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REVIEW

Sequestosome 1/p62: a multi-domain protein with multi-faceted functions

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Abstract **The sequestosome 1/p62 protein has been implicated in the regulation of a multitude of cellular processes such as NF-κB signaling, NRF2-driven oxidative stress response, protein turnover through the ubiquitin-proteasome pathway and the autophagosome/lysosome pathway, apoptosis and cellular metabolism. The domain structure of p62 also reflects this functional complexity since the protein appears to be a mosaic of protein interaction domains and motifs. Deregulation of the level and function of p62 and/or p62 mutations have been linked to a number of human diseases including Paget's disease of the bone, obesity, liver diseases, tumorigenesis and neurodegenerative diseases such as amyotrophic lateral sclerosis and Alzheimer's disease. In this article, we review the current understanding of the involvement of p62 in cellular processes under physiologic and pathological conditions.**

Keywords sequestosome 1/p62, autophagy, ubiquitin-proteasome system, NF-κB signaling, Paget′s disease of bone, amyotrophic lateral sclerosis

Introduction

The p62 protein is a multifunctional protein that plays critical roles in many cellular processes including receptor-mediated signaling (Layfield, 2007; Xu et al., 2009; Goode and Layfield, 2010), NF-κB activation (Moscat et al., 2007), autophagy (Bjørkoy et al., 2005; Pankiv et al., 2007) and cellular metabolism (Rodriguez et al., 2006; Duran et al., 2011). P62 is also called "sequestosome 1" because of its ability to sequester ubiquitinated proteins to cytoplasmic inclusions (Shin, 1998). P62 has also been implicated in a number of diseases, such as Paget's disease of bone (PDB) (Hocking et al., 2004; Layfield, 2007; Najat et al., 2009; Rea et al., 2009; Goode and Layfield, 2010), liver diseases (Komatsu et al., 2007; Komatsu et al., 2010), obesity (Rodriguez et al., 2006; Duran et al., 2011), tumorigenesis (Mathew et al., 2009; Parkhitko et al., 2011) and neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) (Mizuno et al., 2006; Gal et al., 2007; Gal et al., 2009), Alzheimer's disease (AD) (Babu et al., 2005; Ramesh Babu et al., 2008; Kuusisto et al., 2001a), Huntington's disease (Nagaoka et al., 2004) and Parkinson's disease (Kuusisto et al., 2001a). In this review, we will focus on the role of p62 in various cellular processes under physiologic conditions as well as its involvement in different diseases.

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The domain structure of p62

We first illustrate the domain structure of p62, which will help understand its multi-faceted functions. The human p62 gene has 8 coding exons and encodes a protein of 440 amino acid residues. The domain structure of p62 is illustrated in Fig. 1 and it includes the Phox and Bem1 (PB1) domain, the ZZ-type zinc finger, the SOD1 mutant interaction region (SMIR), the TRAF6 binding (TB) motif, the microtubule-associated protein 1 light chain 3B (LC3) interaction region (LIR) and an ubiquitin association (UBA) domain (Moscat et al., 2007; Gal et al., 2009). The details of each domain are discussed below.

PB1 domain

The N-terminal Phox and Bem1 (PB1) domain of p62 can form homo-dimers and homo-oligomers of p62 itself, and can also form heterodimers with the PB1 domain in other proteins (Lamark et al., 2003; Wilson et al., 2003; Gal et al., 2009). The PB1 domain of p62 interacts with the PB1 domain of

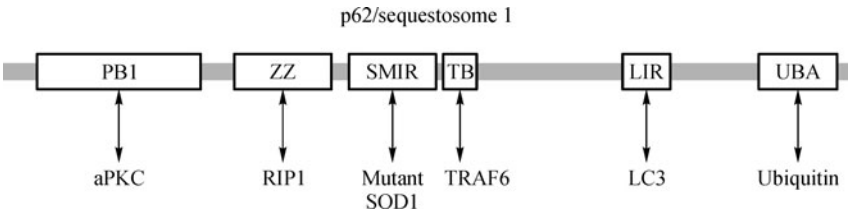

Figure 1 The domain structure of p62. The PB1, ZZ-type zinc finger, SMIR, TB, LIR and UBA domains and the responding molecules interacting with these domains are illustrated.

atypical protein kinase C (aPKC), MAPK/ERK kinase 5 (MEK5) and neighbor of BRCA1 gene 1 (NBR1) (Lamark et al., 2003). In particular, the interaction between p62 and aPKC plays an important role in NF-κB signaling described below (Moscat et al., 2007).

ZZ-type zinc finger

The ZZ-type zinc finger mediates the interaction of p62 with receptor-interacting serine/threonine-protein kinase 1 (also called RIP, RIP1 or RIPK1) (Sanz et al., 1999). This interaction also plays an important role in the TNFα-induced NF-κB signaling pathway (see below).

TB motif

The TRAF6 binding (TB) motif of p62 binds TRAF6, an E3 ubiquitin ligase in the RANKL-induced NF-κB signaling pathway (Sanz et al., 2000). The interaction of p62 with TRAF6 promotes K63-linked polyubiquitination of TRAF6 and of other substrates such as TrkA (Geetha et al., 2005; Wooten et al., 2005; Martin et al., 2006; Moscat et al., 2007).

