Mutant p62P392L Stimulation of Osteoclast Differentiation in Paget’s Disease of Bone

Kumaran Sundaram, Srinivasan Shanmugarajan, D. Sudhaker Rao, and Sakamuri V. Reddy

Charles P. Darby Children’s Research Institute (K.S., S.S., S.V.R.), Medical University of South Carolina, Charleston, South Carolina 29425; and Henry Ford Hospital (D.S.R.), Detroit, Michigan 48202

Paget’s disease of the bone (PDB) is an autosomal dominant trait with genetic heterogeneity, characterized by abnormal osteoclastogenesis. Sequestosome 1 (p62) is a scaffold protein that plays an important role in receptor activator of nuclear factor \( \kappa B \) (RANK) signaling essential for osteoclast (OCL) differentiation. p62P392L mutation in the ubiquitin-associated (UBA) domain is widely associated with PDB; however, the mechanisms by which p62P392L stimulate OCL differentiation in PDB are not completely understood. Deubiquitinating enzyme cylindromatosis (CYLD) has been shown to negatively regulate RANK ligand-RANK signaling essential for OCL differentiation. Here, we report that CYLD binds with the p62 wild-type (p62WT), non-UBA mutant (p62A381V) but not with the UBA mutant (p62P392L) in OCL progenitor cells. Also, p62P392L induces expression of c-Fos (2.8-fold) and nuclear factor of activated T cells c1 (6.0-fold) transcription factors critical for OCL differentiation. Furthermore, p62P392L expression results in accumulation of polyubiquitinated TNF receptor-associated factor (TRAF)6 and elevated levels of phospho-I\( \kappa \)B during OCL differentiation. Retroviral transduction of p62P392L/CYLD short hairpin RNA significantly increased TRAP positive multinucleated OCL formation/bone resorption activity in mouse bone marrow cultures. Thus, the p62P392L mutation abolished CYLD interaction and enhanced OCL development/bone resorption activity in PDB. (Endocrinology 152: 4180–4189, 2011)
p62 has been shown to physically associate with TNF receptor-associated factor (TRAF)6 and involved in receptor-activator of nuclear factor κB (NF-κB) (RANKL) signaling critical for OCL differentiation (13). p62 is a scaffold protein that mediates RANK ligand (RANKL) signaling by activating transcription factors such as NF-κB and activator protein 1 (AP-1), which induces the nuclear factor of activated T cells (NFATc1) transcription factor expression essential for osteoclastogenesis (14–16). p62 knockout mice have impaired osteoclastogenesis in response to parathyroid hormone-related peptide (13). It has been shown that p62 mutant (p62P392L) transgenic mice have increased OCL formation but do not develop focal osteolytic lesions with the characteristics of PDB (17). In contrast, recently, p62 UBA domain mutation (p62P394L) has been shown to be sufficient to cause a Paget’s disease-like disorder in mice (18). Also, truncation of the p62 UBA domain causes aberrant RANK signaling and increased osteoclastogenesis in RAW 264.7 cell cultures (19). Recently, p62P392L mutation has been shown to alter RANKL signaling and induces activation of human OCL (20). However, the molecular mechanisms by which p62P392L stimulates OCL differentiation in PDB are not completely understood.

Pridgeon et al. (21), using an in vitro expression cloning approach, identified several proteins that interact with the p62 UBA domain. The tumor suppressor cylindromatosis (CYLD) gene was first identified in human affected with familial CYLD, a genetic syndrome in which numerous benign tumors of skin develop, principally on the head and neck region. The disease is inherited in an autosomal manner and is caused by germline mutations in the CYLD gene on chromosome 16q12-q13, which predicts truncation or absence of the encoded protein (22). CYLD protein has been shown to physically interact with p62, and it negatively regulates osteoclastogenesis (23). CYLD is a deubiquitinating enzyme that removes the ubiquitin chain from several proteins, particularly TRAF2, TRAF6, and NF-κB essential modulator, and inactivates NF-κB signaling (24–26). The deubiquitination activity of CYLD is highly specific for proteins at lysine-63 (K63)-linked ubiquitin chains in substrate but has been shown to act on K48-linked polyubiquitin chains (27). Polyubiquitination of target protein at K63 influences protein-protein interactions, which play an important role in cell signaling, and ubiquitination at K48 directs to proteasomal degradation via ubiquitin-proteosomal pathway (28). Here, we report that the p62 UBA mutant (p62P392L) abolished the interaction with CYLD, which implicates a potential role in enhanced OCL development in PDB.

