Biomarkers, 2012, 1–5, Early Online

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.3109/1354750X.2011.653986

informa
healthcare

REVIEW ARTICLE

Sequestosome 1/p62: across diseases

Thangiah Geetha, Nilmini Vishwaprakash, Marina Sycheva, and Jeganathan Ramesh Babu

Department of Nutrition, Dietetics, and Hospitality Management, Auburn University, Auburn, AL, USA

Abstract
Sequestosome 1/p62 is a signal modulator or adaptor protein involved in receptor-mediated signal transduction. Sequestosome 1/p62 is gaining attention as it is involved in several diseases including Parkinson disease, Alzheimer disease, liver and breast cancer, Paget's disease of bone, obesity and insulin resistance. In this review, we will focus on the most recent advances on the physiological function of p62 relevant to human diseases.

Keywords: Neurodegenerative diseases, cancer, Paget's disease of bone, obesity, insulin resistance

Introduction

Sequestosome 1/p62 was originally identified as a phosphotyrosine-independent ligand of the src homology 2 (SH2) domain of p56lck (Joung et al. 1996). Since it functions as an intracellular signal modulator or adaptor protein, it plays a major role in receptor-mediated signal transduction. A highly conserved cytosolic 62 kDa protein, it functions as scaffolding that interacts with the atypical PKCs (aPKC; PKCζ and PKCλ/ι) and leads to the activation of nuclear factor-κB (NF-κB), a transcription factor important in several signaling pathways (Moscat & Diaz-Meco 2000). p62 harbors an amino terminal PB1 domain with an SH2 binding domain and an acidic interaction domain (AID) that binds the atypical PKC (aPKC) (Laurin et al. 2002), a ZZ finger, a binding site for the RING-finger protein tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), two peptide sequences rich in proline (P), glutamic acid (E), serine (S) and threonine (T) (PEST sequences), an LC3 (autophagy marker) interacting region (LIR) (Pankiv et al. 2007), and a carboxyl terminal ubiquitin (Ub)-associated (UBA) domain (Seibenhener et al. 2004). Several mutations of p62 are associated with Paget's disease of bone (PDB) (Laurin et al. 2002). In the past decade, studies have shown that p62 is associated with several other diseases including Parkinson disease (PD), Alzheimer disease (AD), liver cancer, breast cancer, obesity and insulin resistance. The purpose of this review is to shed light on the physiological function of p62 in these diseases.

Neurodegenerative diseases

In neurodegenerative diseases, oxidative stress leads to protein misfolding and upon polyubiquitination the misfolded proteins accumulate in cytoplasmic and intracellular inclusions forming protein aggregates (Alves-Rodrigues et al. 1998; Lowe et al. 2001). For example, α-synuclein and parkin are the major protein components of the inclusion bodies found in *PD* brain (Giasson & Lee 2001; Goedert 2001). Other examples of protein aggregates include neurofibrillary tangles in AD, Lewy bodies in PD, Mallory bodies (MBs) in steatohepatitis, and intracytoplasmic hyaline bodies in hepatocellular carcinoma (HCC) (Kuusisto et al. 2001a; Zatloukal et al. 2002). Neuronal cell death or proteasomal dysfunction also leads to the accumulation of misfolded and ubiquitinated proteins (Kuusisto et al. 2001b) and causes increased expression of p62, which protects cells by localizing the misfolded proteins as aggregates in cytoplasmic inclusions (Zatloukal et al. 2002; Nakaso et al. 2004). Parkin, a ubiquitin ligase, polyubiquitinates depolarized mitochondria through its lysine 27 and lysine 63 ubiquitin chains. Ubiquitinated mitochondria shuttle through microtubules to form aggregates in the perinuclear region and are degraded by autophagy (Geisler et al. 2010; Okatsu et al. 2010). Likewise, p62 shuttles ubiquitinated proteins to autophagy for degradation (Komatsu & Ichimura 2010) and is also involved in the clustering and degradation of depolarized mitochondria and formation of aggresomes (Geisler et al.