LC3 interaction region (LIR)

The microtubule-associated protein 1 light chain 3B (LC3) plays a critical role in autophagy, specifically it is essential for autophagosome formation (Kabeya et al., 2000). The interaction between p62 and LC3 was reported and the LC3 interaction region (LIR) of p62 was located (Bjørkøy et al., 2005; Pankiv et al., 2007).

SMIR motif

We identified a motif that is essential for the interaction of p62 with mutants of the Cu/Zn superoxide dismutase (SOD1) linked to familial ALS (Gal et al., 2009). The SOD1 mutant interaction region (SMIR, residues 178-224) is the actual sequence that interacts with mutant SOD1. In particular, the conserved W184, H190 and the positively charged R183, R186, K187, and K189 residues within the SMIR are critical for the interaction because substitution of these residues with alanine significantly impaired the p62-mutant SOD1 interaction. In addition, oligomerization of p62 via the PB1 domain also plays an indispensable role in the p62-mutant SOD1 interaction (Gal et al., 2009). The ubiquitin-independent recognition of misfolded proteins by SMIR is illustrated in Fig. 2.

Ubiquitin association (UBA) domain

The C-terminal ubiquitin association (UBA) domain of p62 is responsible for binding to ubiquitin, which is the mechanism by which p62 interacts with polyubiquitinated proteins (Vadlamudi et al., 1996; Ciani et al., 2003; Seibenhener et al., 2004). When the polyubiquitin chain of a substrate protein binds to p62 via the UBA domain, the substrate can be either targeted for proteasomal or autophagosomal degradation. For the autophagy pathway, the p62-LC3 interaction discussed above is critical to the role of p62 as an adaptor linking substrate proteins to the autophagosomes (Pankiv et al., 2007). In addition, self-oligomerization of p62 by the PB1 domain could facilitate the process by sequestering multiple substrates simultaneously (Seibenhener et al., 2007; Komatsu and Ichimura, 2010). The ubiquitin-dependent recognition of misfolded proteins by UBA is also illustrated in Fig. 2.

The role of p62 in NF-κB signaling

P62 plays a critical role in ligand-induced NF-κB signaling. Perturbations in the signaling function of p62 have been implicated in PDB and cancer and might also play roles in other diseases. In this section we review the involvement of p62 in the TNFα- and RANKL-induced NF-κB signaling pathways. Additionally, p62 was also shown to play a role in the NF-κB component of the IL-1 (Sanz et al., 2000; Feng and Longmore, 2005) and neurotrophin (Wooten et al., 2001; Wooten et al., 2005) signaling pathways.

P62 and the TNFα-induced NF-κB signaling

Tumor necrosis factor α (TNFα) is an important cytokine involved in inflammation, cellular homeostasis, tumor progression and apoptosis (van Antwerp et al., 1998; Heyninck and Beyaert, 2001). TNFα functions through two

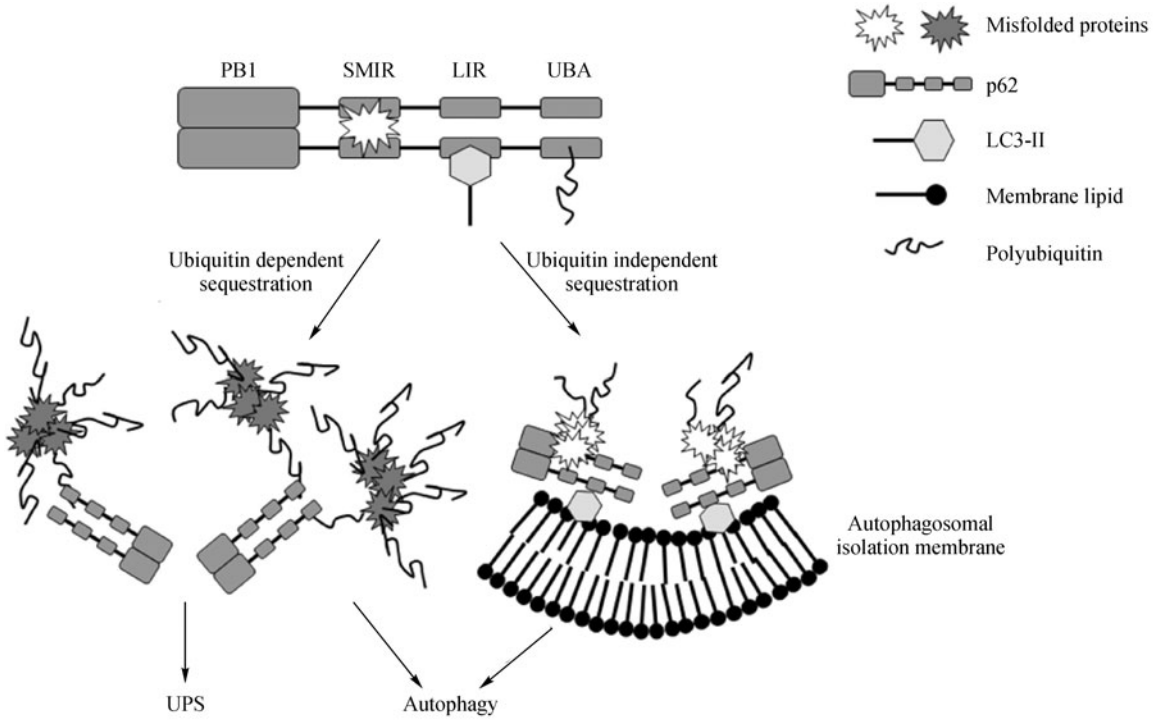

Figure 2 The role of p62 as a substrate receptor in protein turnover. The p62 protein targets substrates both to the ubiquitin-proteasome system and the autophagy-lysosome pathway. Misfolded proteins are recognized by p62 by ubiquitin-dependent or -independent mechanisms. The UBA domain is critical for the ubiquitin-dependent recognition of substrates. The SMIR motif is responsible for recognizing proteins such as familial ALS-related SOD1 mutants in an ubiquitin-independent manner. The LC3 interaction region (LIR) is necessary for targeting the substrates to autophagy.

