patients with PDB (n = 4) compared to normal subjects (Fig. 2B). Taken together, our results indicate that MVNP induce SIRPβ1 expression which may have an important functional role in pathogenesis of PDB.

3.2. SIRPβ1 signaling in preosteoclast cells

SIRPβ1 is a transmembrane glycoprotein associated with an adaptor molecule DAP12 in hematopoietic cells (Kharenkonov et al., 1997). Therefore, to examine MVNP modulates SIRPβ1 interaction with DAP12 in preosteoclast cells, we transduced MVNP retroviral expression plasmid into the human bone marrow derived mononuclear cells and stimulated with M-CSF (10 ng/ml) and RANKL (100 ng/ml) for 48 h. Total cell lysates obtained from these cells were subjected to immunoprecipitation analysis using anti-DAP12 antibody. The immunoprecipitant was subjected to western blot analysis for SIRPβ1 expression. As shown in Fig. 3A, MVNP significantly increased SIRPβ1 interaction with DAP12 with and without RANKL stimulation in these cells. Spleen tyrosine kinase (Syk) is an adaptor molecule for DAP12 (Koga et al., 2004). Therefore, we further
examined the SIRPβ1 participation in the activation of Syk in preosteoclast cells. We transduced MVNP with and without a SIRPβ1 shRNA into human bone marrow mononuclear cells and stimulated with RANKL (100 ng/ml) for 24 h. Total cell lysates obtained from these cells were subjected to western blot analysis for p-syk and syk. We identified that MVNP increased p-syk (4.2-fold) compared to EV transduced cells. Further, shRNA suppression of SIRPβ1 significantly decreased MVNP elevated p-syk level in these cells. In addition, shRNA knockdown of SIRPβ1 inhibits MVNP stimulated c-Fos expression in these cells (Fig. 3B).

DC-STAMP, a cell fusion protein plays an important role in the formation of multinucleated OCLs (Yagi et al., 2005). Recently, it has been shown that connective tissue growth factor (CTGF) interaction with DC-STAMP promotes osteoclastogenesis (Nishida et al., 2011). Therefore, we next examined the effect of SIRPβ1 on DC-STAMP and CTGF expression in preosteoclast cells. We transduced MVNP with and without SIRPβ1 shRNA expression plasmids into bone marrow mononuclear cells and stimulated with MCSF (10 ng/ml) and RANKL (100 ng/ml) for 48 h. Total RNA isolated from these cells was subjected to real-time RT-PCR analysis for DC-STAMP and CTGF mRNA expression. As shown in Fig. 4B, MVNP increased DC-STAMP (3.0-fold) and CTGF (5.0-fold) mRNA expression compared to EV transduced cells. In addition, SIRPβ1 knockdown significantly decreased MVNP stimulated DC-STAMP and CTGF mRNA expression in these cells. These results suggest that MVNP modulation of SIRPβ1 signaling may play an important role in enhanced OCL differentiation in PDB.

3.3. SIRPβ1 participation in MVNP enhanced osteoclast differentiation and bone resorption

Since MVNP regulated SIRPβ1 signaling molecules, we further determined the participation of SIRPβ1 in MVNP induced OCL formation and bone resorption activity. PBMC transduced with EV or MVNP and SIRPβ1 shRNA were cultured in the presence of M-CSF (10 ng/ml) and RANKL (100 ng/ml) for 10 days. Inconsistent with the previous findings, MVNP markedly increased the size and number of OCL formation (Fig. 4A). Interestingly, SIRPβ1 knockdown inhibits MVNP enhanced OCL formation. We next examined the potential of SIRPβ1 in OCL bone resorption activity. SIRPβ1 suppression in MVNP transduced cells demonstrated a significant inhibition of resorption lacunae on dentine slices compared to MVNP alone transduced cultures (Fig. 5B). Thus, our results suggest that SIRPβ1 contributes to MVNP escalated OCL differentiation/resorption activity in PDB.

