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The Role of p62/SQSTM1 in Liver Physiology and Pathogenesis

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Abstract

p62/sequestosome-1/A170/ZIP (hereafter referred to as p62) is a scaffold protein that has multiple functions, such as signal transduction, cell proliferation, cell survival, cell death, inflammation, tumorigenesis, and oxidative stress response. While p62 is an autophagy substrate and is degraded by autophagy, p62 serves as an autophagy receptor for selective autophagic clearance of protein aggregates and organelles. Moreover, p62 functions as a signaling hub for various signaling pathways, including NF-κB, Nrf2 and mTOR. In this review, we discuss the pathophysiological role of p62 in the liver, including formation of hepatic inclusion bodies, cholestasis, obesity, insulin resistance, liver cell death and tumorigenesis.

Keywords

p62/SQSTM1; Autophagy; Liver

Introduction

p62, also known as sequestosome-1(SQSTM1), A170, and zeta-interacting protein (ZIP), is a scaffold protein with multiple domains that functions in signal transduction, cell proliferation, cell survival, cell death, inflammation, tumorigenesis, and in the oxidative stress response [1, 2]. Recent autophagy studies have revealed that p62 is an autophagy substrate that also serves as an autophagy receptor for protein aggregates and damaged or excess organelles as well as for invading microbes [3–5]. At physiological conditions, p62 plays an important role in maintenance of bone and metabolic homeostasis based on studies from p62 knockout (KO) mice [6, 7]. In addition, mutations of p62 have been found in human Paget's disease of bone, which is a chronic metabolic disorder characterized by increased bone turnover, further underscoring the relevance of p62 in human diseases [8]. Due to the rapid research progress on autophagy and p62, p62 has been extensively reviewed in regard to its role in selective autophagy [9, 10], metabolic homeostasis [11] and cancer [1]. Therefore, our current review will discuss the role of p62 in liver physiology and pathology.

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Historical Aspect of p62 and its Association with Autophagy and Liver Diseases

When searching for Src homology 2 (SH2) domain binding proteins, p62 was first identified as an unknown 62 kDa protein that bound to the SH2 domain of $p56^{lck}$ in 1995 by Shin [12]. Shin first cloned human p62 cDNA in 1996 [13]. He also found that p62 preferentially bound to ubiquitinated protein aggregates, which led him to coin the name “sequestosome” (SQSTM1) for p62 [14]. Using differential screening, Ishii *et al.* cloned cDNA encoding a novel oxidative stress protein, which they called A170, from mouse peritoneal macrophages [15]. A170 is approximately 90% identical to human p62. Later, using yeast two-hybrid screening, p62 was discovered to interact with the atypical protein kinase C (aPKC) [16, 17]. Puls *et al.* then successfully cloned the rat orthologue of p62, cytoplasmic ZIP, which functions as a scaffold protein responsible for regulating PKCζ signal transduction [17]. The endogenous and ectopically expressed p62 protein was originally found to display a punctate pattern when anchored together with aPKCs in the endosomal-lysosomal compartment, which was the earliest implication that p62 was associated with the autophagy-lysosomal pathway [16]. Subsequently, p62 was found to co-localize with the autophagy marker, the microtubule light chain 3 (LC3), and is degraded by autophagy [18]. Johansen’s group and Komatsu’s group independently showed that p62 directly binds to LC3 via a conserved LIR (LC3 interacting region) sequence, which is a motif required for autophagic degradation of p62 [19, 20]. Moreover, p62 levels were also highly increased in autophagy-deficient mouse livers, which further confirmed that p62 was an autophagy substrate [21].

When Denk and Zatloukal’s group tried to characterize the nature of intracytoplasmic hyaline bodies (IHBs), which are common inclusions in hepatocellular carcinoma cells, they found that one of the major components of the IHBs had an approximate molecular weight of 62 kDa by using two-dimensional gel electrophoresis [22]. Sequence analysis revealed that this 62 kDa protein was identical to p62, which was reported by Shin’s group [13]. This study was the first to link the accumulation of p62 to human pathological disease. The same group subsequently discovered that p62 was also a major component in Mallory-Denk bodies (MDBs), which are often associated with alcoholic and non-alcoholic steatohepatitis [23, 24]. In addition, they discovered that p62 was a component of α1-antitrypsin deficiency aggregates in hepatocytes, as well as in non-liver tissues, such as Lewy bodies in Parkinson’s disease and neurofibrillary tangles in Alzheimer’s disease [24]. Whether p62 is required for the formation of the inclusion bodies in the liver is currently not clear. It is possible that p62 is in these inclusions to serve as an autophagy receptor for their autophagic removal. Moreover, it has been shown that impaired clearance of p62 positive inclusions may promote liver tumorigenesis (see below discussion).

Structure and Multiple Functions of p62

Ongoing studies continue to discover functional roles of p62 as novel interaction domains are defined (Figure 1). The protein weight of 62 kDa gave p62 its name. Six distinct functional domains have been defined within the p62 protein structure and include the following: Phox/Bemp1 (PB1) domain, ZZ zinc finger region, tumor necrosis factor (TNF)

associated receptor-6 (TRAF6) binding (TB) domain, LIR, Kelch-like ECH-associated protein 1 (Keap1) interacting region (KIR) and ubiquitin binding domain (UBA) [4, 25]. p62 also possesses two regions that are required for interaction with p38 mitogen-activated protein kinase (MAPK), which are within residues 173–182 and 335–344, respectively [26, 27]. The p62 protein has two nuclear localization signal (NLS) sequences, a nuclear export signal (NES) sequence [28], and two PEST sequences rich in proline (P), glutamic acid (E), serine (S) and threonine (T) with putative phosphorylation sites [13, 15, 17], which are hypothesized to act as signaling peptides for protein degradation. The nuclear shuttling of p62 allows p62 to localize to nuclear protein aggregates such as promyelocytic leukemia bodies and may recruit proteasomes to the nuclear inclusions for their degradation [28]. These various domains reveal that p62 has multiple roles in signaling amplification and suppression, protein and organelle quality control, and response to oxidative stress. As discussed above, p62 was originally found to interact with aPKC through its N-terminal PB1 domain [6, 17, 29]. The PB1 domain also interacts with other protein kinases including ERK, MEKK3, and MEK5 [1, 9, 30, 31] and plays a role in p62 self- and hetero-oligomerization with other PB1 domain containing proteins, such as the neighbor of BRCA gene 1 protein (NBR1) [9]. Moreover, PB1-mediated p62 self-oligomerization is essential for its localization to the autophagosome formation site, a process that is independent of LC3 localization [32]. It has been suggested that p62 may serve as an adaptor protein to recruit Atg proteins for initiation of autophagosome formation. However, this is controversial because starvation-induced autophagosome formation is normal in p62 KO cells [21].

The ZZ zinc finger region is located next to the PB1 domain and is responsible for binding to the receptor interacting protein (RIP), a TNF-α signaling adaptor protein [33, 34]. RIP is a serine/threonine kinase that regulates TNF-α receptor 1-mediated activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) through its interaction with TNF receptor type 1-associated death domain protein (TRADD) [33]. In addition to the ZZ domain, the TB domain interacts with TRAF-6, a lysine 63 (K63) E3 ligase that also regulates NF-κB activation. In response to interleukin-1 (IL-1) and Receptor Activator of NF-κB ligand (RANKL), p62 undergoes self-oligmerization through its PB1 domain, which promotes K63 ubiquitination of TRAF-6 and results in NF-κB activation and a subsequent inflammatory response [6, 29].

Pankiv *et al.* [20] and Ichimura *et al.* [19] independently identified the autophagy effecter protein LC3 and other members of this family of proteins, such as gamma-aminobutyric acid receptor-associated protein (GABARAP) and GABARAP-like molecules, as binding partners for p62. The LIR is a short acidic 11 peptide sequence located within amino acids 337–343 that is required for p62-LC3B interaction. The LIR sequence was further characterized and found to contain conserved aspartic acid and tryptophan residues, which are required for interaction with LC3B. This LC3B binding motif was defined as D(D/E) (D/E)WT and is located near the C-terminus. The LIR is required for autophagic degradation of p62, and impairment of p62-LC3 interaction leads to formation of ubiquitin and p62 positive aggregates [19, 20].