receptors, TNF-R1 and TNF-R2 (Aggarwal, 2003). TNF-R2 is exclusively expressed on endothelial and immune cells. TNF-R1 is expressed in many cell types, and has been studied more extensively than TNF-R2 (Wu and Zhou, 2010). TNFα binds TNF-R1, leading to the recruitment of the adaptor protein TNF-R1-associated death domain protein (TRADD) which in turn recruits TRAF2 and the RIP1 protein kinase (Hsu et al., 1996; Micheau and Tschopp, 2003). RIP1 interacts with p62 through the ZZ-type zinc finger of p62 (Sanz et al., 1999). The atypical protein kinase C (aPKC) is activated by its interaction with p62 through the PB1 domain (Puls et al., 1997), phosphorylating and activating IKKβ (Lallena et al., 1999; Sanz et al., 1999; Sanz et al., 2000). TRAF2 is an E3 ubiquitin ligase (Shi and Kehrl, 2003; Habelhah et al., 2004) that polyubiquitinates RIP1 upon TNFα stimulation (Lee et al., 2004). Polyubiquitinated RIP1 plays a role in the recruitment of the TAK1/TAB1/TAB2/TAB3 complex that also phosphorylates IKK, leading to IKK activation (Blonska et al., 2005; Ea et al., 2006; Li et al., 2006). Activated IKK phosphorylates IκB, and in turn, IκB is polyubiquitinated and degraded by the proteasome. Transcription factor NF-κB is then released from IκB and translocates from the cytosol to the nucleus, activating the transcription of target genes (Wu and Zhou, 2010). The TNFα-induced NF-κB signaling is illustrated in Fig. 3.

P62 and the RANKL-induced NF-κB signaling

Receptor activator of nuclear factor kappa-B ligand (RANKL) is a cytokine that is highly expressed in bone marrow (Teitelbaum and Ross, 2003). RANKL-induced NF-κB activity controls normal osteoclastogenesis as well as the bone resorbing function of mature osteoclasts (Jimi et al., 2004; Teitelbaum and Ross, 2003; Layfield, 2007). Therefore, upregulation of RANKL-induced NF-κB signaling could at least in part explain the increase in osteoclastic activity in PDB (Layfield, 2007).

The binding of RANKL to RANK receptor induces the recruitment of TNF receptor-associated factor 6 (TRAF6) to the RANK receptor (Darnay et al., 2007; Lamothe et al., 2007). TRAF6 is a RING domain-containing E3 ubiquitin ligase (Lamothe et al., 2007; Yang et al., 2010; de Bie and Ciechanover, 2011). P62 is recruited to TRAF6 through its TRAF6 binding (TB) motif (Sanz et al., 2000), leading to aPKC activation, IKK phosphorylation, IκB phosphorylation and degradation and NF-κB nuclear translocation as detailed above for TNFα signaling. Furthermore, p62 was reported to facilitate the K63-linked polyubiquitination of TRAF6 by itself (Deng et al., 2000; Wooten et al., 2005). Polyubiquitinated TRAF6, similarly to RIP1, activates the TAB1/TAB2/TAK1 complex that activates IKK (Wang et al., 2001). The pathway is illustrated in Fig. 3.