Address for Correspondence: Jeganathan Ramesh Babu, Department of Nutrition, Dietetics, and Hospitality Management, Auburn University, Auburn, AL 36849, USA, Tel.: (334) 844-3840. Fax: (334) 844-3268. E-mail: jeganrb@auburn.edu

(Received 28 December 2011; revised 00 00 0000; accepted 28 December 2011)

2010; Okatsu et al. 2010). The neurosecretory cells of the hypothalamic and brainstem paraventricular nuclei were found to be p62 immunoreactive (Braak et al. 2011) and p62 protects these neurons from PD by degrading misfolded proteins and blocking the aggregate formation (Braak et al. 2011).

In *AD*, the major histopathological lesions are neurofibrillary tangles (NFTs) and the neurite plaques (NPs) that contain amyloid β (Terry et al. 1999). NFTs accumulate with tau, a hyperphosphorylated microtubule associated protein. NFTs and distorted neuritis of NPs were found to be associated with ubiquitin (Perry et al. 1987). Tau accumulated in a polyubiquitinated form (Morishima-Kawashima et al. 1993) and the ubiquitin mediated proteasome function was attenuated in AD (Keller et al. 2000). In 2002, Kuusisto et al. (2002) discovered the ubiquitin binding protein p62 accumulated and co-localized with ubiquitin and tau aggregates in NFTs in AD hippocampus and cortex (Kuusisto et al. 2001a; Kuusisto et al. 2002). We have shown that the inclusion bodies from AD brain contained p62, ubiquitin, phosphorylated tau, and TRAF6 (Babu et al. 2005). TRAF6 is a ubiquitin ligase E3 that can ubiquitinate several substrates (Joazeiro & Weissman 2000). p62 is known to bind polyubiquitinated substrates through its UBA domain and shuttles them to the proteasome for degradation through its PB1 domain (Seibenhener et al. 2004). Interestingly, tau was a K63 polyubiquitinated substrate of TRAF6 that binds to the UBA domain of p62 (Babu et al. 2005). Degradation of tau is ubiquitin-proteasome or p62 dependent and p62 is required to shuttle tau to the proteasome (Babu et al. 2005). Since its absence impairs degradation of tau and leads to the accumulation of insoluble K63 polyubiquitinted aggregates (Wooten et al. 2008), p62 deficient mice accumulate aggregated, K63 polyubiquitinated and hyperphosphorylated tau, and develop neurofibrillary tangles and neurodegeneration (Babu et al. 2008). AD affects memory, thinking, behavior, and cognitive skills such as judgment. Mice deficient in the p62 gene exhibit AD-like characteristics (Babu et al. 2008). Since deletion of the mouse p62 gene revealed disturbances in short-term memory, increased anxiety, and depression similar to that observed in human AD (Babu et al. 2008). In neurodegenerative diseases, oxidative damage to the p62 promoter reduced its expression (Du et al. 2009). Overexpression of p62 in brain may be a novel way to prevent or treat neurodegeneration (Du et al. 2009).

Cancer

Many studies found correlations between p62 protein expression and cancer, but no direct links have been reported. Autophagy deficient mice, however, develop multiple liver tumors and overexpress p62 protein in *malignant tumor* cells (Takamura et al. 2011). An abundance of p62 protein is associated with *breast tumors* and *liver cirrhosis* as well (Lu et al. 2001; Thompson et al. 2003). In addition, abnormal expression of both fetal RNA-binding protein and p62 is found in *liver cancer* and *liver cirrhosis* (Lu et al. 2001), and p62 has been identified as an important NF-κB mediator in *tumorigenesis* (Duran et al. 2004). A study by Mathew et al. (2009) shows that p62 was eliminated when autophagy suppresses tumorigenesis. The ubiquitin–proteasome pathway can be the target of cancer-related deregulation and can lead to the transformation of normal cells to cancer cells, increased drug resistance, and tumor progression (Spataro et al. 1998). p62 non-covalently binds free ubiquitin (Vadlamudi & Shin 1998; Shin 1998) and may play a significant role in an ubiquitination-mediated regulatory mechanism during cell proliferation and differentiation.

