Introduction

The maintenance of cellular homeostasis depends on an exquisite balance between the biosynthesis and degradation (catabolism) of macromolecules. This balance is controlled by finely tuned mechanisms triggered by environmental signals. In eukaryotic cells, two main systems are responsible for protein degradation. The first system is the ubiquitin-proteasome system that allows for the degradation of proteins with a short half-life (generally less than 5 hours). Conversely, the second system, which is strictly dependent on the lysosome and represents approximately 99% of cellular proteins, is involved in the degradation of proteins with a half-life of up to 5 hours [1].

The latter system includes the targeting of proteins to the lysosomes and the further degradation of these proteins by lysosomal hydrolases. The major process for the delivery of proteins to the lysosome is orchestrated by a specific and highly conserved mechanism called autophagy or macro-autophagy [2].

Macro-autophagy, hereafter referred to simply as autophagy, is currently the most well studied of these degradation pathways, which is likely due to its important and paradoxical role in the control of cell death and survival. Active at the basal level in the resting cell, autophagy is induced under conditions of stress and thus behaves as an adaptative survival mechanism, more particularly through amino acid recycling following the degradation of damaged organelles and macromolecules. In mammal cells, autophagy is a multistep process, including an initiation step, a nucleation step, an elongation step and, finally, a maturation step (Figure 1). The initiation and nucleation steps both converge to isolate de novo a portion of the intracellular membrane called a phagophore. The phagophore then invaginates, and its ends can fuse to generate a double-membraned vesicle referred to as the autophagosome. The autophagosomal structure is delimited by several
lipidic layers that sequester the cytoplasmic content and/or organelles [2]. The strict origin of the autophagosome is still unknown, but it is thought that several cellular compartments, including the endoplasmic reticulum (ER), the Golgi/trans-Golgi apparatus and the plasma membrane, can participate in the genesis of the autophagosome. Finally, following an additional maturation step, the autophagosome becomes an amphisome after it fuses with multivesicular endosomes (Figure 1). During this step, the amphisome is acidified via the activation of proton pumps contributed by the endosomes. Ultimately, this amphisome will fuse with a lysosome to become an autolysosome in which the internal content is degraded by lysosomal enzymes (Figure 1).

From yeast to mammals, all of the processes of autophagosome biogenesis are controlled by the specific interaction of several protein complexes composed of Atg proteins (autophagy-related genes). Of a total of 30 Atg proteins identified to date, approximately 50% play an essential role in the formation and elongation of the phagophore. With the exception of Atg9,
none of these proteins possesses a transmembrane domain. Once recruited into the cytoplasm, Atg proteins bind transiently to the membranes of the phagophore (or the pre-autophagosomal structure, PAS in yeast) and the autophagosome [3].

The Atg proteins can be classified into three functional groups according to the autophagy step in which they participate (Figure 1): 1) The complex composed of the serine/threonine kinase ULK1 (or its yeast homolog Atg1), mAtg13, FIP200 and Atg101 are involved in the initiation step of the phagophore. 2) The Atg6/Beclin 1-Atg14L-Vps34-Vps15 and Beclin 1-UVRAG-Vps34-Vps-15 complexes are required for phagophore nucleation. 3) The two conjugation systems composed of Atg5-Atg12 and Atg8/LC3-PE are essential for the elongation and closure of the autophagosome [2]. Notably, the mAtg9/Atg9 complex is important during the induction of autophagy, although its exact role in the autophagic process remains unknown. Atg9 does not belong to any of the above-mentioned functional groups, but it appears to be important during all steps of autophagosome biogenesis [4].

In contrast to what was initially thought, macroautophagy is not only a non-selective mechanism for the degradation of cellular constituents, but it is also now widely known that this form of autophagy is involved in the specific targeting of organelles within the cell. This targeting is called mitophagy in the case of mitochondria, pexophagy for peroxisomes and ER-phagy for the endoplasmic reticulum. Finally, the mechanism of autophagy responsible for the degradation of unfolded or aggregated proteins is referred to as aggrephagy [5].