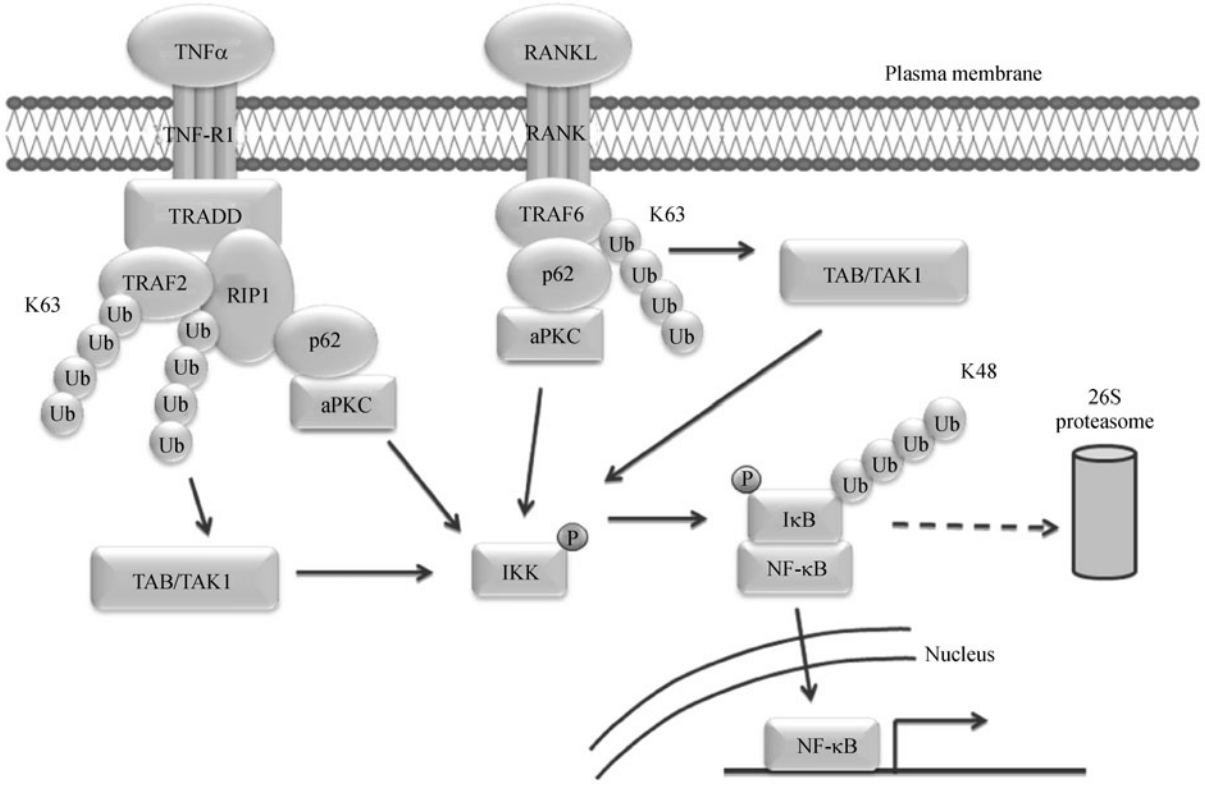

Figure 3 The role of p62 in the TNFα- and RANKL-induced NF-κB signaling pathways. The binding of TNFα to the TNF receptor induces the recruitment of TRADD to the receptor, which in turn recruits TRAF2 and RIP1. RIP1 recruits p62 and activates the phosphorylation of the IKK complex by aPKC. The binding of RANKL to RANK leads to the recruitment of TRAF6 which in turn recruits p62 and activates the aPKC-dependent phosphorylation of IKK and subsequent NF-κB nuclear translocation and activation. It is noted that polyubiquitinated RIP1 and TRAF6 can both activate the IKK complex through a p62 independent pathway involving the TAB/TAK1 complex.

The role of p62 in protein degradation

P62 plays a critical role in both the ubiquitin-proteasome system (UPS) and autophagy, the two major known protein degradation pathways in mammalian cells. P62 was reported to be a shuttling factor to the proteasome (Seibenhener et al., 2004; Babu et al., 2005; Geetha et al., 2008). Increasing amount of evidence suggests that p62 plays an important role in multiple steps of autophagy. Moreover, p62 can mediate the cross-talk between the UPS and autophagy. It was proposed that p62 accumulation after autophagy inhibition could further suppress the clearance of ubiquitinated proteins destined for proteasomal degradation (Korolchuk et al., 2010).

The p62 protein plays a critical role in autophagy as a cargo receptor. P62 is frequently detected in protein inclusions related to human diseases (Kuusisto et al., 2001a; Kuusisto et al., 2002; Zatloukal et al., 2002; Nagaoka et al., 2004). The depletion of p62 inhibited autophagic degradation of aggregation-prone polyglutamine-expanded huntingtin inclusions while p62 protected cells from cell death induced by polyglutamine-expanded huntingtin (Bjørkøy et al., 2005). Inhibition of autophagy caused elevated levels of p62 and induced more and larger p62 inclusions (Bjørkøy et al., 2005; Komatsu et al., 2007; Yue, 2007). It was found that p62 actually regulates the formation and autophagic removal of protein inclusions (Bjørkøy et al., 2005; Komatsu et al., 2007). p62 binds directly to LC3 through its LC3 interaction region (LIR) (Bjørkøy et al., 2005; Pankiv et al., 2007) that is critical to its ability to shuttle substrates to autophagosomes for degradation (Pankiv et al., 2007; Ichimura et al., 2008; Shvets et al., 2008). The C-terminal ubiquitin association (UBA) domain can interact with polyubiquitin chains (Vadlamudi et al., 1996). It was proposed that p62 targets ubiquitinated protein aggregates to autophagy through an interaction between its UBA domain and polyubiquitin (Pankiv et al., 2007). However, we found that the UBA domain of p62 was dispensable for the recognition of familial ALS-related mutant SOD1 (Gal et al., 2009). Instead, an internal sequence motif, the SMIR plays a critical role in mutant SOD1 recognition, suggesting that p62 might also target protein cargos for autophagic degradation via ubiquitin-independent mechanisms (Gal et al., 2009).

The steady-state level of p62 has recently been used as a marker of autophagic degradation activity. For instance, an elevated level of p62 would be interpreted as inhibition or

failure of autophagic activity (Klionsky et al., 2008). However, this involves the critical assumption that p62 biosynthesis is not itself regulated. It has been reported that p62 can be induced at the transcriptional level by various stresses including oxidative stress (Ishii et al., 1996; Jain et al., 2010) or proteasome inhibition (Kuusisto et al., 2001b). Thus, caution should be exercised when using the p62 level as a marker of autophagic activity.