MBs arise because of a hepatocellular disorder that is a consequence of chronic alcoholic liver disease. In this condition, p62 is rapidly induced in hepatocytes and directly increases MB formation by associating with abnormal keratins (Zatloukal et al. 2002; Stumptner et al. 2002). p62 is up-regulated when the proteasome is inhibited (Kuusisto et al. 2002) and several studies have documented impairment of the proteasome in alcoholic liver disease (Fataccioli et al. 1999; Bardag-Gorce et al. 2004; Donohue et al. 2004). Autophagy is a major pathway for degradation of cytoplasmic proteins and has been implicated in tumor suppression. The size of the $Atg7^{-/-}$ liver tumors is reduced by deletion of p62 suggesting that autophagy is important for the suppression of spontaneous tumorigenesis and that accumulation of p62 contributes to tumor progression (Takamura et al. 2011). Overproduction of p62 or autophagy deficiency competes with the interaction between Nrf2 and Keap1, resulting in stabilization of Nrf2 and transcriptional activation of Nrf2 target genes (Copple et al. 2010; Jain et al. 2010; Komatsu et al. 2010; Lau et al. 2010; Riley et al. 2010). Induction of Nrf2 target genes has been observed in many human cancers (Hayes et al. 2009) that also exhibit accumulation of p62 (Zatloukal et al. 2002). Liver-specific Atg7 knockout mice develop hepatocellular adenoma accompanied by excess accumulation of p62 and then Nrf2 activation. The persistent activation of Nrf2 through p62 contributes to development of human *HCC* (Inami et al. 2011). The loss of p62 reduces liver damage in Atg7 knockout mice (Komatsu et al. 2007; Jin et al. 2009), whereas characterization of liver-specific p62 overexpression in transgenic mice revealed a phenotype of a fatty liver with microvesicular fat distribution in p62 transgenic mice (Tybl et al. 2011). Results suggest that p62 plays a role in hepatic pathophysiology and might serve as a diagnostic and therapeutic marker.

Paget's disease of bone

PDB involves abnormal bone destruction and regrowth. The phenotypic analysis of genetically modified mice lacking p62 shows that it regulates osteoclastogenesis and bone homeostasis through the E3 ubiquitin ligase TRAF6 by acting as an important intermediary of the receptor activator of nuclear factor κB (RANK) pathway (Duran et al. 2004). This is consistent with the finding that p62 mutations are associated with this disorder

