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REVIEW ARTICLE

Sequestosome 1/p62: across diseases

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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.

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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.

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Declaration of interest

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