Three independent groups reported that p62 directly interacts with the Kelch-repeat domain of Keap1, and the term KIR was coined by Komatsu's group [19, 35, 36]. The KIR contains the specific conserved amino acid sequence, DPSTGE, and it is located immediately following the LIR in the C-terminus between amino acids 347–352. The DPSTGE sequence is very similar to the DEETGE sequence in nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which is responsible for interaction with Keap1's kelch domain. Under normal conditions, Keap1 negatively regulates Nrf2 by promoting its ubiquitination, resulting in the rapid degradation of Nrf2 by the ubiquitin proteasome system [37]. p62 competes with Nrf2 for binding to Keap1, and interaction of p62 with Keap1 results in dissociation of Nrf2 from Keap1, which allows for Nrf2 translocation to the nucleus where it induces transcription of detoxification enzymes and oxidative stress response genes [19, 35, 36]. Therefore, p62 serves as a positive regulator for Nrf2 activation. The role of p62 in regulation of Nrf2 is discussed in more detail in a later section.

Vadlamudi *et al.* [38] first demonstrated that p62 interacts with ubiquitin using a yeast two-hybrid system and ubiquitin-conjugated Sepharose beads. Between amino acids 386–434 within its C-terminus, p62 binds non-covalently to both mono- and poly-ubiquitinated proteins via its UBA domain. Because of its unique binding to ubiquitinated proteins together with its ability to bind LC3, p62 has been demonstrated to be an important receptor protein for selective autophagic clearance of ubiquitinated protein aggregates and organelles [5, 9]. The importance of the UBA domain of p62 has also been underscored by the findings that mutations of the UBA domain are frequently associated with familial and sporadic Paget's disease of bone, which is a chronic and metabolic disorder characterized by increased bone turnover [39–41]. It is well known that RANKL and its receptor RANK play an important role in regulating osteoclast differentiation, activity, and survival by activating NF-κB [6]. It has been suggested that p62 forms a complex with TRAF-6 and aPKC, which is critical for RANKL-induced NF-κB activation [6, 42, 43]. Mutations in the UBA domain results in loss of UBA function and also activate TRAF6–NF-kB signaling, which results in increased osteoclastogenesis [8, 39, 44]. p62 KO mice also show a similar phenotype reminiscent of Paget's disease with impaired NF-κB activation [6], further supporting that p62 is an important mediator regulating bone homeostasis.

Transcriptional and Post-translational Regulation of p62

Sqstm1 has been shown to be transcriptionally regulated by Nrf2 [35, 45] and Farnesoid X Receptor (FXR) [46]. It has also been suggested that *Sqstm1* is a target gene of NF-kB and AP-1 [38]. *Nrf2* binds to an antioxidant response element (ARE) within the *Sqstm1* gene promoter region and induces its transcription during oxidative stress [35, 45]. In addition to regulation of *Sqstm1* gene expression by Nrf2, p62 protein accumulation stabilizes Nrf2 activation, which allows for nuclear translocation and induction of target gene transcription by Nrf2, as previously discussed. Therefore, p62 establishes a positive feedback loop for regulating its own gene transcription through stabilizing Nrf2 activation [35].

The nuclear receptor FXR also regulates *Sqstm1* gene transcription. We previously found that FXR binds to the *Sqstm1* gene in both the liver and ileum using chromatin immunoprecipitation (ChIP). In addition, luciferase activity was enhanced when HepG2

cells were transfected with a *Sqstm1* fragment containing the FXR binding site, and this luciferase activity was lost when cells were transfected with a *Sqstm1* fragment containing a mutant FXR binding site. However, the FXR agonist GW4064 induced *Sqstm1* mRNA and protein expression in the ileum but not in the liver, which suggests that FXR regulates *Sqstm1* in a tissue-specific manner [46].

The *Sqstm1* gene has been shown to contain binding sites for several other transcription factors involved in mitogen signaling, monocyte differentiation, and inflammation. For example, the *Sqstm1* gene contains binding sites for *c-myc* and Sp1 [47]. The promoter region also contains three TPA response elements (TRE) for binding of Fos- and Jun-related bZip proteins or Jun homodimers along with two distinct binding sites for Ets-1 family transcription factors, which are induced by mitogenic signal transduction pathway proteins or serum, as well as by monocyte differentiation pathway factors [38]. However, transcriptional activation of *Sqstm1* by these transcription factors has not been shown, and it would be interesting to investigate if these transcription factors also regulate expression of *Sqstm1*/p62 in the liver.

Expression of *Sqstm1*/p62 mRNA and protein is also induced pharmacologically. For example, proteasome inhibitors and hydrogen peroxide have both been shown to induce *Sqstm1* expression in Neuro-2a cells and murine macrophages, respectively [48, 49]. Moreover, it was reported that Resveratrol increases the expression of *Sqstm1* by activating the JNK pathway in chronic myelogenous leukemia (CML) cells, although it is not clear how JNK increased *Sqstm1* expression [50].

In addition to transcriptional regulation, p62 has also been found to be posttranslationally regulated. For example, p62 has been found to be phosphorylated by casein kinase 2 (CK2) on S403 within the UBA domain, which enhances the binding of p62 to ubiquitin chains and leads to increased autophagic clearance of ubiquitinated proteins [51]. Moreover, p62 is phosphorylated by cyclin- dependent kinase 1 (CDK1) at T269 and S272, which is necessary to maintain appropriate cyclin B1 levels for cell cycle regulation. In response to Ras-induced transformation, the nonphosphorylatable mutant of p62 leads to a faster exit from mitosis and enhanced cell proliferation and tumorigenesis [52].

Furthermore, intracellular p62 protein levels can be regulated by two intracellular protein degradation pathways: autophagy and the ubiquitin proteasome system (UPS). As discussed above, p62 directly interacts with LC3 through its LIR and is degraded during autophagy induction [18, 20]. Moreover, p62 protein levels are accumulated in autophagy-deficient mouse livers and neurons, indicating that basal autophagy may constitutively degrade p62 [21, 53]. In the UPS pathway, p62 recruits ubiquitinated proteins and targets them for degradation by proteasomes, and p62 is also degraded in this process. p62 shuttles ubiquitinated proteins to the proteasome, and a decrease in p62 levels leads to impaired proteasomal degradation and subsequent accumulation of ubiquitinated proteins [54]. These data argue that p62 plays a role in recruiting ubiquitinated proteins not only to autophagosomes, but also to proteasomes. Taken together, intracellular p62 expression levels are regulated transcriptionally and posttranslationally, and posttranslational modifications of p62 can further enhance p62-

mediated removal of ubiquitinated proteins via autophagy. In addition, p62 protein levels are regulated by both autophagy and the proteasome.

p62 Serves as a Receptor for Protein Aggregates and Damaged or excess Organelles

Autophagy was generally thought to be a non-selective lysosomal degradation pathway; however, accumulating evidence now supports that autophagy can be selective. Selective autophagic degradation of intracellular misfolded proteins and damaged/or excess organelles plays an important role in regulating cellular homeostatic function [5, 9, 10, 55]. Selective autophagy is mediated by autophagy receptors. An ideal autophagy receptor is able to interact directly with both the cargo that is targeted for degradation and the autophagy machinery. Increasing evidence now demonstrates that protein ubiquitination is a prerequisite for cargo to be “tagged” for autophagic recognition. An autophagy receptor protein is defined as a protein that is able to bind to ubiquitinated cargos, such as protein aggregates, and LC3 protein through its LIR [9]. p62 has been identified as one of many autophagy receptors and p62 may promote autophagic degradation of cargos in at least three mechanisms. First, p62 is directly associated with early autophagosome formation by recruiting Atg proteins to the autophagosome formation site through its PB1 domain independent of its LIR [32]. Second, p62 binds to ubiquitinated proteins to form protein aggregates via its UBA domain. Third, p62 also directly interacts with LC3 via its LIR motif. Therefore, p62 may facilitate delivery of autophagic cargos to the autophagosome formation site due to its association with the early autophagosome membrane. p62 may also further recruit ubiquitinated cargos to the autophagosome via its interaction with both ubiquitin and LC3 for selective degradation [9, 20]. These selective cargos include protein aggregates, peroxisomes, mitochondria and invading intracellular bacteria [9, 56, 57].