The selectivity of autophagy results in alternative molecular mechanisms that connect with and engage the autophagic machinery to use this machinery to digest specific substrates. This process requires key scaffold proteins, which have only been partially identified, that may be associated with several autophagic components to influence the selective degradation of some substrates. Importantly, several recent studies have highlighted the role of two of these adaptor proteins, p62 and NBR1, whose function is to specifically address ubiquitinated and sometimes aggregated protein substrates for autophagic degradation [6]. Both of these proteins exhibit a high level of homology, and, although the role of NRB1 is better understood than that of p62, both proteins appear to share common functions. The present review will first focus on the structure/function relationship of p62 and then describe how p62 is regulated via several oncogenic or tumor-suppressor signaling pathways.

As a molecular adaptor between the autophagic machinery and its substrates, p62 is degraded during this autophagic process. This property led some authors to use this protein as an index for the autophagic flux measurement. We will discuss more deeply the ambiguity that occurs when using this protein as a hallmark of autophagic flux.

Finally, we will broaden our investigation into the role of autophagy in tumor development, and we will assess the impact of p62 as a molecular link between autophagy and cancer.

Structure and function relationship

p62/SQSTM1 (Sequestosome 1, ZIP3) contains multiple major domains such as PB1 (aa. 21–103) and ZZ (aa. 128-163) domains that confer the ability to interact with key components involved in essential signaling pathways. Two other very important domains, the UBA (aa. 386-440) and LIR (aa. 321-345) domains, cause p62 to function as an adaptor between autophagy and ubiquitinated proteins (Figure 2).

Interaction of p62 with major signaling pathways via the Phox and B pem 1 (PB1) and the central zinc finger (ZZ) domains

P62 is a multifunctional protein characterized initially by its ability to bind atypical protein kinase C (PKC zeta and iota) via its N-terminal PB1 (Phox and B pem1) domain (Figure 2). Heterodimerization of p62 with atypical PKC is crucial for the regulation of the NFκB pathway. Indeed, following binding of the RANK ligand (RANK-L) to its cognate receptor RANK in osteoclasts, the association of p62 with either atypical PKC or with TRAF6 via its TRAF6-binding domain (TBD, aa. 225-250) contributes to the activation of IKK (Ik-B Kinase) and to the nuclear translocation of the transcription factor NFκB [7]. It has been recently reported that the MAP kinase kinase 3 (MEKK3) can activate TRAF6 to promote nuclear relocalization of
NFκB by binding to the PB1 domain of p62 [8]. Conversely, p62 can recruit, via its PB1 domain, CYLD, a deubiquitin ligase that acts as an inhibitor of TRAF6 following NFκB activation [9]. This modulation of the NFκB pathway by p62 is essential for proper osteoclastogenesis. Finally, it has been reported that the PB1 domain of p62 can also interact with ERK1 to promote adipogenesis [10].

Importantly, this PB1 domain is also required for the homodimerization of the protein [11, 12] or its association with NBR1, which allows its oligomerization. For example, in ALK-positive large B cell lymphoma, which is characterized by p62-ALK translocation, homodimerization of p62 via its PB1 domain has been suggested to be decisive for the constitutive activity of the ALK kinase that is responsible for this hematopoietic malignancy [13]. In addition to the PB1 domain, p62 possesses a central zing finger domain (ZZ domain) that interacts with RIP
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(receptor-interacting protein) to modulate the NFκB pathway in conjunction with atypical PKC [14].

In conclusion, a recent report has identified a new region of p62 located between the ZZ and TBD domains that is responsible for the interaction of p62 with the mTOR regulator Raptor [15] (Figure 2). In mammalian cells, mTOR is part of two distinct complexes: mTORC1 (formed by the mTOR, Raptor, and GBL proteins) and mTORC2 (formed by mTOR, Rictor, mSin1, GBL, and Protor) [16]. mTORC1 is highly sensitive to the nutrient deprivation that leads to the inactivation of the serine/threonine kinase mTOR. Conversely, mTOR is activated following normal nutrient input. The interaction of p62 with Raptor and, subsequently, mTORC1 is dependent on the uptake of amino acids that participate in the lysosomal membrane localization of mTORC1 to promote Rag GTPase-mediated mTOR activation [15].

p62/SQSTM1, an adaptor between autophagic machinery and ubiquitinated proteins via its Ubiquitin-Binding (UBA) and LC3-Interacting (LIR) regions

The ubiquitin-binding domain (UBA): The C-terminal ubiquitin-binding domain (UBA) of p62 binds with a moderate affinity to the ubiquitin bound to the lysine residues in position 48 and with a strong affinity to the lysine in position 63 (Figures 2 and 3). The affinity of the UBA domain for ubiquitinated lysine residues is drastically increased following the caseine kinase 2-mediated phosphorylation of serine 403 [17] (Figure 3).