The role of p62 in the regulation of the oxidative stress response

The NF-E2-related factor 2 (NRF2) - Kelch-like ECH-associated protein 1 (KEAP1) pathway is generally considered as one of the major cellular defense mechanisms against oxidative and electrophilic stresses (Itoh et al., 1997; Itoh et al., 1999; Wakabayashi et al., 2003). An important role for p62 in the regulation of NRF2 was reported recently by several groups (Copple et al., 2010; Jain et al., 2010; Komatsu et al., 2010; Lau et al., 2010). The target genes of NRF2 include antioxidant proteins and detoxification enzymes (Motohashi and Yamamoto, 2004) and p62 itself is also under NRF2 regulation (Jain et al., 2010). KEAP1 is a component of the Cullin-3-type ubiquitin ligase that ubiquitinates NRF2, targeting NRF2 to the 26S proteasome for degradation (McMahon et al., 2003; Cullinan et al., 2004; Kobayashi et al., 2004). P62 interacts with KEAP1 through a motif immediately C-terminal of the LIR motif named KEAP1 interacting region or "KIR" (Jain et al., 2010; Lau et al., 2010). The KIR motif within p62 interacts with the NRF2 binding site on KEAP1, thus competing with the interaction between NRF2 and KEAP1. Consequently, NRF2 level is elevated and transcription of the NRF2 target genes is activated (Jain et al., 2010; Komatsu et al., 2010; Lau et al., 2010). Moreover, the p62-KEAP1 interaction also sequesters KEAP1 to aggregates and promotes its degradation (Copple et al., 2010; Jain et al., 2010; Komatsu et al., 2010; Lau et al., 2010). Conversely, knockdown of p62 resulted in increased level of KEAP1, decreased level of NRF2 and decreased levels and function of several NRF2-targeted cell defense genes (Copple et al., 2010).

P62 and apoptosis

There is a rather complex relationship between autophagy and apoptosis (Gump and Thorburn, 2011). The cross-talk between the two processes is in part mediated by p62. Caspases are proteases that play essential roles in apoptosis. During apoptosis induction, caspases are activated in a sequential manner. Caspase-8 is an upstream protease in apoptotic activation cascades. The full activation of caspase-8 depends on molecular aggregation events. It was reported that p62 and the polyubiquitination of caspase-8 by CUL3 promoted caspase-8 aggregation, leading to its full activation and cell death (Jin et al., 2009). The crosstalk between autophagy and apoptosis might be complex since it was shown that p62 is a target for caspase-6 and –8 cleavage (Norman et al., 2010) and that caspase-8 can be degraded by autophagy (Hou et al., 2010).

The role of p62 in metabolic regulation

It was reported that p62 knockout (KO) mice ($p62^{-/-}$) developed mature-onset obesity, with the characteristics of leptin resistance, impaired glucose handling and insulin intolerance (Rodriguez et al., 2006). In $p62^{-/-}$ non-obese mice, the metabolic rate was significantly reduced compared with wild-type mice (Rodriguez et al., 2006). The mechanism leading to obesity in p62 KO mice has been proposed to be associated with the ERK pathway. The extracellular signal-regulated kinases (ERKs) are involved in signaling pathways which regulate cell proliferation and differentiation (Lewis et al., 1998; Bost et al., 2005). It was shown that early states of adipocyte differentiation in embryonic stem cells require the ERK signaling cascade (Bost et al., 2002). In ERK1 KO mice (Pagès et al., 1999), it was found that the adipogenesis was impaired in embryo fibroblasts and preadipocytes isolated from adult adipose tissue (Bost et al., 2005). Altogether, it was suggested that ERK1 contributes to adipocyte differentiation and adiposity (Bost et al., 2005). It was also reported that the basal activity of ERK was elevated in the fat from non-obese p62 KO mice (Rodriguez et al., 2006). In addition, it was found that embryo fibroblasts from p62 KO mice were more likely to differentiate into adipocytes compared with wild-type controls, which was disrupted by using inhibitors of the ERK pathway (Rodriguez et al., 2006). Altogether, it was proposed that p62 negatively regulates the basal ERK activity and adipocyte differentiation and loss of p62 results in the hyperactivation of ERK which leads to adipogenesis and obesity (Rodriguez et al., 2006).

P62, mTORC1 and autophagy were proposed to play important roles in adipogenesis (Rodriguez et al., 2006; Zhang et al., 2009; Moscat and Diaz-Meco, 2011). Several studies have shown that mTORC1 could positively modulate adiposity and lower energy expenditure (Um et al., 2004; Polak et al., 2008; Zhang et al., 2009; Moscat and Diaz-Meco, 2011). It was reported that p62 interacted with mTOR and raptor, components of mTORC1 (Kim et al., 2002; Duran et al., 2011; Moscat and Diaz-Meco, 2011). Furthermore, the interaction was nutrient-dependent and could activate mTORC1 (Duran et al., 2011), thus p62 is critical for mTORC1 activation by nutrients. The mTOR pathway also suppresses autophagy (Ravikumar et al., 2004; Jung et al. 2010), resulting in p62 accumulation, mTORC1 activation, and a positive feed-forward loop.