How are misfolded/mutant proteins and protein aggregates selectively removed by autophagy? It has been proposed that a cargo receptor complex, which includes the tagged substrate, a receptor, a scaffold protein and an Atg8 family protein, is involved in the selective removal of misfolded/mutant protein aggregates [9]. p62 has been demonstrated to concentrate ubiquitinated proteins to form aggregates before they become degraded by autophagy. LC3 and p62 form a shell around huntingtin protein aggregates, suggesting that p62 may serve to mark these aggregates for autophagic degradation [18]. Indeed, over-expression of p62 reduced TAR DNA binding protein-43 (TDP-43) protein aggregates, a signature protein in inclusions seen in neurodegeneration related diseases. In contrast, siRNA knockdown of p62 levels or over-expression of a p62 deletion mutant lacking the UBA domain increased apoptosis in cells expressing mutant human huntingtin protein [18]. In addition to huntingtin protein, it is well-known that p62 is associated with MDBs, the most common inclusion bodies in the liver as discussed above. However, the role of p62 in regulating the formation of MDBs is not clear. Experiments using p62 KO mice would help to elucidate whether p62 may also help remove protein aggregates in the liver as a protective mechanism.

In addition to p62, two other p62 interacting partners, the neighbor of Brca1 gene (NBR1) and autophagy linked FYVE protein (ALFY), are also needed for constitutive autophagic

degradation of ubiquitinated protein aggregates [9, 58]. NBR1 is a 966-amino acid protein that has similar domain organization to p62, which also has an N-terminal PB1 domain, a ZZ-type zinc finger, and a C-terminal UBA domain. Unlike p62, NBR1 cannot polymerize via its PB1 domain, but it binds to p62 via this domain. Endogenous NBR1 was found in the same complex with p62 and Atg8 family proteins, and it also accumulates in Atg7 and Atg7/p62-double KO (DKO) mouse livers as well as in human alcoholic Mallory bodies [59]. ALFY is a giant 400 kDa scaffold protein that contains a FYVE domain, which is ubiquitously expressed and conserved from yeast, nematodes, and flies to mammals [60]. While ALFY binds with PtdIns(3)*P*, which is similar to other FYVE-domain proteins, ALFY was not found on endosomes and was found to be redistributed from the nucleus to autophagic membranes in response to starvation or proteasome inhibition [60]. Unlike p62 and NBR1, ALFY does not accumulate in autophagy-deficient cells, suggesting that ALFY is not a selective autophagy substrate. ALFY is highly expressed in the brain and has been shown to play a role in the clearance of mutant α-synuclein. Drosophila lacking the ALFY homolog Blue Cheese (bchs) accumulate ubiquitin positive inclusions and have a neurodegenerative phenotype and reduced life span [61]. Both p62 and ALFY are found in promyelocytic leukemia nuclear bodies (PML-NBs), and the localization of ALFY to PML-NBs is dependent on p62 and vice versa [61]. Although no LIR region was identified in ALFY, ALFY interacts with the Atg5-Atg12 complex and may stimulate LC3-PE conjugation. Moreover, the FYVE domain on ALFY may also recognize PI3P membranes and perhaps plays a role in autophagosome membrane recruitment [58]. Therefore, it has been suggested that ALFY might work as a scaffold protein to facilitate the assembly of p62 positive aggregates and target these protein aggregates for autophagic degradation [58, 61]. The role of ALFY in the formation of liver protein aggregates is currently unclear, but ALFY is expressed in the liver [60], indicating that it may indeed play a role in liver protein aggregate formation.

Histone deacetylase 6 (HDAC6) mainly resides in the cytoplasm and is another crucial component required for selective protein aggregate clearance [62–64]. HDAC6 possesses two functional deacetylase domains. It also contains a ubiquitin-binding zinc-finger domain of the BUZ class that binds with ubiquitin and is essential for HDAC6-mediated selective degradation of protein aggregates [63, 64]. Ubiquitin-positive protein aggregates are readily found in HDAC6-deficient mouse brains leading to neurodegeneration in these animals, indicating that HDAC6 plays an important role in removing cellular protein aggregates [63]. It is well known that lysine (K) 48-linked polyubiquitination directs proteins for proteasome-mediated degradation, whereas K63-linked polyubquitination is recognized by both HDAC6 and p62 and is preferentially degraded by autophagy [5, 64, 65]. Unlike p62 and NBR1, HDAC6 does not contain a LIR but can interact with microtubules and F-actin cytoskeleton via its ubiquitin binding domain [64]. It has been shown that the interaction of HDAC6 with the cytoskeleton plays a critical role for transporting aggregated proteins to the peri-nuclear microtubule-organizing center (MTOC), which is where most lysosomes are located [63]. While HDAC6 per se is not essential for autophagy induction, it controls the fusion of autophagosomes with lysosomes by regulating the actin network [63]. Therefore, it seems that HDAC6 regulates protein aggregate degradation by not only transporting protein aggregates to the MTOC, but also by regulating autophagy at the late autophagosome maturation

step [63, 64]. Taken together, it seems that p62 can coordinately work with ALFY and HDAC6 to regulate selective clearance of protein aggregates by autophagy (Figure 2).

In addition to the removal of ubiquitinated protein aggregates, p62 also plays an important role in selective removal of mitochondria [66, 67], peroxisomes [56], midbody ring structures [68], and bacteria [57, 69]. When mitochondria are depolarized by uncouplers such as CCCP, the ubiquitin E3 ligase Parkin is recruited from the cytosol to damaged depolarized mitochondria [66, 70]. The recruitment of Parkin to mitochondria also requires the PTEN-induced putative protein kinase 1 (PINK1), a serine/threonine kinase that contains a mitochondrial targeting sequence, which is degraded in healthy mitochondria by PARL, a mitochondria intramembrane protease [71–73]. Although it remains unclear how PINK1 recruits Parkin to mitochondria, it seems that the kinase activity of PINK1 is required for Parkin mitochondrial translocation and for increasing ubiquitination activity of Parkin [74, 75]. Following its mitochondrial translocation, Parkin induces two types of polyubiquitin chains, which appear to have different functions. Parkin-mediated K48 chains on specific outer mitochondrial membrane proteins trigger proteasome-dependent degradation of these proteins, which include Miro, Mitofusin1/2, hFis1, and Tom20 as well as others [76, 77]. The Parkin-mediated proteasome-dependent degradation of these outer mitochondrial membrane proteins seems to be required, but it is not sufficient for mitophagy. For example, proteasomal degradation of Mitofusin1/2 causes mitochondrial fragmentation, which may make them more readily enveloped by autophagosomes and thus, promote their clearance by mitophagy [55, 76]. In a second model, Parkin also induces K63 and K27 ubiquitin chains that initiate a signaling cascade to activate mitophagy, which may involve an autophagy receptor such as p62 [67]. VDAC1, a component of the mitochondrial permeability transition pore, has been reported to be a target for Parkin-mediated K63 and K27 poly-ubiquitination and promotes mitophagy [67]. However, the role of VDAC1 ubiquitination in mitophagy is controversial because it was found that VDAC1 is dispensable for mitophagy by another study [78]. It is possible that the presence of ubiquitination on the mitochondria, rather than a particular ubiquitinated protein on the mitochondria, is important and sufficient for the induction of mitophagy. This notion is supported by observations that p62 is recruited to depolarized mitochondria in a Parkin-dependent manner, while VDAC1 and VDAC3 are dispensable for p62 mitochondrial translocation [78]. Once on the mitochondria, p62 serves as a receptor to further recruit LC3-positive autophagosomes to p62-ubiquitin decorated mitochondria for their degradation. However, the role of p62 in mitophagy is still controversial. While studies from siRNA-mediated knockdown of p62 suggested that it plays a role in Parkin-mediated mitophagy, studies using genetic p62 KO cells suggest that p62 is required for Parkin-induced mitochondrial clustering, but not for mitophagy [78]. The discrepancy could be due to the lack of reliable quantitative assays for mitophagy because immunostaining for the mitochondrial outer membrane protein Tom20 was used for a marker for mitophagy in these studies [66, 67, 78]. It is now known that Tom20 is largely degraded through Parkin-mediated proteasome-dependent degradation and not by autophagy [76, 77]. While more reliable quantitative assays for mitophagy are desperately needed, it seems that the degradation of mitochondria matrix proteins, such as heat shock protein (HSP60), is more dependent on autophagy [76]. Moreover, a recent study using adipocyte-specific p62 KO mice suggested that p62 may regulate mitochondrial homeostasis