characterized by aberrant osteoclastogenic activity (Laurin et al. 2002). PDB is caused by genetic mutation of p62 where the ubiquitin binding-associated (UBA) domain is either truncated or has somehow lost its function (Layfield et al. 2006). Understanding how loss of the ubiquitin-binding function of p62 impacts on signal transduction events in osteoclasts will undoubtedly further our understanding of the molecular mechanism of PDB (Layfield et al. 2006). When compared to wild-type cells, the p62 UBA domain deletion mutant (p62ΔUBA) significantly enhanced osteoclastogenesis in vitro (Laurin et al. 2002). Overexpressed p62ΔUBA enhanced the receptor activator of nuclear factor-κB (NF-κB) ligand that induced activation of nuclear factor-κB, NFAT, and ERK phosphorylation. Deletion of the p62 UBA domain reduced its association with TRAF6 in the proteasomal compartment, suggesting that the UBA domain may encode the regulatory elements for the receptor activator of NF-κB ligand-induced osteoclast formation and bone resorption and may be directly associated with the onset of PDB. Mutation of the p62 UBA domain impairs the ubiquitination and NF-κB signaling that might impact osteoclastogenesis and osteoclast activity (Cavey et al. 2006; Goode & Layfield 2010). In PDB, mutations in the UBA domain of p62 are P392L, S399P, M404V/T, G411S, and G425R (Cavey et al. 2006; Layfield et al. 2004; Visconti et al. 2010; Michou et al. 2006). Most PDB patients have the P392L mutation, which did does not affect the ubiquitin binding ability of p62 (Garner et al. 2011). The severity of PDB in patients is somehow related to the dysfunction in the ubiquitin binding of p62 mutant proteins and remains to be determined. PDB is characterized by increased osteoclast activity followed by osteoblast response (Morales-Piga et al. 1995). The tumor suppressor cylindromatosis (CYLD) gene is a deubiquitinase enzyme that can interact with p62 and negatively regulate osteoclastogenesis (Jin et al. 2008). CYLD disrupts the ubiquitin chains from several substrates and inhibits the activation of NF-κB (Trompouki et al. 2003; Brummelkamp et al. 2003; Kovalenko et al. 2003). Interestingly, the deubiquitinase activity of CYLD is dependent upon p62 (Wooten et al. 2008) since its interaction with the p62 mutant P392L was impaired and increased the osteoclast activity in PDB (Sundaram et al. 2011). Thus, p62 is critical to the development of PDB.

Obesity and insulin resistance

Obesity is associated with an increased risk of developing insulin resistance and type 2 diabetes. Five-month-old p62 knockout mice had a significant increase in body fat (Rodriguez et al. 2006) and were heavier as well as larger than control mice. The amount of food eaten by p62 knockout and control mice was same, but p62 knockout mice drank more water than the control mice suggesting that they may be diabetic. The size and weight of the liver, spleen, and heart tissues were increased in p62 knockout mice that presented with impaired glucose and insulin tolerance. Deletion of the p62 gene increased ERK activation and adipogenesis could lead to obesity and insulin and leptin resistance (Rodriguez et al. 2006). Recently, p62 is found to interact with mTOR and raptor (Duran et al. 2011). p62 connects autophagy and mTORC1 activity to control adipogenesis (Moscat & Diaz-Meco 2011). An α-glucosidase inhibitor, acarbose has been used to treat type 2 diabetes (Chiasson et al. 2002), by increasing insulin sensitivity and reducing the blood sugar (Chiasson et al. 1996). After 10 weeks of acarbose treatment, obese and insulin resistant p62 knockout mice showed reduced body fat and weight gain as well as lower blood glucose and cholesterol (Okada et al. 2009). p62 may prove to be useful for therapeutic treatment of obesity and type 2 diabetes.

Conclusions

Sequestosome 1/p62 has roles in neurodegenerative diseases such as PD and ADs. Evidence suggests that it may be a factor in breast cancer, liver cancer, PDB, obesity, and insulin resistance. p62 may prove to be useful for therapeutic treatment of obesity and type 2 diabetes.

Acknowledgments

We thank Dr. Catherine Wernette for editing this manuscript.

Declaration of interest

This work was supported by the New Faculty Start-up Fund from Auburn University and Alabama Agriculture Experimental Station Hatch Funding to JRB.

References

Alves-Rodrigues A, Gregori L, Figueiredo-Pereira ME. (1998). Ubiquitin, cellular inclusions and their role in neurodegeneration. *Trends Neurosci* 21:516–520.

Babu JR, Geetha T, Wooten MW. (2005). Sequestosome 1/p62 shuttles polyubiquitinated tau for proteasomal degradation. *J Neurochem* 94:192–203.

Bardag-Gorce F, Riley NE, Nan L, Montgomery RO, Li J, French BA, Lue YH, French SW. (2004). The proteasome inhibitor, PS-341, causes cytokeratin aggresome formation. *Exp Mol Pathol* 76:9–16.

Braak H, Thal DR, Del Tredici K. (2011). Nerve cells immunoreactive for p62 in select hypothalamic and brainstem nuclei of controls and Parkinson's disease cases. *J Neural Transm* 118:809–819.