A more recent study showed that autophagy inhibition in adipose-specific Atg7 KO mice resulted in decreased obesity

and adiposity (Zhang et al., 2009). The results support that autophagy positively regulates adipogenesis. Since impaired autophagy causes p62 accumulation, p62 appears to negatively regulate adipogenesis, which is consistent with the earlier finding that loss of p62 leads to obesity (Rodriguez et al., 2006). However, the interplay of p62, mTORC1 and autophagy and their exact roles in adipogenesis are rather complicated and further investigation is necessary to fully elucidate the underlying mechanisms.

The role of p62 in human diseases

In addition to the obesity association discussed above, p62 is involved in a number of other diseases. These include PDB (Hocking et al., 2004; Layfield, 2007; Najat et al., 2009; Rea et al., 2009; Goode and Layfield, 2010), liver injury (Komatsu et al., 2010), cancer and neurodegenerative diseases such as ALS (Nakano et al., 2004; Mizuno et al., 2006; Gal et al., 2007; Gal et al., 2009; Fecto et al., 2011) and AD (Babu et al., 2005; Ramesh Babu et al., 2008). We will focus on discussing the role of p62 in neurodegenerative diseases and PDB in detail.

P62 and neurodegenerative diseases

P62 and ALS

The p62 protein is commonly present in pathological inclusions in ALS patients (Nakano et al., 2004; Mizuno et al., 2006). P62 immunopositive inclusions were reported in familial ALS cases caused by mutations in CHMP2B (Parkinson et al., 2006; Filimonenko et al., 2007; Rusten and Simonsen, 2008), ANG/angiogenin (Seilhean et al., 2009) and FIG4 (Ferguson et al., 2009). More recently, p62 immunopositivity was demonstrated in TDP-43 (Maekawa et al., 2009; Braak et al., 2010) and FUS inclusions (Deng et al., 2010; Matsuoka et al., 2010; Tateishi et al., 2010). We reported that p62 co-localized with SOD1- and ubiquitin-positive inclusions in the G93A SOD1 transgenic ALS mouse model (Gal et al., 2007).

It is noted that p62 is not always co-localized with ubiquitin in pathologic protein inclusions. In frontotemporal lobar degeneration with motor neuron disease (FTLD-MND) that shows overlapping symptoms with ALS, p62 was reported to co-localize with TDP-43 inclusions, but not with ubiquitin (Hiji et al., 2008). The result again suggests that p62 may participate in disease-related processes independent of polyubiquitin.

In the case of SOD1-mediated familial ALS, we found significant and age-dependent accumulation of the p62 protein in the G93A SOD1 transgenic mice (Gal et al., 2007). However, only a moderate trend of elevated p62 mRNA levels with marginal statistical significance was found in the spinal cords of ALS mice using real-time PCR (J. Gal and H. Zhu, unpublished). The p62 mRNA level was only approximately 20% higher in the G93A mice than in the WT SOD1 transgenic mice at the end stage of the disease. The moderate elevation of the p62 mRNA level did not match the accumulation of the p62 protein. Thus, the evidence suggested that the accumulation of p62 in ALS mice was probably due mostly to slowing of its turnover, which was indicative of a suppressed autophagy degradation activity.

We have reported that SOD1 positive inclusions in the spinal cord motor neurons in the G93A SOD1 transgenic mice were also positive for p62 and ubiquitin (Gal et al., 2007). In addition, p62 was co-immunoprecipitated with familial ALS mutants but not with WT SOD1 from both transgenic mouse spinal cord extracts and transfected cells (Gal et al., 2007; Gal et al., 2009). Since p62 is a critical adaptor protein targeting proteins to autophagic degradation, it is important to better define the mechanism by which p62 recognizes autophagic substrates such as mutant SOD1. It has been generally believed that p62 recognizes polyubiquitinated proteins via the interaction between its UBA domain and the polyubiquitin chain (Kim et al., 2008). However, to the best of our knowledge, ubiquitination of SOD1 has not been demonstrated to be required for its autophagic degradation. Interestingly, the UBA domain of p62 was dispensable for the p62-mutant SOD1 interaction, suggesting that p62 can recognize familial ALS mutants of SOD1 via an ubiquitin-independent mechanism (Gal et al., 2007; Gal et al., 2009).

We further identified two distinct regions within p62 that were essential to its binding to mutant SOD1: the N-terminal PB1 domain and a separate internal region we termed as "SOD1 mutant interaction region" or SMIR (Gal et al., 2009) (Fig. 1). The PB1 domain is required to ensure oligomeric status of p62. The SMIR is the region that interacted with mutant SOD1 and several conserved amino acid residues within SMIR were also demonstrated to be critical to the interaction. We also demonstrated that the SMIR and LIR were independent of each other, thus p62 can function effectively as an adaptor to target mutant SOD1 to autophagosomes via LC3. The existence of mutant SOD1 in acidic autolysosomes indeed decreased in cells lacking p62, suggesting the hypothesis that p62 functions as an adaptor between mutant SOD1 and the autophagy machinery (Gal et al., 2009). The ubiquitin-dependent and independent mechanisms by which p62 can recognize misfolded proteins such as mutant SOD1 are illustrated in Fig. 2.

Although we have only demonstrated the ubiquitin-independent interaction mechanism between p62 and the familial ALS mutants of SOD1, we speculate that this may represent a more general mechanism recognizing other misfolded or aggregated autophagic substrates. This hypothesis remains to be tested in future studies.