through a p38-mediated transcriptional program [7]. More studies are definitely needed to further understand the role of p62 in mitophagy, preferably when more reliable quantitative assays are available *in vivo*. Moreover, we recently demonstrated that Parkin is ubiquitously expressed in many tissues including liver in mice [55], but the exact role of Parkin-p62 in mitophagy in the liver is not clear.

p62 in Liver physiology and Pathology

Liver is one of the most dynamic and metabolically active organs, and increasing evidence indicates that autophagy in the liver plays an important role in maintaining the levels of blood glucose and amino acids. As discussed above, p62 was found to be a major component of the liver protein aggregates, MDBs, which are the signature manifestation of alcoholic liver disease [22, 79]. Thus, p62 may play an important role in the regulation of protein aggregates in the liver [80]. As a major signaling adapter protein, p62 has also been shown to regulate the Nrf-2-mediated antioxidant response, as previously discussed. In addition, p62 has been shown to regulate NF-κB activation, mTOR and MAPK signaling pathways. As a result, p62 has emerged as a major factor in regulating obesity, insulin sensitivity, energy homeostasis, cholestasis and liver tumorigenesis. The role of p62 in these individual pathologies is discussed in more detail below.

p62 Regulation of Protein Aggregates in the Liver: MDBs and IHBs

MDBs are intracellular protein aggregates that are composed mainly of p62, ubiquitin, chaperones, proteasomal subunits, and intermediate filaments [24, 80]. MDBs are hallmarks of liver pathogenesis, such as chronic alcoholic steatohepatitis and non-alcoholic steatophepatitis [23]. Furthermore, other cytoplasmic inclusions, such as IHBs, are the hallmark of hepatocellular carcinoma, and sometimes exist alongside MDBs [22]. MDBs are also a manifestation of copper toxicity diseases including Wilson's disease, Indian childhood cirrhosis, and idiopathic copper toxicosis. These diseases are also characterized by hepatocyte ballooning, inflammation, and cytoskeletal alternations resulting in development of micronodular cirrhosis [81].

It has been suggested that MDBs are degraded by both the ubiquitin-proteasome system (UPS) and autophagy, and p62 may play a role in this process [23, 24]. Proteasome inhibitors induce MDB-like structures in cultured cells and in mouse liver [82], whereas liver-specific Atg7 KO mice have increased ubiquitin and p62 positive protein aggregates [21]. These findings support the notion that both proteasome and autophagy regulate MDB formation. Indeed, it has been shown that induction of autophagy by rapamycin significantly suppresses MDB formation both *in vitro* and *in vivo*[83]. Currently, the role of p62 in liver pathogenesis associated with MDBs is not clear. p62 plays a role in reducing protein misfolding caused by copper toxicity induced-oxidative stress by sequestering the misfolded protein into MDBs [81]. This suggests that p62 likely participates in cellular rescue mechanisms during oxidative hepatocyte injury exerted by copper toxicity [81]. Moreover, it has also been suggested that soluble protein aggregates are more detrimental to cells, and p62 may play a protective role by packing these aggregates into insoluble and less toxic "aggresomes" [64, 65, 84]. However, this theory does not explain why Atg7/p62 double KO

(DKO) mice are actually protected against the liver injury found in Atg7 liver-specific KO mice [21]. It is possible that p62-mediated persistent activation of Nrf2 plays a more important role in this liver injury than soluble protein aggregates because Atg7/Nrf2 DKO mice also have less liver injury than Atg7 liver-specific KO mice [85].

Cholestasis

Immunohistochemistry analysis of liver tissues from primary biliary cirrhosis (PBC) patients showed increased LC3 expression and p62 positive aggregates in biliary epithelial cells in damaged small bile ducts, which indicates that p62 may have a role in cholestasis [86, 87]. Moreover, MDBs have also been found in chronic cholestatic conditions, particularly in patients with PBC and primary sclerosing cholangitis [80, 88, 89]. It has also been suggested that elevated levels of bile acids plays a role in MDB formation. For example, cholic acid feeding and common bile duct ligation result in formation of MDBs in drug-primed mice, suggesting that bile acids may drive the formation of MDBs in cholestatic liver disease [88]. Indeed, MDBs in PBC livers were found in acinar zone 1, where bile acids levels are highest [89]. Furthermore, drug-induced MDBs by diethyl 1,4,-dihydro-2,4,6,-trimethyl-3,5-pyridine dicarboxylate (DDC) or griseofulvin also display cholestasis and elevated serum bile acids [88, 90, 91]. We recently found that there was a significant increase of p62 protein levels in FXR-deficient mouse livers compared to wild type mice (Manley *et al.*, manuscript in preparation), and FXR-KO mice also have increased hepatic bile acid levels. Based on the above evidence, it is possible that p62 is involved in the pathogenesis of cholestasis, although further investigation is definitely needed.

Obesity and Insulin Resistance

Obesity is a global-wide health issue that is implicated in metabolic syndrome, which includes insulin resistance, type II diabetes and hepatocellular carcinoma (HCC) [92, 93]. p62 KO mice develop mature-onset obesity in addition to impaired glucose and insulin tolerance [94]. Studies from genetic p62 KO mice suggest that p62 plays an important role in metabolic syndrome development and obesity pathogenesis by regulating ERK, JNK, and Akt signaling pathways.

The ERK1/2 signaling pathway is a subgroup of MAPK signaling cascades responsible for adipogenesis [95, 96]. Mice fed with high fat diet have increased ERK activity in their adipose tissue. Moreover, patients with type II diabetes display enhanced ERK activity in adipocytes [96]. Furthermore, increased basal ERK activity is observed in fat tissues of both obese and non-obese p62 KO mice, suggesting that p62 could be a negative regulator of ERK activity [94]. Indeed, p62 interacts with and sequesters ERK through its PB1 domain and subsequently suppresses ERK enzymatic activity and function. Consistent with this notion, p62 is induced during adipocyte differentiation as a negative feedback mechanism to down regulate ERK mediated adipogenesis by inhibiting expression of downstream targets of ERK, C/EBPβ and PPARγ [94]. ERK1/p62 DKO mice do not have p62-deficiency-induced adipogenesis, indicating that the increased adipogenesis seen in p62 KO mice is due to the lack of negative regulation of ERK activity by p62 [97]. Interestingly, adipocyte-specific, but not hepatocyte-specific, p62 KO mice have increased body fat, hepatic steatosis, impaired

glucose tolerance and decreased insulin sensitivity [98], which suggests a tissue-specific role for p62 in regulating adipogenesis and energy expenditure.