Materials and Methods

Reagents and antibodies

Cell culture and DNA transfection reagents were purchased from Invitrogen Corp. (Carlsbad, CA). RANKL and macrophage colony-stimulating factor (M-CSF) were obtained from R&D Systems, Inc. (Minneapolis, MN). Rabbit-anti-CYLD antibody was purchased from Abcam (Cambridge, MA). Rabbit-anti-NFATc1, anti-c-Fos, anti-hemagglutinin (HA) tag, and peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). pNFAT, pNFκB, and pAP-1-Luc cis-reporter plasmids were obtained from Stratagene (La Jolla, CA). SuperSignal enhanced chemiluminescence reagent was obtained from Amersham Bioscience (Piscataway, NJ), and nitrocellulose membranes were purchased from Millipore (Bedford, MA). A luciferase reporter assay system was obtained from Promega (Madison, WI).

p62 mutagenesis

A plasmid (pcDNA3-HA-p62) containing the full-length human p62 cDNA (>85% homologous to murine p62) was kindly provided by Jorge Moscat (Sanford-Burnham Medical Research Institute, La Jolla, CA), and the P392L mutation in UBA domain (exon-8) and A381V in non-UBA domain (exon-7) were introduced by site-directed mutagenesis using QuikChange II XL Site-Directed Mutagenesis kit (Stratagene, Inc., La Jolla, CA) as described earlier (29). The mutagenized p62 cDNA was sequenced verified for correct introduction of P392L and A381V mutations (Fig. 1A).

p62 and CYLD short hairpin RNA (shRNA) retroviral expression

The p62WT and mutant cDNA were excised from the pcDNA3-HA-p62 plasmids by digestion with EcoRI and subcloned into the pLXSN retroviral vector (Clontech Laboratories, Inc., Palo Alto, CA). The resulting plasmid construct transcribes p62 mRNA expression under the control of 5′ long terminal repeat viral promoter elements. The recombinant p62 constructs and CYLD shRNA retroviral plasmid (Open Biosystems, Rockford, IL) were transfected into the PT67 amphotropic packaging cell line using lipofectamine (Invitrogen Corp.), Stable clonal cell lines producing p62 recombinant retrovirus at high titer (1 × 10^6 virus particles/ml) were established by selecting for resistance to neomycin (600 μg/ml). Similarly, a control retrovirus producer cell line was established by transfecting the cells with the pLXSN empty vector (EV). Producer cell lines were maintained in DMEM containing 10% fetal calf serum (FCS), 100 U/ml each of streptomycin and penicillin, 4 mM l-glutamine, and high glucose (4.5 g/liter). Retroviral supernatants from the producer cell cultures were collected and filtered (0.45 μm pore diameter) for immediate use. Mouse bone marrow-derived nonadherent cells were transduced with p62WT, p62P392L, or p62A381V and CYLD shRNA 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 (30).

OCL culture and bone resorption assay

Mouse bone marrow-derived nonadherent cells were dispersed into α-MEM containing 10% FCS and were seeded in 96-well plates at 6 × 10^4 cells/well in 0.2 ml of medium. Cells
Sundaram cells were removed from the dentine disc, using 1M NaOH and 10 d on dentine slices. At the end of the culture period, adherent MO). TRAP positive multinucleated cells containing three or more nuclei were scored as OCL cells under a microscope. Mouse bone marrow-derived nonadherent cells were transduced with retroviral expression plasmids of p62WT, p62A381V, or p62P392L, and cells were stimulated with M-CSF (10 ng/ml) and RANKL (100 ng/ml) for 48 h. Total cell lysates obtained were subjected to immunoprecipitation using rabbit anti-HA antibody. The immunoprecipitants were analyzed by Western blotting using rabbit anti-CYLD antibody.