Since p62 positive inclusions have been observed in many subsets of ALS patients, it becomes tempting to speculate whether mutations in p62 could possibly be responsible for a subset of familial ALS. Mutations in p62 have been

implicated in Paget's disease of bone (see below), in which protein inclusions are also a pathological feature (Helfrich and Hocking, 2008). Recently, in a genetic screen of p62 in familial and sporadic ALS patients, nine missense mutations and one in-frame deletion mutation were found that were missing in a cohort of control subjects (Fecto et al., 2011). The mutations did not cluster in any apparent hotspot. Interestingly, the mutations found in the UBA domain, P392L, G411S and G425R were also reported in PDB (see below). It is intriguing that the coexistence of PDB and ALS symptoms was reported before (Varelas et al., 1997). These findings suggest that p62 mutations might represent a causative or risk factor in ALS.

Another link between ALS and PDB is the AAA ATPase valosin-containing protein (VCP) that is involved in vesicle trafficking and proteasomal degradation (Rabouille et al., 1995; Zhong et al., 2004). Mutations in VCP are a cause of inclusion body myopathy with early onset PDB and frontotemporal dementia (IBMPFD) (Watts et al., 2004). Defects in VCP are also the cause of amyotrophic lateral sclerosis type 14 with or without frontotemporal dementia (ALS14) (Johnson et al., 2010). Since both p62 and VCP are involved in protein degradation pathways and they are both implicated in ALS and PDB, it is consistent with the notion that clearance of misfolded proteins is critical and failure of maintaining protein homeostasis could be the underlying mechanism for the two diseases.

P62 and AD

Neurofibrillary tangles (NFTs) containing hyperphosphorylated and polyubiquitinated tau protein are generally believed to be a hallmark of Alzheimer's disease (AD) (Babu et al., 2005; Ramesh Babu et al., 2008). It was reported that in the AD hippocampus and cortex, p62 co-localized with ubiquitin and tau in NFTs (Kuusisto et al., 2002). In addition, TRAF6 and p62 co-localized with tau in the AD brain and p62 interacted with polyubiquitinated tau through its UBA domain (Babu et al., 2005). As described earlier, TRAF6 is an E3 ubiquitin ligase enzyme which catalyzes K63-linked polyubiquitination of itself and that of other proteins, thus it is within the expectation that tau was shown to be K63-polyubiquitinated by TRAF6 (Babu et al., 2005). It was also demonstrated that p62 was required for the targeting of tau for proteasomal degradation (Babu et al., 2005). Accordingly, it was proposed that decreased p62 level and proteasome activity might contribute to the accumulation of insoluble and aggregated K63-linked polyubiquitinated tau. This model is supported by the accumulation of aggregated insoluble K63-linked polyubiquitin chains in the brain of p62 KO mice (Wooten et al., 2008) and specifically by the abnormal accumulation of aggregated K63-ubiquitinated tau (Ramesh Babu et al., 2008).

CYLD is a deubiquitinating enzyme that inhibits TRAF6 ubiquitination (Jin et al., 2008). The interaction between CYLD and TRAF6 relies on p62 (Jin et al., 2008; Wooten et al., 2008). Therefore, it was proposed that loss of p62 might result in decreased activity of CYLD and increased K63-linked polyubiquitination of proteins which require TRAF6 as an E3 ubiquitin ligase enzyme (Wooten et al., 2008). Thus, it is plausible that in the brain of p62 KO mice, the hyperactivation of tau polyubiquitination might also be due to decreased CYLD activity.

P62 and Paget's disease of bone (PDB)

The most common genetic mutations found in classical PDB patients are in the p62 gene, located on chromosome 5 at the PDB3 locus (Watts et al., 2004; Layfield, 2007). Mutations in p62 have been associated with familial and sporadic PDB in up to 40% of the cases (Helfrich and Hocking, 2008). To date, over 20 PDB-associated p62 mutations have been identified (Goode and Layfield, 2010). Most of the p62 PDB mutations are either missense or truncating mutations in the ubiquitin-associated (UBA) domain (Layfield et al., 2004). There are few p62 PDB mutations outside the UBA domain (Goode and Layfield, 2010). Interestingly, one of the PDB-associated p62 mutations, D335E, was found in the LIR (Falchetti et al., 2009).

As we discussed earlier, p62 plays a critical role in the RANKL-induced NF-κB pathway. RANKL plays crucial roles in the differentation, survival and bone resorption activity of osteoclasts (Lacey et al., 1998; Quinn et al., 1998; Hsu et al., 1999; Durán et al., 2004). Accordingly, much of the attention in PDB research in recent years was focused on the potential involvement of p62 PDB mutations in RANKL signaling.

Many of the PDB mutations reside in the UBA domain of p62. They include P387L (Johnson-Pais et al., 2003), P392L (Hocking et al., 2002; Laurin et al., 2002), L394X (Hocking et al., 2004), E396X (Johnson-Pais et al., 2003), S399P (Cavey et al., 2006), M404T (Cavey et al., 2006), M404V (Falchetti et al., 2004; Hocking et al., 2004), G411S (Hocking et al., 2004) and G425R (Falchetti et al., 2004; Hocking et al., 2004). The three-dimensional structure of the p62 UBA domain (residues 387-436) was determined by protein NMR in 2003 (Ciani et al., 2003; Layfield and Hocking, 2004), which provided structural insights into the potential perturbation caused by the PDB mutations. Moreover, it was demonstrated that almost all of these mutations impaired K48-linked polyubiquitin binding by p62 *in vitro* (Cavey et al., 2006). Therefore, it was proposed that the disease mechanism in PDB involves a common loss of ubiquitin binding of p62.