c-Jun-N-terminal kinase (JNK) is another subgroup of the MAPK pathway that is activated in response to diverse stimuli including cytokines, reactive oxygen species, xenobiotics, and metabolic changes. JNK also plays a role in hepatic steatosis, fibrosis and HCC. Both mice fed with high-fat diet (HFD) and genetically obese (*ob/ob)* mice display enhanced JNK activity [99, 100]. Deletion of JNK1 prevents diet-induced obesity and increases insulin sensitivity in mice fed with HFD [99, 101, 102]. JNK has also been shown to have a role in activation of autophagy. JNK is required for induction of proteasome inhibitor induced-autophagy downstream of IRE-1 [103]. Moreover, JNK is also involved in starvation-induced autophagy through JNK-mediated phosphorylation of Bcl-2 to release Beclin-1 [104] or through *c-jun*-mediated transcription of *Beclin-1* expression [105]. While it is not clear if p62 regulates JNK activation, JNK may regulate p62 protein indirectly through activation of autophagy, which causes p62 degradation. In addition, we can speculate that JNK-deficiency may lead to increased p62 levels due to impaired autophagy. If this is true, increased p62 levels may contribute to the decreased lipogenesis and steatosis observed in JNK1 KO mice fed with HFD by down regulating ERK and p38 activity, as previously discussed.

p62 also regulates the insulin signaling pathway by interacting with insulin receptor substrate-1 (IRS-1) [106]. Over-expression of p62 increases phosphorylation of Akt at S473 and T308, which is a downstream target of IRS-1, and promotes the translocation of GLUT4 to the cell membrane for glucose uptake [106]. Conversely, tyrosine phosphorylation of both IRS-1 and its downstream target Akt in response to insulin injection was severely decreased in both muscle and fat tissues of p62 KO mice [94]. These results suggest that p62 may play a positive role in insulin sensitivity by enhancing the insulin signaling pathway, and p62 could be a potential target for diabetes.

p62 Regulates Liver Tumorigenesis by Activating Multiple Signaling Pathways

Increasing evidence now supports a strong association of p62 protein levels with cancer. Increased expression of p62 has been found in various cancer tissues, including HCC [22, 107], breast cancer [108], lung cancer [109] and colon cancer [110]. The level of p62 has also been found to be associated with poor prognosis for patients with lung carcinoma. Mice with specific deletion of either Atg7 or Atg5 in the liver have spontaneous development of liver adenoma, and high levels of p62 protein have been found in these mouse livers [52, 107]. More importantly, Atg7/p62 DKO mice have significantly decreased liver tumorigenesis [52], which convincingly support the notion that p62 promotes tumorigenesis. As discussed above and below, p62 has multiple functions and sits at the crossroads for several signaling pathways including NF-κB, Nrf2, mTOR and autophagy, which are all important for cell proliferation and tumoral transformation.

p62 Regulation of NF-κB and its Role in Liver Tumorigenesis

p62 regulates NF-κB by interacting with multiple players through its different domains, including interaction with aPKC through its PB1 domain, RIP through its ZZ domain and TRAF6 through its TB domain [111]. As discussed above, p62 interacts with RIP and bridges RIP to aPKCs resulting in the activation of NF-κB by the TNF-α signaling pathway [33]. Inhibition of aPKC and down-regulation of p62 decreases NF-κB activation by IL-1, indicating that p62 promotes NF-κB activation [29]. In addition, p62 interacts with TRAF6 to induce TRAF6 polyubiquitination, and the formation of a p62-TRAF6-IKKβ-aPKC signaling complex activates NF-κB by nerve growth factor [43]. Deletion or mutation of the UBA domain, deletion of the N-terminal dimerization PB1 domain, and elimination of the TRAF6 binding site all induce abolishment of TRAF6 polyubiquitination [43]. Furthermore, the rear end acidic cluster region of the p62 PB1 domain binds the front end basic region of MAPK kinase kinase 3 (MEKK3) to promote NF-κB activation [112]. Conversely, the PB1 domain of p62 also interacts with CYLD, a deubiquitinase, which inhibits TRAF6 polyubiquitination and serves as a negative regulator for RANK-mediated NF-κB activation and osteoclastogenesis [113]. Although it is not clear how p62 exactly balances positive and negative regulators of NF-κB activation, it appears that p62 modulates multiple pathways to exquisitely regulate activation of NF-κB.

HCC is one of the most common cancers worldwide and is frequently linked with chronic hepatitis characterized by liver inflammation, hepatocyte apoptosis and compensatory liver regeneration [114]. However, the mechanisms underlying these sequential events are poorly understood. Recently, accumulating evidence has suggested that NF-κB signaling plays an important role in the development of liver tumors. Depending on the model used, NF-κB activation has been shown to inhibit or promote liver tumorigenesis. Mdr2-deficient mice develop HCC in the context of chronic bile duct inflammation. Tumor progression in Mdr2-deficient mice was inhibited by overexpression of a non-degradable IκBα super-repressor that blocks NF-κB activation [115]. In contrast, liver-specific KO of IKKβ increased tumor formation in a mouse model of chemical carcinogen-induced HCC [116]. Moreover, mice with conditional ablation of NEMO in parenchymal liver cells develop spontaneous liver cancer, suggesting that NF-κB inhibits liver tumorigenesis [117]. The contradicting findings from these studies could be due to the use of different tumor models and different approaches for blocking NF-κB. Therefore, the function of NF-κB in liver cancer seems to be complex, acting either as a tumor promoter or as a tumor suppressor.

While NF-κB has been implicated in liver cancer development, the role of p62-mediated NF-κB activation in liver tumorigenesis is still elusive. In a Ras-induced mouse lung adenocarcinoma model, p62 was found to be induced by Ras and activated IKKβ by polyubiquitination of TRAF6. More importantly, p62 KO mice were resistant to Ras-induced lung adenocarcinoma due to increased ROS levels as a result of decreased NF-κB activation [42]. This has been thought to lead to enhanced cell death and subsequent decreased tumorigenicity of Ras. In contrast, using autophagy-and apoptosis-defective immortalized baby mouse kidney (iBMK) cells, it was found that overexpression of p62 inhibited NF-κB activation and promoted tumorigenesis [118]. These findings are similar to the previously discussed carcinogen-induced liver tumorigenesis in IKKβ- and NEMO-deficient

hepatocytes, suggesting that defective autophagy and deregulation of p62 can be associated with suppression of the canonical NF-κB pathway. This NF-κB suppression may in turn promote tumorigenesis through the non-canonical pathway. The discrepancy regarding the role of p62 in NF-κB activation has been suggested to be due to the metabolic stress conditions used for culture of the iBMK cells, in which overexpression of p62 may drive the NF-κB components into huge p62 positive aggregates. It would be interesting to determine the NF-κB status in Atg7 or Atg5 liver-specific KO mouse livers, which have accumulated p62 levels in their hepatocytes and are more physiologically relevant than cultured iBMK cells. Nevertheless, even though there is unresolved controversy regarding the role of p62 in NF-κB activation, the evidence that accumulation of p62 promotes tumorigenesis in the liver, as well as other tissues, is consistent.

p62 Regulation of the Non-Canonical Nrf2 Pathway

Nrf2, a basic leucine zipper (bZIP) transcription factor, is activated during stress and induces expression of antioxidant enzymes [37, 119]. In the canonical pathway, Nrf2 is negatively regulated by Keap1, an adaptor for the cullin-3-type ubiquitin ligase, which targets Nrf2 for its ubiquitination and proteasome-mediated degradation. In response to oxidative stress, the activity of this E3 ubiquitin ligase is inhibited due to modification of cysteine residues in Keap1, which results in stabilization and activation of Nrf2 [37].