FIG. 1. p62 interaction with CYLD. A, Site-directed mutagenesis of p62 non-UBA (p62A381V) and UBA (p62P392L) mutations associated with PDB. A plasmid containing the full-length human p62 cDNA was used as template for site-directed mutagenesis of UBA mutant, p62P392L (C-to-T transition), and a non-UBA mutant, p62A381V (C-to-T transition), as described in Materials and Methods. The mutagenized p62 cDNA sequenced to verify correct introduction of the P392L, A381V mutations as circled. B, p62 UBA mutation (p62P392L) abolished interaction with CYLD in pre-OCL cells. Mouse bone marrow-derived nonadherent cells were transduced with retrovirally expressing plasmids of p62WT, p62A381V, or p62P392L, and cell lysates were collected in a lysis buffer [50 mM HEPES (pH 7.5), 250 mM NaCl, 0.2 mM EDTA, 10 μM NaF, and 0.5% Nonidet P-40] were immunoprecipitated using anti-HA antibody as described earlier (29). Immunocomplexes were subjected to Western blot analysis for CYLD using rabbit anti-CYLD antibody.

Quantitative real-time RT-PCR
NFATc1 mRNA expression was measured by real-time RT-PCR as described previously (29). Briefly, total RNA was isolated from pre-OCL cells transfected with p62WT, p62A381V, or p62P392L and were stimulated with and without RANKL (100 ng/ml) for 48 h, using RNAzol reagent (Biotec Laboratories, Houston, TX). A RT reaction was performed using a cDNA synthesis kit (Bio-Rad, Hercules, CA) in a 25-μl reaction volume containing total RNA (2 μg), 1X PCR buffer, and 2 mM MgCl2, at 42 C for 15 min followed by 95 C for 5 min. The quantitative real-time PCR was performed using iQ SYBR Green Supermix in an iCycler (iCycler iQ Single-color Real Time PCR Detection System; Bio-Rad). The primer sequences used to amplify glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were 5’-CCCTCCTCCCAAATGTATCCGTTGTG-3’ (sense) and 5’-GGAGGAATGGGAGTTGCTGTT-3’ (antisense) and for mouse NFATc1 mRNA were 5’-GGGGCGAACAGACTACAGTTA-3’ (sense) and 5’-GAGATACCCGGGTTGAC-3’ (antisense). Thermal cycling parameters were 94 C for 3 min, followed by 40 cycles of amplifications at 94 C for 30 sec, 60 C for 1 min, 72 C for 1 min, and 72 C for 5 min as the final elongation step. Relative levels of NFATc1 mRNA expression were normalized in all the samples analyzed with respect to GAPDH amplification.

NF-κB, AP-1, and NFAT-Luc reporter gene assay
RAW 264.7 cells were cultured in DMEM supplemented with 10% FCS in a humidified atmosphere with 5% CO2 at 37 C. DNA transfections were performed using lipofectamine transfection reagent (Invitrogen Corp.) according to the manufacturer’s protocol. RAW 264.7 cells were transfected with NF-κB, AP-1, and NFAT-Luc reporter plasmids and coexpressed with p62WT, p62P392L, or p62A381V. Cells were cultured in the presence or absence of RANKL (100 ng/ml) for 48 h. The cell monolayer was washed twice with PBS and incubated at room temperature for 15 min with 0.3 ml lysis buffer. The monolayer was scraped and spun briefly in a microfuge to pellet the debris. Then, a 20-μl aliquot of each sample was mixed with 100 μl of the luciferase assay reagent. Light emission was measured for 10 sec of integrated time using Sirius Luminometer (Promega). The transfection efficiency was normalized by cotransfection with 0.2 μg of pRSV β-gal plasmid and measuring β-galactosidase activity in the cell lysates. LacZ cytotoxicity assay (Invitrogen Corp.) indicated a DNA transfection efficiency (>80%) in RAW 264.7 cells.