Brummelkamp TR, Nijman SM, Dirac AM, Bernards R. (2003). Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-κB. *Nature* 424:797–801.

Cavey JR, Ralston SH, Sheppard PW, Ciani B, Gallagher TR, Long JE, Searle MS, Layfield R. (2006). Loss of ubiquitin binding is a unifying mechanism by which mutations of SQSTM1 cause Paget's disease of bone. *Calcif Tissue Int* 78:271–277.

Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M; STOP-NIDDM Trail Research Group. (2002). Acarbose for

prevention of type 2 diabetes mellitus: The STOP-NIDDM randomised trial. *Lancet* 359:2072–2077.

Chiasson JL, Josse RG, Leiter LA, Mihic M, Nathan DM, Palmason C, Cohen RM, Wolever TM. (1996). The effect of acarbose on insulin sensitivity in subjects with impaired glucose tolerance. *Diabetes Care* 19:1190–1193.

Copple IM, Lister A, Obeng AD, Kitteringham NR, Jenkins RE, Layfield R, Foster BJ, Goldring CE, Park BK. (2010). Physical and functional interaction of sequestosome 1 with Keap1 regulates the Keap1-Nrf2 cell defense pathway. *J Biol Chem* 285:16782–16788.

Donohue TM Jr, Kharbanda KK, Casey CA, Nanji AA. (2004). Decreased proteasome activity is associated with increased severity of liver pathology and oxidative stress in experimental alcoholic liver disease. *Alcohol Clin Exp Res* 28:1257–1263.

Du Y, Wooten MC, Wooten MW. (2009). Oxidative damage to the promoter region of SQSTM1/p62 is common to neurodegenerative disease. *Neurobiol Dis* 35:302–310.

Duran A, Amanchy R, Linares JF, Joshi J, Abu-Baker S, Porollo A, Hansen M, Moscat J, Diaz-Meco MT. (2011). p62 is a key regulator of nutrient sensing in the mTORC1 pathway. *Mol Cell* 44:134–146.

Durán A, Serrano M, Leitges M, Flores JM, Picard S, Brown JP, Moscat J, Diaz-Meco MT. (2004). The atypical PKC-interacting protein p62 is an important mediator of RANK-activated osteoclastogenesis. *Dev Cell* 6:303–309.

Fataccioli V, Andraud E, Gentil M, French SW, Rouach H. (1999). Effects of chronic ethanol administration on rat liver proteasome activities: Relationship with oxidative stress. *Hepatology* 29:14–20.

Garner TP, Long J, Layfield R, Searle MS. (2011). Impact of p62/SQSTM1 UBA domain mutations linked to Paget's disease of bone on ubiquitin recognition. *Biochemistry* 50:4665–4674.

Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, Springer W. (2010). PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol* 12:119–131.

Giasson BI, Lee VM. (2001). Parkin and the molecular pathways of Parkinson's disease. *Neuron* 31:885–888.

Goedert M. (2001). α-synuclein and neurodegenerative diseases. *Nat Rev Neurosci* 2:492–501.

Goode A, Layfield R. (2010). Recent advances in understanding the molecular basis of Paget disease of bone. *J Clin Pathol* 63:199–203.

Hayes JD, McMahon M. (2009). NRF2 and KEAP1 mutations: Permanent activation of an adaptive response in cancer. *Trends Biochem Sci* 34:176–188.

Inami Y, Waguri S, Sakamoto A, Kouno T, Nakada K, Hino O, Watanabe S, Ando J, Iwadate M, Yamamoto M, Lee MS, Tanaka K, Komatsu M. (2011). Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells. *J Cell Biol* 193:275–284.

Jain A, Lamark T, Sjøttem E, Larsen KB, Awuh JA, Øvervatn A, McMahon M, Hayes JD, Johansen T. (2010). p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. *J Biol Chem* 285:22576–22591.