However, more PDB-associated p62 mutations have been identified outside the UBA domain in recent years. A D335E missense mutation is located in the LC3 interaction region (LIR) of p62, about 50 amino acids away from the UBA domain (Falchetti et al., 2009; Goode and Layfield, 2010).

Other non-UBA mutations include P364S (Rea et al., 2009), A381V (Falchetti et al., 2009; Najat et al., 2009) and Y383X (Falchetti et al., 2009).

Several studies have reported that some PDB mutations, such as P364S (Rea et al., 2009), K378X (Rea et al., 2006), P392L (Rea et al., 2006; Chamoux et al., 2009) and E396X (Rea et al., 2006) increased the RANKL-induced NF-κB signaling. It was further suggested that increased NF-κB signaling rather than the impairment of ubiquitin binding may be essential in the pathogenesis of PDB associated with p62 mutations (Chamoux et al., 2009; Rea et al., 2009).

The exact mechanism by which p62 mutants increase NF-κB signaling is not well understood. A recent study reported that the de-ubiquitination enzyme CYLD interacted with wild type and the non-UBA mutant A381V p62 in osteoclast progenitor cells, but not with the UBA mutant P392L p62 (Sundaram et al., 2011). Expression of p62 P392L also resulted in increased levels of polyubiquitinated TRAF6 and phospho-IκB during osteoclast differentiation. These findings suggest that at least some p62 PDB mutations might perturb NF-κB signaling by altering CYLD activity and TRAF6 polyubiquitination.

In light of the above studies and our own unpublished work, we propose a model integrating the role of PDB-associated p62 mutants in both NF-κB signaling and autophagy. In the normal osteoclast cells under physiologic conditions, wild-type p62 has twofold functions that maintain a delicate balance. Wild-type p62 facilitates TRAF6 polyubiquitination and NF-κB signaling as described earlier. On the other hand, p62 also interacts with CYLD that reduces TRAF6 polyubiquitination and decrease NF-κB signaling (Jin et al., 2008). In the PDB patients or patients with complex symptoms associated with other diseases (such as IBMPFD discussed earlier), deficiency in UPS and/or autophagy is likely the underlying mechanism for inclusions of misfolded proteins (Helfrich and Hocking, 2008). Consequently, p62 is accumulated and NF-κB signaling is hyper-activated. Therefore, PDB-associated p62 mutations contribute to disease progression by synergistically affecting both NF-κB signaling and autophagy.

P62 and liver injury

P62 is a major component of the pathological protein inclusions found in a range of liver diseases (Denk et al., 2006). It has been proposed that the impairment of autophagy is responsible for the formation of cytoplasmic protein inclusions and liver injury. Knocking out p62 in mice suppressed the appearance of protein inclusions in hepatocytes and markedly attenuated liver injury caused by autophagy deficiency (Komatsu et al., 2007). As discussed earlier, p62 is a selective substrate of autophagy and regulates autophagy activity as well. Autophagy deficiency would cause the accumulation of p62 and subsequent stabilization of NRF2, which will activate the transcription of NRF2 target genes (Copple et al., 2010; Jain et al., 2010; Komatsu et al., 2010; Lau et al., 2010). It was proposed that the toxicity of p62 accumulation in the liver might be associated with the chronic hyperactivation of NRF2 target genes (Komatsu et al., 2010).

The role of p62 in tumorigenesis

Autophagy and p62 play important roles in maintaining the balance between cell death and survival that is critical to determine the outcome of tumorigenesis. Autophagy deficient mice develop multiple tumors and p62 accumulation contributes to tumor progression (Takamura et al., 2011). Similarly, it was found that the tumorigenesis in tuberous sclerosis complex (TSC), a tumor suppressor syndrome characterized by benign tumors in multiple organs, is also dependent on p62 (Parkhitko et al., 2011). As discussed earlier, p62 plays an important role in the regulation of NF-κB signaling and most of the target genes upregulated by NF-κB promote cellular survival (Duran et al., 2008). Thus, p62 is an important NF-κB mediator in tumorigenesis. It was also reported that the elevated p62 level caused by the autophagy defects commonly found in human tumors is sufficient to alter NF-κB regulation and downstream gene expression and to promote tumorigenesis (Mathew et al., 2009).

Future perspectives

The multi-domain protein p62 clearly plays a critical role in many important intertwined cellular processes, particularly in autophagy and NF-κB signaling. Dysregulation of p62 function can cause a number of human diseases. However, many important questions about p62 still require better understanding. These include, but are not limited to, the following: which proteins are targeted by p62 to autophagosomes for degradation? Do all p62 UBA domain mutations and non-UBA mutations affect the NF-κB signaling in a similar fashion or not? What are the cellular consequences of p62 PDB mutations, aside from perturbed NF-κB signaling and autophagy? Through what mechanisms do p62 mutations cause PDB and ALS? What is the exact role of p62 and autophagy in adipogenesis? Addressing these questions would help us better understand the function and regulation of p62 and its roles in the related diseases. Considering that p62 is associated with an increasing number of diseases, fully understanding the molecular functions of p62 may help to find new therapeutic approaches to treat these diseases in the future.

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