TRAF6 ubiquitination assay
Mouse bone marrow-derived nonadherent cells were seeded in six-well plates (5 × 105 cells/well), transduced with p62WT,
p62<sup>P392L</sup>, or p62<sup>A381V</sup> retroviral expression vectors, and stimulated with M-CSF (10 ng/ml) and RANKL (100 ng/ml). After 48 h, total cell lysates were collected in a lysis buffer [50 mM HEPES (pH 7.5), 250 mM NaCl, 0.2 mM EDTA, 10 mM NaF, and 0.5% Nonidet P-40]. TRAF6 was immunoprecipitated using rabbit-anti-TRAF6 antibody, and the ubiquitin-conjugated TRAF6 was detected by Western blotting using rabbit-antiubiquitin antibody.

### Statistical analysis

Results are presented as mean ± SD for three independent experiments and were compared by Student’s t test. Values were considered significantly different for a and b, P < 0.05.

### Results

#### p62 UBA mutation (p62<sup>P392L</sup>) abolished interaction with CYLD in pre-OCL cells

To examine the functional role of p62<sup>P392L</sup> in OCL differentiation, we developed HA-tagged p62<sup>WT</sup>, non-UBA mutant (p62<sup>A381V</sup>), and UBA mutant (p62<sup>P392L</sup>) retroviral expression vectors as described in Materials and Methods. Mouse bone marrow-derived nonadherent cells were transduced with p62<sup>WT</sup>, p62<sup>A381V</sup>, or p62<sup>P392L</sup> expression vectors and stimulated with M-CSF (10 ng/ml) and RANKL (100 ng/ml) for 48 h. p62 was then immunoprecipitated from the total cell lysates using anti-HA antibody. Western blot analysis of the immunocomplex revealed that CYLD coimmunoprecipitated with p62<sup>WT</sup> and non-UBA mutant p62<sup>A381V</sup> but not with UBA mutant p62<sup>P392L</sup>. In contrast, a control nonspecific rabbit IgG did not immunoprecipitate CYLD (Fig. 1B). These results suggest that the p62 UBA mutation (p62<sup>P392L</sup>) abolished interaction with CYLD in pre-OCL cells.

#### p62<sup>P392L</sup> enhances TRAF6 ubiquitination during OCL differentiation

TRAF6 is an adaptor molecule involved in RANK signaling, and polyubiquitination of TRAF6 plays an important role in OCL differentiation. To determine whether the p62<sup>P392L</sup> mutant modulates TRAF6 ubiquitination during OCL differentiation, nonadherent mouse bone marrow cells were transduced with p62<sup>WT</sup>, non-UBA mutant (p62<sup>A381V</sup>), or UBA mutant (p62<sup>P392L</sup>) and stimulated with RANKL (100 ng/ml) and M-CSF (10 ng/ml) for 48 h. Total cell lysates obtained were subjected to immunoprecipitation of TRAF6 using rabbit anti-TRAF6 antibody. Western blot analysis of the immunocomplex revealed accumulation of polyubiquitinated TRAF6 in p62<sup>P392L</sup> transduced pre-OCL cells with and without RANKL stimulation compared with p62<sup>WT</sup> and p62<sup>A381V</sup> transduced cells. However, a modest increase in TRAF6 ubiquitination was observed in p62<sup>WT</sup> and p62<sup>A381V</sup> transduced cells. Therefore, we examined whether p62<sup>P392L</sup>/ and p62<sup>A381V</sup> compared with control EV-transduced pre-OCL cells (Fig. 2A). We next examined whether shRNA suppression of CYLD in the presence of p62<sup>WT</sup> and mutants modulate TRAF6 ubiquitination in pre-OCL cells. Mouse bone marrow-derived nonadherent cells were transduced with p62<sup>WT</sup>, p62<sup>A381V</sup>, or p62<sup>P392L</sup> in the presence and absence of CYLD shRNA. Cells were transduced with RANKL (100 ng/ml) and M-CSF (10 ng/ml) for 48 h, and total cell lysates obtained were subjected to immunoprecipitation of TRAF6. As shown in Fig. 2B, shRNA suppression of CYLD expression significantly increased polyubiquitination of TRAF6 in the presence of p62<sup>WT</sup>, p62<sup>A381V</sup>, and p62<sup>P392L</sup> compared with nonspecific control shRNA transduced pre-OCL cells. These data suggest that p62<sup>P392L</sup>/CYLD modulates TRAF6 ubiquitination during OCL differentiation.