Jin W, Chang M, Paul EM, Babu G, Lee AJ, Reiley W, Wright A, Zhang M, You J, Sun SC. (2008). Deubiquitinating enzyme CYLD negatively regulates RANK signaling and osteoclastogenesis in mice. *J Clin Invest* 118:1858–1866.

Jin Z, Li Y, Pitti R, Lawrence D, Pham VC, Lill JR, Ashkenazi A. (2009). Cullin3-based polyubiquitination and p62-dependent aggregation of caspase-8 mediate extrinsic apoptosis signaling. *Cell* 137:721–735.

Joazeiro CA, Weissman AM. (2000). RING finger proteins: Mediators of ubiquitin ligase activity. *Cell* 102:549–552.

Joung I, Strominger JL, Shin J. (1996). Molecular cloning of a phosphotyrosine-independent ligand of the p56lck SH2 domain. *Proc Natl Acad Sci USA* 93:5991–5995.

Keller JN, Hanni KB, Markesbery WR. (2000). Impaired proteasome function in Alzheimer's disease. *J Neurochem* 75:436–439.

Komatsu M, Ichimura Y. (2010). Physiological significance of selective degradation of p62 by autophagy. *FEBS Lett* 584:1374–1378.

Komatsu M, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Ichimura Y, Sou YS, Ueno I, Sakamoto A, Tong KI, Kim M, Nishito Y, Iemura S, Natsume T, Ueno T, Kominami E, Motohashi H, Tanaka K, Yamamoto M. (2010). The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat Cell Biol* 12:213–223.

Komatsu M, Waguri S, Koike M, Sou YS, Ueno T, Hara T, Mizushima N, Iwata J, Ezaki J, Murata S, Hamazaki J, Nishito Y, Iemura S, Natsume T, Yanagawa T, Uwayama J, Warabi E, Yoshida H, Ishii T, Kobayashi A, Yamamoto M, Yue Z, Uchiyama Y, Kominami E, Tanaka K. (2007). Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. *Cell* 131:1149–1163.

Kovalenko A, Chable-Bessia C, Cantarella G, Israël A, Wallach D, Courtois G. (2003). The tumour suppressor CYLD negatively regulates NF-κB signalling by deubiquitination. *Nature* 424:801–805.

Kuusisto E, Salminen A, Alafuzoff I. (2001a). Ubiquitin-binding protein p62 is present in neuronal and glial inclusions in human tauopathies and synucleinopathies. *Neuroreport* 12:2085–2090.

Kuusisto E, Salminen A, Alafuzoff I. (2002). Early accumulation of p62 in neurofibrillary tangles in Alzheimer's disease: Possible role in tangle formation. *Neuropathol Appl Neurobiol* 28:228–237.

Kuusisto E, Suuronen T, Salminen A. (2001b). Ubiquitin-binding protein p62 expression is induced during apoptosis and proteasomal inhibition in neuronal cells. *Biochem Biophys Res Commun* 280:223–228.

Lau A, Wang XJ, Zhao F, Villeneuve NF, Wu T, Jiang T, Sun Z, White E, Zhang DD. (2010). A noncanonical mechanism of Nrf2 activation by autophagy deficiency: Direct interaction between Keap1 and p62. *Mol Cell Biol* 30:3275–3285.

Laurin N, Brown JP, Morissette J, Raymond V. (2002). Recurrent mutation of the gene encoding sequestosome 1 (SQSTM1/p62) in Paget disease of bone. *Am J Hum Genet* 70:1582–1588.

Layfield R, Cavey JR, Najat D, Long J, Sheppard PW, Ralston SH, Searle MS. (2006). p62 mutations, ubiquitin recognition and Paget's disease of bone. *Biochem Soc Trans* 34:735–737.