As discussed above, p62 can interact with Keap1 through its KIR motif, which releases Keap1 from Nrf2 and, in turn, promotes Nrf2 stabilization and activation. As a result, Nrf2 is persistently activated in the autophagy-deficient mouse liver. Activation of Nrf2 leads to the expression of a number of antioxidants, phase II detoxifying enzymes, and hepatic transporters, which are essential for cytoprotection against oxidative stress [37, 120, 121]. Paradoxically, autophagy-deficient mice show severe liver injury as demonstrated by hepatomegaly, accumulation of damaged mitochondria and elevated serum hepatic enzymes, even though these mice have persistent activation of Nrf2 due to the aberrant accumulation of p62 [21]. The liver injury in liver-specific Atg7 KO mice was rescued by the deletion of either p62 or Nrf2, suggesting that both p62 and Nrf2 could be responsible for the liver injury found in the autophagy-deficient mice [21, 85]. More recently, Yamamoto's group generated Atg7/keap1 DKO mice, which exhibited more severe liver injury than Atg7 liver-specific single KO mice [122]. Interestingly, further deletion of Nrf2 in Atg7/keap1 DKO mice was more protective against liver injury compared to further deletion of p62 in these mice [122]. While these results suggest that Nrf2 activation as a result of p62 accumulation plays a dominant role in liver injury in autophagy-deficient mice, it is possible that other mechanisms could also be involved.

In addition to activating Nrf2, p62 has also been shown to increase apoptosis by promoting caspase-8 activation. It was shown that cullin3 (CUL3) E3 ligase-mediated caspase-8 polyubiquitination is required for the activation of caspase-8 in response to death receptor activation, and this process was promoted by p62 [123]. However, recent evidence suggests that caspase-8 forms a complex with Atg5 and colocalizes with LC3 and p62. Moreover, the LC3 positive autophagosome membrane serves as a platform for caspase-8 aggregation and activation [124, 125]. LC3 positive membranes are likely more important than the presence of

p62 because the activation of caspase-8 was significantly reduced in Atg5-deficient cells in which p62 levels were accumulated, but no LC3 positive autophagosome membranes were formed [126]. However, it should also be noted that these studies were conducted *in vitro* in cultured cells. The role of p62 for the activation of caspase-8 *in vivo* is not clear yet. We found that both caspase-8 and caspase-3 are activated in liver-specific Atg5 KO mice, which have aberrant p62 accumulation without LC3-positive autophagosomes (Ni et al., Manuscript in preparation). These results suggest that p62 protein alone may be sufficient to induce caspase-8 activation in autophagy-deficient livers, although more work is needed to further elucidate whether this would involve CUL3-mediated caspase-8 polyubiquitination. Currently, it is not clear how Nrf2 activation increases liver injury. A recent study suggests that the Nrf2-dependent stress response, which results in accumulation of ubiquitin conjugates, may be involved [127]. Further studies are needed to further elucidate the role of Nrf2 in the liver injury observed in autophagy-deficient mice.

While persistent activation of Nrf2 induces liver injury in autophagy-deficient mice, p62-mediated Nrf2 activation attenuates drug or lipogenic stress-induced liver injury that involves oxidative stress [53, 128]. Acetaminophen (APAP) is a safe drug at therapeutic levels, but an overdose can cause severe liver injury in animals and man by inducing necrosis [129]. We found that pharmacological induction of autophagy by rapamycin significantly protects against APAP-induced liver injury [130]. However, to our surprise, liver-specific Atg5 KO mice were more resistant to APAP-induced liver injury. Subsequently, we found that persistent Nrf2 activation resulted in upregulation of glutathione synthase and drug detoxification genes in liver-specific Atg5 KO mice, which was accountable for the resistance to APAP-induced liver injury [53]. In another study, it was shown that sestrins, which are p53 target genes involved in protection of cells from oxidative stress, interact with Keap1, p62 and the ubiquitin ligase, Rbx1 [128]. It was found that the antioxidant effects of sestrins are mediated through Nrf2 activation by p62-mediated autophagic degradation of Keap1. Interestingly, sestrin-mediated Nrf2 activation protected against liver injury induced from the acute lipogenic stimulus elicited by re-feeding after food deprivation [128]. These results suggest that persistent activation of Nrf2 plays a dual role in the regulation of cell injury in the liver. While persistent activation of Nrf2 itself is detrimental to the autophagy-deficient liver, it renders these mice more resistant to drug- and oxidative stress-induced further liver damage.

In addition to regulating liver injury, the non-canonical Nrf2 pathway activated by the accumulation of p62 may also play a critical role in liver tumorigenesis. Nrf2 target genes are critical to eliminate toxicants or carcinogens to maintain redox homeostasis, so it has long been thought that activation of Nrf2 would be a promising strategy to prevent carcinogenesis and tumorigenesis. Many natural dietary compounds and synthetic small molecules that activate Nrf2 have been found to have chemo preventative effects in cell culture and animal models [131]. In line with this notion, Nrf2-KO mice have increased sensitivity to carcinogenesis due to blunted efficacy of chemo preventive compounds [132, 133]. Paradoxically, increasing evidence also suggests that activation of Nrf-2 promotes tumorigenesis and/or tumor progression as well as chemo resistance [131, 134]. It is not surprising that tumors may take advantage of the Nrf2 pathway to promote tumor cell

survival by increasing the expression of antioxidant proteins as well as detoxifying enzymes. Somatic mutations of KEAP1 and Nrf2 have been found in patients with lung cancer or head and neck cancers, causing constitutive Nrf2 activation in these patients [134, 135]. Up-regulation of Nrf2 target genes, including heme oxygenase-1 (HMOX-1), peroxiredoxin (Prx) and NAD(P)H quinone oxidoreductase-1 (NQO-1), has been found in many cancers and may contribute to chemo resistance and tumor progression [131]. As we discussed above, autophagy-deficient mouse livers have aberrant accumulation of p62 resulting in persistent activation of Nrf2 and eventual development of liver adenoma [107]. Importantly, Komatsu's group found that more than 25% of human HCC (26 of 102 patients) had increased p62 protein levels [107]. A recent cohort study of patients with lung cancer found that 34% and 37% of non-small-cell lung cancer (NSCLC) patients had an accumulation of Nrf2 and p62, respectively. Interestingly, accumulation of p62 is found to be associated with a poor diagnosis for these patients, although accumulation of Nrf2 seems to be unrelated to accumulation of p62 in these patients [109]. Taken together, it is possible that p62-mediated persistent activation of Nrf2 would promote the liver tumorigenesis seen in Atg7 liver-specific KO mice. Studies on Nrf2 and Atg7 liver DKO mice would help to answer this question.

p62 Regulating mTOR Complex 1 (mTORC1)

It is well-known that autophagy is negatively regulated by the mammalian target of rapamycin (mTOR), a serine/theornine kinase [136, 137]. Meijer's group first discovered that rapamycin inhibits mTOR and activates autophagy in isolated rat hepatocytes [138]. mTOR exists in two heteromeric complexes, mTOR complex 1 (mTORC1) and mTORC2. It is known that rapamycin-sensitive mTORC1 plays a major role in the regulation of autophagy and cell growth, although there is evidence that rapamycin-insensitive mTORC2 may also regulate autophagy in certain tissues [139–141]. Growth factors or insulin activates class-I-phosphatidylinosital-3 kinase (PI3K) and AKT, which leads to mTOR activation through the tuberous sclerosis complex protein (TSC)-Rheb signaling cascade [142]. Activation of mTOR then shuts down autophagy, which is a cellular catabolic process, by regulating the ULK1-FIP200-Atg13 complex [139]. Meanwhile, activation of mTOR also activates the anabolic process of protein synthesis by directly phosphorylating the translational initiation factor 4E (eIF4E)-binding protein (4E-BP1) and S6 kinase 1 (S6K1) [143, 144]. Moreover, mTORC1 also plays a role in controlling mammalian lipid metabolism, including fatty acid and cholesterol synthesis by regulating the sterol regulatory element-binding protein1/2 (SREBP1/2) transcription factor, lipid oxidation, transport, lipolysis and adipocyte differentiation [145, 146].