#### p62<sup>P392L</sup> modulation of downstream effectors of RANK signaling

p62<sup>P392L</sup> has been shown to increase OCL differentiation (17, 20). Therefore, we examined whether p62<sup>P392L</sup>/
CYLD modulates downstream effectors of RANK signaling during OCL differentiation. Mouse bone marrow-derived nonadherent cells were transduced with p62WT, p62A381V, or p62P392L and stimulated with M-CSF (10 ng/ml) and RANKL (100 ng/ml) for different time points (0–60 min). Western blot analysis of total cell lysates obtained from pre-OCL cells revealed that p62P392L induced high level expression of phospho-IkB with and without RANKL stimulation compared with p62WT and p62A381V transduced cells. However, we observed a modest increase in p-IkB expression in p62WT and p62A381V compared with EV transduced cells (Fig. 3A). Furthermore, pre-OCL cells stimulated with RANKL and M-CSF for 48 h had increased (2.8-fold) c-Fos expression in p62P392L compared with p62WT or p62A381V transduced cells (Fig. 3B). To further examine the role of p62P392L/CYLD on c-Fos expression, mouse bone marrow-derived nonadherent cells were transduced with CYLD shRNA coexpressed with p62WT, p62A381V, or p62P392L and stimulated with RANKL and M-CSF for 48 h. Western blot analysis of total cell lysates identified that CYLD knockdown significantly increased c-Fos expression in p62WT, p62A381V, or p62P392L mutant transduced cells without RANKL stimulation compared with control shRNA transduced cells (Fig. 3C). shRNA suppression (~80%) of CYLD expression was confirmed by Western blot analysis (Fig. 3D).
These results further suggest that p62P392L enhanced c-Fos gene expression during OCL differentiation. c-Fos plays an important role in expression of NFATc1, a critical transcription factor essential for OCL differentiation and bone resorption. Therefore, we next examined the functional impact of p62P392L in NFATc1 expression during OCL differentiation. Real-time PCR analysis of total RNA isolated from pre-OCL cells showed that p62P392L significantly increased (4.0-fold) NFATc1 mRNA expression without RANKL stimulation compared with p62WT or p62A381V transduced cells. In contrast, no significant changes in NFATc1 expression were observed in p62WT and p62A381V transduced cells compared with EV transduced cells (Fig. 3E).

To further confirm that p62 mutant modulates NF-κB, AP-1, and NFAT transcriptional activity, pNF-κB- Luc, AP-1-Luc, and NFAT-Luc reporter gene plasmids were transfected with p62WT, p62A381V, or p62P392L and stimulated with and without RANKL (100 ng/ml) for 48 h. Total cell lysates prepared were assayed for luciferase activity. The transfection efficiency was normalized by β-galactosidase activity coexpressed in these cells. Values are expressed as mean ± s.d for three independent experiments (a and b, P < 0.05; compared with EV with and without RANKL stimulation, respectively).