Layfield R, Ciani B, Ralston SH, Hocking LJ, Sheppard PW, Searle MS, Cavey JR. (2004). Structural and functional studies of mutations affecting the UBA domain of SQSTM1 (p62) which cause Paget's disease of bone. *Biochem Soc Trans* 32:728–730.

Lowe J, Mayer J, Landon M, Layfield R. (2001). Ubiquitin and the molecular pathology of neurodegenerative diseases. *Adv Exp Med Biol* 487:169–186.

Lu M, Nakamura RM, Dent ED, Zhang JY, Nielsen FC, Christiansen J, Chan EK, Tan EM. (2001). Aberrant expression of fetal RNA-binding protein p62 in liver cancer and liver cirrhosis. *Am J Pathol* 159:945–953.

Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, Chen HY, Bray K, Reddy A, Bhanot G, Gelinas C, Dipaola RS, Karantza-Wadsworth V, White E. (2009). Autophagy suppresses tumorigenesis through elimination of p62. *Cell* 137:1062–1075.

Michou L, Collet C, Laplanche JL, Orcel P, Cornélis F. (2006). Genetics of Paget's disease of bone. *Joint Bone Spine* 73:243–248.

Morales-Piga AA, Rey-Rey JS, Corres-González J, García-Sagredo JM, López-Abente G. (1995). Frequency and characteristics of familial aggregation of Paget's disease of bone. *J Bone Miner Res* 10:663–670.

Morishima-Kawashima M, Hasegawa M, Takio K, Suzuki M, Titani K, Ihara Y. (1993). Ubiquitin is conjugated with amino-terminally processed tau in paired helical filaments. *Neuron* 10:1151–1160.

Moscat J, Diaz-Meco MT. (2000). The atypical protein kinase Cs. Functional specificity mediated by specific protein adapters. *EMBO Rep* 1:399–403.

Moscat J, Diaz-Meco MT. (2011). Feedback on fat: P62-mTORC1-autophagy connections. *Cell* 147:724–727.

Nakaso K, Yoshimoto Y, Nakano T, Takeshima T, Fukuhara Y, Yasui K, Araga S, Yanagawa T, Ishii T, Nakashima K. (2004). Transcriptional activation of p62/A170/ZIP during the formation of the aggregates: Possible mechanisms and the role in Lewy body formation in Parkinson's disease. *Brain Res* 1012:42–51.

Okada K, Yanagawa T, Warabi E, Yamastu K, Uwayama J, Takeda K, Utsunomiya H, Yoshida H, Shoda J, Ishii T. (2009). The α-glucosidase inhibitor acarbose prevents obesity and simple steatosis in sequestosome 1/A170/p62 deficient mice. *Hepatol Res* 39:490–500.

Okatsu K, Saisho K, Shimanuki M, Nakada K, Shitara H, Sou YS, Kimura M, Sato S, Hattori N, Komatsu M, Tanaka K, Matsuda N. (2010). p62/SQSTM1 cooperates with Parkin for perinuclear clustering of depolarized mitochondria. *Genes Cells* 15:887–900.

Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, Øvervatn A, Bjørkøy G, Johansen T. (2007). p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem* 282: 24131–24145.

Perry G, Friedman R, Shaw G, Chau V. (1987). Ubiquitin is detected in neurofibrillary tangles and senile plaque neurites of Alzheimer disease brains. *Proc Natl Acad Sci USA* 84:3033–3036.

Ramesh Babu J, Lamar Seibenhener M, Peng J, Strom AL, Kemppainen R, Cox N, Zhu H, Wooten MC, Diaz-Meco MT, Moscat J, Wooten MW. (2008). Genetic inactivation of p62 leads to accumulation of hyperphosphorylated tau and neurodegeneration. *J Neurochem* 106:107–120.

Riley BE, Kaiser SE, Shaler TA, Ng AC, Hara T, Hipp MS, Lage K, Xavier RJ, Ryu KY, Taguchi K, Yamamoto M, Tanaka K, Mizushima N, Komatsu M, Kopito RR. (2010). Ubiquitin accumulation in autophagy-deficient mice is dependent on the Nrf2-mediated stress response pathway: A potential role for protein aggregation in autophagic substrate selection. *J Cell Biol* 191:537–552.