It should be noted that in addition to growth factors, amino acids have been known to activate mTORC1, although the mechanisms of activation has only been recently revealed. When amino acids are abundant, active mTORC1 localizes on the lysosomes and late endosomes, which is activated by a group of GTPase proteins called Ragulators [147]. Thus, amino acids activate mTORC1 independent of TSC and do not involve the insulin/PI3K–AKT signaling pathway. Since activation of autophagy may transiently increase cellular amino acid levels due to lysosomal degradation of proteins, amino acids from lysosomal

degradation may serve as a negative feedback mechanism to inhibit autophagy by activating mTORC1 [148].

As discussed above, it is well known that p62 is an autophagy substrate and normally degraded during autophagy activation. Interestingly, p62 can also negatively regulate autophagy by activating mTORC1. p62 can promote mTORC1 activation by directly interacting with Raptor, a key component of mTORC1, but not Rictor or any other component of mTORC2 [149]. It was found that the region between the ZZ and TB domains (amino acids 167–230) of p62 is required for the interaction between p62 and Raptor, while the UBA, TB, PB1, and ZZ-type zinc finger domains are not essential for p62-Raptor interaction. In response to stimulation with amino acids, deletion of p62 results in reduced mTORC1 activity. Conversely, over-expression of p62 enhanced mTORC1 activation [149]. In addition, p62 also activates the Ragulator complex to promote mTORC1 activation. p62 preferentially interacts with Rag C, and this interaction is further enhanced in the presence of Raptor. Moreover, p62 may serve as a docking site and is required for mTORC1 localization to the lysosomal membrane in response to amino acid stimulation [149]. Therefore, p62-mTORC1 signaling may also establish another line of feedback regulating p62 levels: decreased autophagy leads to the accumulation of p62, and p62 activates mTORC1 to suppress autophagy resulting in more p62 accumulation.

It is not surprising that activation of mTORC1 promotes tumorigenesis. mTORC1 activation increases protein synthesis while blocking autophagy, and autophagy is a well-known process to suppress tumorigenesis. Indeed, liver-specific TSC1 KO mice have persistent activation of mTORC1 and develop spontaneous HCC [150]. Pathological analysis indicates that these mice have the primary etiologies of HCC in humans, including liver damage, inflammation, necrosis, and regeneration [150]. Therefore, p62-mediated mTORC1 activation may also contribute to tumorigenesis. Taken together, current evidence support that p62 may work as a tumor promoter by modulating multiple signaling pathways including NF-κB, Nrf2, mTOR and autophagy.

Concluding remarks and future directions

As a multifunctional adaptor protein, p62 has emerged as a critical player in liver physiology and pathology (Figure 3). Although the major physiological role of p62 is to regulate metabolic homeostasis and bone formation, p62 may also play a role in cholestasis, diabetes, obesity, insulin resistance and liver tumorigenesis.

p62 is an autophagy substrate and is degraded during autophagy activation, but p62 can also regulate autophagy by activating mTORC1. Suppression of autophagy leads to accumulation of p62, and p62 further inhibits autophagy, which in turn leads to more p62 accumulation. This forms a positive feedback loop for more p62 accumulation and autophagy suppression.

However, many questions remain to be answered. While it is clear that p62 may serve as a receptor for selective autophagic removal of protein aggregates, its role in selective mitophagy is still controversial due to the lack of reliable mitophagy makers and quantitative assays. Moreover, the role of p62 in mitophagy *in vivo* has not been explored. In addition, the mechanisms for liver toxicity induced by p62-mediated Nrf2 activation are still not clear.

Nevertheless, as understanding of the pathophysiological roles of p62 increases, it seems that targeting p62 may be a promising strategy for treating diabetes, obesity and liver hepatosteatosis.

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Abbreviations

4E-BP1	Translational initiation factor 4E (eIF4E)-binding protein
ALFY	autophagy linked FYVE protein
APAP	Acetaminophen
AP-1	Activator protein 1
aPKC	Atypical protein kinase C
ARE	Antioxidant response element
bchs	Blue cheese
bZIP	Basic leucine zipper
C/EBPβ	CCAAT-enhancer-binding protein β
CCCP	Carbonyl cyanide *m*-chlorophenyl hydrazone
CDK1	Cyclin-dependent kinase 1
ChIP	Chromatin immunoprecipitation
CK2	Casein kinase 2
CUL3	Cullin3
DDC	Diethyl 1,4,-dihydro-2,4,6,-trimethyl-3,5-pyridine dicarboxylate
DKO	Double knockout
ERK	Extracellular-signal-regulated kinase
FXR	Farnesoid X Receptor
GABARAP	Gamma-aminobutyric acid receptor-associated protein
HCC	Hepatocellular carcinoma
HDAC6	Histone deacetylase 6
HFD	High-fat diet
HMOX-1	heme oxygenase-1
HSP60	Heat shock protein 60

iBMK	immortalized baby mouse kidney
IHBs	Intracytoplasmic hyaline bodies
IKKβ	Inhibitor of κB kinase
IL-1	Interleukin-1
IRE-1	Inositol-requiring protein 1
IRS-1	insulin receptor substrate-1
JNK	c-Jun N-terminal kinase
K	Lysine
K27	Lysine 27
K48	Lysine 48
K63	Lysine 63
Keap1	Kelch-like ECH-associated protein 1
KIR	Keap1 interacting region
KO	Knockout
LC3	Microtubule light chain 3
LIR	LC3 interacting region
MAPK	Mitogen-activated protein kinase
MDBs	Mallory-Denk Bodies
Mdr2	Multidrug resistance protein 2
MEKK3	MAPK kinase kinase 3
MEK5	MAPK kinase 5
MTOC	Microtubule-organizing center
mTOR	Mammalian target of rapamycin
mTORC1	mTOR complex 1
NBR1	Neighbor of BRCA gene 1
NEMO	NF-κB essential modulator/IKKγ
NES	Nuclear export signal
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NLS	Nuclear localization signal
NQO-1	NAD(P)H quinone oxidoreductase-1
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
NSCLC	non-small-cell lung cancer

PB1	Phox/Bemp1
PBC	Primary biliary cirrhosis
PEST	Proline (P), glutamic acid (E), serine (S), and threonine (T) rich sequence
PI3K	Class-I-phosphatidylinosital-3 kinase
PINK1	PTEN-induced putative protein kinase 1
PML-NBs	promyelocytic leukemia nuclear bodies
PPARγ	peroxisome proliferator-activated receptor γ
Prx	Peroxidredoxin
RANK	Receptor Activator of NF-κB
RANKL	Receptor Activator of NF-κB ligand
RIP	Receptor interacting protein
S6K1	S6 kinase 1
SH2	Src-homology 2
Sp1	Specificity protein 1
SQSTM1	Sequestosome 1
SREBP1/2	Sterol regulatory element-binding protein1/2
TB	TRAF-6 binding
TDP-43	TAR DNA binding protein-43
TNF	Tumor necrosis factor
TOM20	Translocase of outer mitochondrial membrane 20
TRADD	TNF receptor type 1-associated death domain protein
TRAF6	TNF-associated receptor-6
TRE	TPA response element
TSC	Tuberous sclerosis complex protein
UBA	Ubiquitin-associated
UPS	Ubiquitin proteasome system
ZIP	Cytoplasmic zeta-interacting protein