Discussion

p62 has been shown to interact with the atypical protein kinase C and TRAF6 to modulate RANK signaling and
Osteoclastogenesis (13). Ubiquitination of RANK adaptor protein, TRAF6, plays an important role in activation of NF-κB during OCL differentiation (31). The UBA domain truncated p62 has been shown to increase RANKL induced NFAT expression and ERK phosphorylation during osteoclastogenesis of RAW 264.7 cells (19). Deubiquitinating enzyme, CYLD, has been shown to inhibit NF-κB activation through deubiquitinatin of TRAF2 and TRAF6 (24). Furthermore, CYLD interacts with the p62 UBA domain to inhibit TRAF6 ubiquitination and negatively regulates RANK signaling and osteoclastogenesis (23). In the present study, we identify that in contrast to p62WT and non-UBA mutant (p62A381V), UBA mutant (p62P392L) abolished CYLD interaction, and this indicates that RANK signaling essential for osteoclastogenesis is modulated in PDB. We consistently observed accumulation of polyubiquitinated TRAF6 in p62P392L transduced pre-OCL cells.

Ubiquitination of signaling molecules by E3 ubiquitin ligases has been shown to modulate NF-κB signaling (32). Because CYLD negatively regulates different signaling pathways by deubiquitination of K63-linked polyubiquitin chains from several substrates, it is possible that lack of p62P392L interaction with CYLD may affect the ubiquitination status of other RANK signaling molecules that may also play a role in enhanced osteoclastogenesis. Elevated p-IκB expression in p62P392L mutant transduced pre-OCL cells suggests p62P392L activation of NF-κB in these cells. However, a modest increase observed in TRAF6 ubiquitination and p-IκB expression in p62WT and p62A381V compared with control EV transduced cells is more likely due to overexpression of p62 in these cells. Therefore, the mutant p62P392L in contrast to p62WT and p62A381V stimulates NF-κB activity in pre-OCL cells, and this suggests that lack of p62P392L interaction with CYLD may have a specific functional role in enhanced osteoclastogenesis in PDB. Previously, p62 has been shown to be involved in NF-κB activation (13). Further, p62P392L mutation has been shown to elevate NF-κB activation as well as p38 MAPK and ERK1/2 signaling in OCL precursors stimulated with RANKL (17). However, Rea et al. (11) have shown that p62 wild type reduces and p62P392L mutant
increases NF-κB activation in HEK293 and Cos-1 cells. Similarly, both p62 non-UBA (A381V) and UBA mutant (P392L) have been shown to increase NF-κB reporter gene activity in HEK293 cells (12). The variability in p62 regulation of NF-κB activity could be due to cell specificity and response to RANKL stimulation. p62 (440 amino acids) contains various domains that mediate protein-protein interactions (33). Several proteins that interact with p62 UBA domain, which includes calmodulin kinase II, nuclear receptor co-receptor I, heat shock protein 70, FK506 binding protein 14, homeobox protein Meis2, and Unc51 like kinase II, have been identified (21). In addition, p62 protein has been shown to interact with several signaling proteins, such as mitogen-activated protein kinases, atypical protein kinase C, p56lk, receptor-interacting protein, TRAF6, ubiquitin, ubiquitinating, and deubiquitinating enzymes (33). Therefore, it is possible that p62A381V may affect interaction with signaling molecules other than CYLD and modulates NF-κB activation in a cell-specific manner. Furthermore, our results that p62P392L expression/shRNA knockdown of CYLD increased NF-κB, AP-1, and NFAT reporter gene activity indicate that mutant p62P392L modulates NF-κB target gene expression required for enhanced osteoclastogenesis in PDB. This is further confirmed by the results that CYLD knockdown significantly increased c-Fos expression in p62WT, p62A381V, and p62P392L, mutant transduced cells without RANKL stimulation. Consistently, up-regulation of c-Fos gene expression in pagetic OCL and osteoblast cells has been reported (34). NFATc1 is a critical transcription factor for osteoclastogenesis (14), and our findings that p62P392L up-regulated NFATc1 expression in pre-OCL cells favors increased osteoclastogenesis associated with PDB. Gene expression profiles in OCL revealed no significant change in p62 and CYLD expression in PDB (35). Inhibition of CYLD resulted in NF-κB activation and apoptotic resistance in hepatocellular carcinoma cells (36). Therefore, it is possible that the p62P392L mutant may have a functional role in proliferation/survival of OCL progenitor cells, which have been shown to be increased in PDB (37). Also B-cell lymphoma 2, antiapoptotic gene expression has been shown to be up-regulated in pagetic bone (38). OCL formed in pagetic bone marrow cultures have high levels of IL-6 and TRAP activity (39). We identified that p62P392L expression increased TRAP mRNA expression in pre-OCL cells (data not shown). p62P392L modulation of c-Fos and NFATc1 expression implicated a potential role in enhanced osteoclastogenesis in PDB. Confirming this, we found that p62P392L expression or shRNA knockdown of CYLD resulted in increased OCL formation and bone resorption activity in mouse bone marrow cultures. Therefore, lack of p62 UBA mutant interaction with CYLD results in increased OCL formation. Mutations in valosin-containing protein, which also contains the UBA domain, have been linked to inclusion body myopathy that is associated with PDB and frontotemporal dementia (IBMFD) (40). Valosin-containing protein is a multiubiquitin chain targeting factor for proteosome degradation, and it also plays an important role in regulating the NF-κB signaling cascade (41). Therefore, identification of p62P392L regulated gene expression profiling is important to better understand the pathogenesis of PDB. Transgenic mice harboring p62P394L mutation (equivalent to human p62P392L) showed increased osteoclastogenic potential due to increased RANKL expression in marrow stromal cells in the bone microenvironment. The OCL precursors from these mice also demonstrated increased sensitivity to RANKL but not to 1,25(OH)2D3 (42). Furthermore, p62 UBA mutant (p62P394L) and MVNP coexpression in mice developed OCL with pagetic phenotype and increased IL-6 production (43). In contrast, others have recently shown that mice with p62P394L mutation have focal lesions with increased OCL number, size, and some nuclear inclusions (18). Consistently, the OCL precursors from these mice are hypersensitive to RANKL. These studies have also suggested that p62P394L mutation up-regulates autophagy, a cellular process for lysosomal degradation of damaged/dysfunctional organelles and protein aggregates (44). However, p62 null mice have a normal skeletal phenotype with no alterations were found in the trabecular size and number of OCL, suggesting that basal osteoclastogenesis is not affected by the loss of p62 (13). Therefore, p62 mutant protein-protein interactions play an important role in enhanced OCL development in PDB.