Rodriguez A, Durán A, Selloum M, Champy MF, Diez-Guerra FJ, Flores JM, Serrano M, Auwerx J, Diaz-Meco MT, Moscat J. (2006). Mature-onset obesity and insulin resistance in mice deficient in the signaling adapter p62. *Cell Metab* 3:211–222.

Seibenhener ML, Babu JR, Geetha T, Wong HC, Krishna NR, Wooten MW. (2004). Sequestosome 1/p62 is a polyubiquitin chain binding protein involved in ubiquitin proteasome degradation. *Mol Cell Biol* 24:8055–8068.

Shin J. (1998). P62 and the sequestosome, a novel mechanism for protein metabolism. *Arch Pharm Res* 21:629–633.

Spataro V, Norbury C, Harris AL. (1998). The ubiquitin-proteasome pathway in cancer. *Br J Cancer* 77:448–455.

Stumptner C, Fuchsbichler A, Heid H, Zatloukal K, Denk H. (2002). Mallory body–a disease-associated type of sequestosome. *Hepatology* 35:1053–1062.

Sundaram K, Shanmugarajan S, Rao DS, Reddy SV. (2011). Mutant p62P392L stimulation of osteoclast differentiation in Paget's disease of bone. *Endocrinology* 152:4180–4189.

Takamura A, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, Eishi Y, Hino O, Tanaka K, Mizushima N. (2011). Autophagy-deficient mice develop multiple liver tumors. *Genes Dev* 25:795–800.

Terry RD, Masliah E, Hansen LA. (1999). The neuropathology of Alzheimer disease and the structural basis of its cognitive alteractions. In: Terry RD, Katzman R, Bick KL, Sisodia SS (eds). *Alzheimer Disease*, 2nd edn, New York: Lippincott Williams & Wilkins, pp. 187–206.

Thompson HG, Harris JW, Wold BJ, Lin F, Brody JP. (2003). p62 overexpression in breast tumors and regulation by prostate-derived Ets factor in breast cancer cells. *Oncogene* 22:2322–2333.

Trompouki E, Hatzivassiliou E, Tsichritzis T, Farmer H, Ashworth A, Mosialos G. (2003). CYLD is a deubiquitinating enzyme that negatively regulates NF-κB activation by TNFR family members. *Nature* 424:793–796.

Tybl E, Shi FD, Kessler SM, Tierling S, Walter J, Bohle RM, Wieland S, Zhang J, Tan EM, Kiemer AK. (2011). Overexpression of the IGF2-mRNA binding protein p62 in transgenic mice induces a steatotic phenotype. *J Hepatol* 54:994–1001.

Vadlamudi RK, Shin J. (1998). Genomic structure and promoter analysis of the p62 gene encoding a non-proteasomal multiubiquitin chain binding protein. *FEBS Lett* 435:138–142.

Visconti MR, Langston AL, Alonso N, Goodman K, Selby PL, Fraser WD, Ralston SH. (2010). Mutations of SQSTM1 are associated with severity and clinical outcome in paget disease of bone. *J Bone Miner Res* 25:2368–2373.

Wooten MW, Geetha T, Babu JR, Seibenhener ML, Peng J, Cox N, Diaz-Meco MT, Moscat J. (2008). Essential role of sequestosome 1/p62 in regulating accumulation of Lys63-ubiquitinated proteins. *J Biol Chem* 283:6783–6789.

Zatloukal K, Stumptner C, Fuchsbichler A, Heid H, Schnoelzer M, Kenner L, Kleinert R, Prinz M, Aguzzi A, Denk H. (2002). p62 Is a common component of cytoplasmic inclusions in protein aggregation diseases. *Am J Pathol* 160:255–263.