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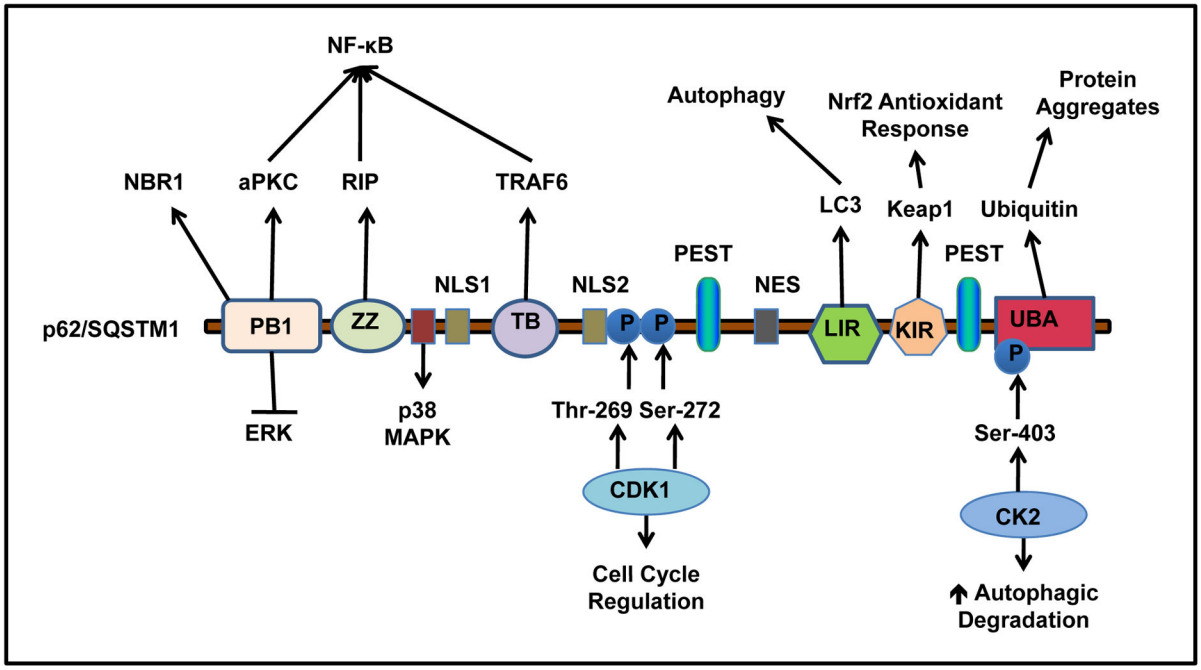

Figure 1. p62 structure, binding partners and functions

p62 has Six distinct functional domains: PB1, ZZ, TB, LIR, KIR and UBA. PB1 domain self- and hetero- oligomerizes with other PB1 containing proteins, such as NBR1, ERK and aPKC. p62 binds with RIP at ZZ zinc finger region, and TRAF6 at TB domain, which regulates NF-κB activation. At residues 173–182, p62 interacts with p38 MAPK. p62 interacts with LC3 through the LIR, and keap1 through the KIR. The c-terminal UBA domain of p62 binds to ubiquitin. p62 has two PEST sequences that are targets for post translation modifications and degradation. p62 is phosphorylated by CDK1 at T269 and S272 that regulates cell cycle. p62 is phosphorylated by CK2 at S403 that enhances autophagic degradation of ubiquitinated proteins. p62 also contains two NLS sequences and a NES sequence which allow p62 shuttling into and out of nucleus.

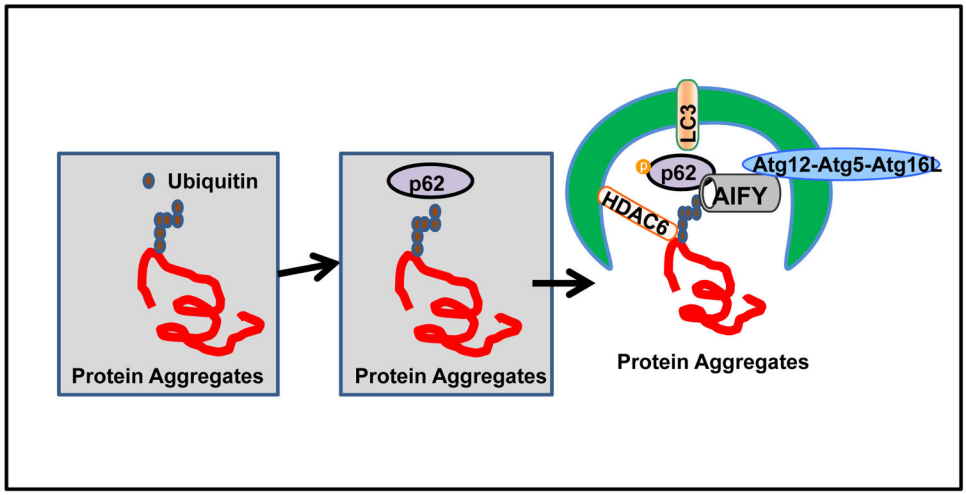

Figure 2. Autophagy receptor complex for the degradation of protein aggregates

Specific ubiquitin (ub) chains are incorporated into the protein aggregates and recruit p62 via its UBA domain, which further delivers ubiquitinated protein aggregates to autophagosomes by interacting with LC3 via its LIR domain. Ubiquitin also recruits HDAC6, which facilitates the transportation of protein aggregates to the MTOC as well as fusion of autophagosomes with lysosomes for the subsequent degradation of protein aggregates. ALFY functions as a scaffold protein for p62 positive protein aggregates that targets them for degradation by autophagy via interaction with the Atg5–Atg12 complex.

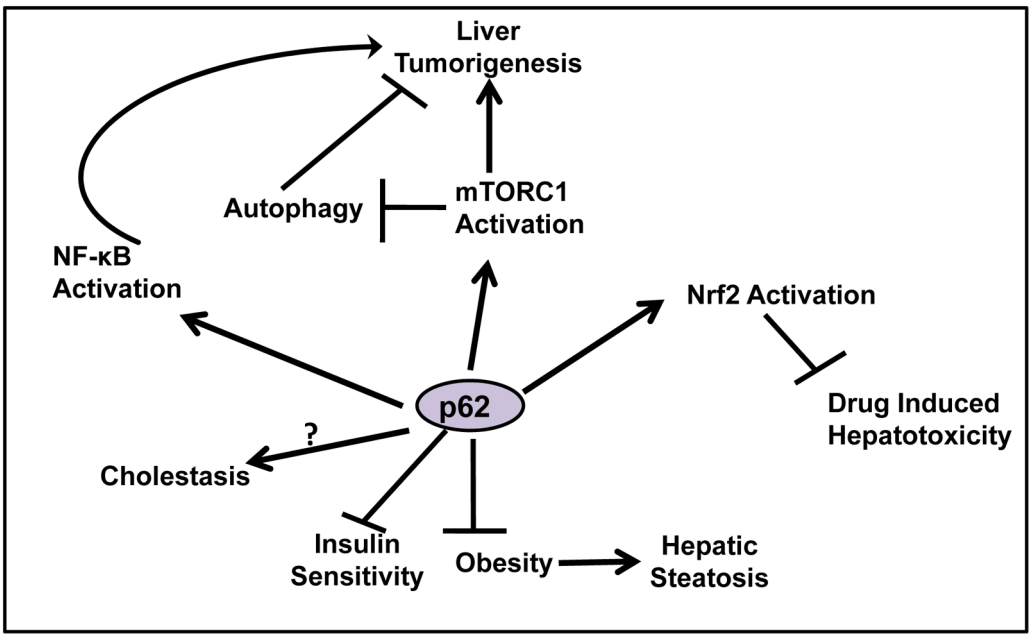

Figure 3. Pathophysiologcal roles of p62 in the liver

p62 negatively regulates adipogenesis and inhibits obesity. p62 also plays a positive role in insulin sensitivity. The role of p62 in regulating cholestasis is currently not clear. p62 promotes liver tumorigenesis by regulating NF-κB, Nrf2 and mTOR activation. p62-mediated Nrf2 activation may also cause resistance to drug-induced liver injury. Finally, p62-Nrf2 also forms a positive feedback loop, which accumulated p62 activates Nrf2 and Nrf2 activation increases transcription for p62. The role of p62 in regulating cholestasis is currently not clear.