PDB patients with p62 mutations displayed polyostotic involvement, indicating severity of the disease (45, 46). However, the familial history of PDB is about 15–40% patients with a first degree relative and of which only 20–30% have a p62 mutation. Thus, p62 mutations occur in 5–10% of patients with PDB. Therefore, p62 mutant stimulation of OCL formation/bone resorption is associated with a very limited patient population. Recently, genome-wide association studies in individuals without p62 mutations have further identified genetic variants CSF1, OPTN, and TNFRSF11A as risk factors that predispose to PDB; however, their functional role in pathogenesis of PDB is yet to be elucidated (47). Presence of nuclear inclusions in pagetic OCL suggested a viral etiology for PDB; however, no infectious virus is isolated. Despite the controversy about the identity of paramyxoviral nuclear inclusions and MVNP expression in pagetic OCL, it has been shown that targeted expression of MVNP to OCL lineage develops pagetic-like bone lesions in mice (48). Therefore, both genetic and environmental factors, such
as paramyxoviruses, play an important role in pathogenesis of PDB. In conclusions, p62 UBA mutation (p62P392L) abolished interaction with CYLD and contributed to enhanced OCL development and excess bone resorption associated with PDB.

Acknowledgments

We thank Danielle Mumford for technical assistance.

Address all correspondence and requests for reprints to: Sakamuri V. Reddy, Ph.D., Charles P. Darby Children’s Research Institute, Medical University of South Carolina, 173 Ashley Avenue, Charleston, South Carolina 29425. E-mail: reddysv@musc.edu

This work was supported by the Department of Defense Medical Research Award PR080480.

Disclosure Summary: The authors have nothing to disclose.

References