4. Discussion

Several costimulatory factors associated with immune signaling have been shown to play an essential role in OCL differentiation (Koga et al., 2004). In this study, microarray analysis demonstrated the pathological significance of MVNP through induction of SIRPβ1 signaling molecule expression in preosteoclast cells. Our findings are consistent that bone marrow monocytes derived from patients with PDB also showed elevated levels of SIRPβ1 expression. These results suggested that MVNP modulation of SIRPβ1 may have a functional significance in PDB. MVNP induced IL-6 in association with widely occurred p62 mutation (P392L) has been shown to contribute to the pathogenesis of PDB suggested that interaction of both viral and genetic factors may play a role in disease severity (Kurihara et al., 2011; Singer, 2011). Geno-
wide association studies on PDB have further identified OPTN, CSF1 and TNFRSF11A gene loci (Albagha et al., 2010). Therefore, genetic abnormalities associated with PDB might influence SIRPβ1 to modulate OCL activity and disease susceptibility. It has been reported that murine macrophage cells persistently infected with RSV showed the altered gene expression profile of cytokines, transmembrane proteins and cell survival (Rivera-Toledo and Gomez, 2012). Though the paramyxoviruses could alter cellular gene expression profile, a functional role for MVNP modulation of SIRPβ1 signaling is evident through enhancing the interaction with DAP12 which play an important role in OCL differentiation. Previously, it has also been shown that SIRPβ1 interaction with DAP12 is involved in cellular activation (Dietrich et al., 2000). Further, Syk kinase activation is associated with multiple biological functions, including OCL bone resorption (Mocsai et al., 2004; Zou et al., 2007). This study demonstrated that SIRPβ1 mediates MVNP induced Syk activation during OCL differentiation.

The c-Fos is essential for OCL differentiation and expressed at high levels in pagetic OCLs (Beedles et al., 1999). It has also been reported that Fos related protein Fra-2 deficiency results in giant OCL formation in mice and that leukemia inhibitory factor (LIF) signaling is critical in this process (Bozec et al., 2008). We previously demonstrated that MVNP stimulate c-Fos expression during OCL differentiation (Shanmugarajan et al., 2008). This study further show that SIRPβ1 knockdown suppress MVNP induced c-Fos expression suggesting a functional role for SIRPβ1 in MVNP induced pagetic OCL development. It has been shown that both MVNP and mutant p62 expression results in OCL precursors hyper-sensitive to RANKL and TNF-α. In addition, MVNP also sensitizes the cells to vitamin D3 (Galson and Roodman, 2017).
2014). We show that SIRPβ1 participates in MVNP induced c-Fos expression as down-stream effector of RANK signaling. Therefore, these results suggest that SIRPβ1 may as well contribute to the hyper-responsivity of OCL precursors to RANK and vitamin D3. CTGF expressed in preosteoclast cells has been shown to induce and interact with DC-STAMP to promote osteoclastogenesis (Nishida et al., 2011). Therefore, our findings that SIRPβ1 silencing inhibit both CTGF and DC-STAMP expression further attests a functional role for SIRPβ1 in MVNP enhanced OCL differentiation in PDB. Preosteoclast fusion is described as a heterogeneous process. Molecules such as OC-STAMP and syncytin-1 retroviral fusion protein have also been implicated in osteoclast precursor fusion (Hobolt-Pedersen et al., 2014; Wittwicka et al., 2015). Therefore, it is possible that MVNP expression or SIRPβ1 signaling may regulate other molecules involved in OCL precursor’s fusion process. Interestingly, SIRPβ1 knockdown only affected the MVNP enhanced OCL formation, size and expression of fusion molecules DC-STAMP and CTGF. However, there was no significant change in the empty vector transduced control cells. This suggests that SIRPβ1 may not be necessary for basal osteoclastogenesis in vitro because there may be other co-receptors available for DAP12. These findings further suggest that upregulation of SIRPβ1 might play a role in other pathologies with inflammatory enhancement of osteoclasts formation.

In contrast, MVNP expression has been shown to decrease FoxO3/Sirt1 signaling to enhance the levels of IL-6 production in pagetic OCLs contributing to the pathogenesis of PDB (Wang et al., 2013). Also, TBK1 (TANK-binding kinase 1) has been shown to mediate the effects of MVNP to induce IL-6 expression and pagetic OCL differentiation (Sun et al., 2014). More recently, it has been demonstrated that MVNP to induce IL-6 expression and pagetic OCL differentiation from patients with Paget’s disease. J. Bone Miner. Res. 17 (1), 145–151. Galson, D.L., Roodman, G.D. 2014. Pathobiology of Paget’s disease of bone. J. Bone Metab. 21 (2), 85–98.

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