The UBA domain of p62 is frequently mutated in Paget’s disease. This pathology is characterized by an increase in the number and the activity of osteoclasts, leading to excessive bone resorption and anomalies of the bone architecture. Although the molecular mechanisms involved in this excessive osteoclastogenesis are currently unknown, 14 mutations of the p62 gene have been reported to occur in this disease [18]. The first discovered point mutation leads to a recurrent proline-to-leucine substitution (P392L) not only in familial forms of Paget’s disease but also in patients with no known familial history. Subsequently, stop (K378X, A390X, L394X, E396X) and non-sense (A381V, P387L, S399P, M404T, M404V, G411S, L413F, G425R) mutations have been identified in the UBA domain coding sequence of the p62 gene [18]. It is assumed that approximately 40% of familial forms and 8% of sporadic cases of patients suffering Paget’s disease harbor a mutation in the gene
encoding p62. To date, no link has been established between the mutations found in the UBA domain of p62 and the etiology of Paget’s disease [19].

The LC3-interacting region (LIR) domain: As mentioned above, the LC3 (Atg8) proteins, which can be divided into seven members, are key components of the autophagy machinery required for the elongation and closure of the autophagosome. To assure their proper functions, LC3 proteins are specifically cleaved at their C-termini by the Atg4 proteases to expose a C-terminal glycine residue, which is then conjugated to a phosphatidylethanolamine (PE) group to form LC3-II [3]. This maturate form of LC3 is tightly bound to the autophagosomal membranes. The targeting of p62 via the autophagic machinery is dependent on the association of this protein with all isoforms of the LC3 protein via an 11 amino-acid portion of LIR located in the 332-343 region [12] (Figure 2). More recently, NBR1 has also been identified as an LC3 binding partner. The overall organization of the NBR1 protein is similar to that of p62, with the noticeable exception of two coiled-coil (CC1 and CC2) domains that allows for the oligomerization of NBR1 following its linkage to ubiquitinated proteins [6] (Figures 2 and 3). The LIR domain of the LC3 protein family can also interact with NDP52, BNIP3, NIX and TP53INP1 [20-23]. Whereas BNIP3 and NIX are key regulators of mitophagy, particularly during erythroid differentiation [20, 21], TP53INP1 regulates autophagy-dependent cell death (also called type II cell death) [22].

The interaction of p62 with LC3-II is required for the autophagy-mediated elimination of unfolded ubiquitinated long-half-life proteins (Figure 3). Thus, it has been shown that the knock-down of the LC3 protein triggers the intracytoplasmic accumulation of ubiquitinated protein aggregates bound to p62 and/or NBR1 proteins [6, 24]. In addition, another study demonstrated that the ubiquitination of soluble cytosolic proteins or organelles (especially proteins embedded in peroxysomal membranes) are sufficient to target these substrates for autophagic degradation [25]. These data clearly demonstrate that p62 is a receptor for the specific elimination of ubiquitinated proteins via autophagy. Because it has only recently been identified, the biological function of NBR1 is not yet fully understood. It is thought, however, that this protein can interact directly with p62 within the same ubiquitinated protein aggregates [6, 26, 27].

Conversely, p62 is not only required for the proper elimination of ubiquitinated proteins but also participates in the autophagic clearance of non-ubiquitinated substrates. This fact is emphasized in a recent publication by Watanabe et al. in which the authors assessed the impact of the overexpression of an isoform of STAT5A, STAT5AΔE18, which has the ability to form non-ubiquitinated aggregates [28]. Despite the absence of ubiquitinated residues in STAT5AΔE18 aggregates, p62 is able to bind to these aggregates via its PB1 domain to mediate the elimination of these aggregates by autophagy [28].

Influence of the PB1 and UBA domains on the autophagic clearance of p62/SQSTM1 targets: the case of neurodegenerative diseases

The PB1 and UBA domains are both required for the proper localization of p62 within cytoplasmic aggregates (either ubiquitinated or not) that are referred to as p62-bodies. Thus, the inhibition of autophagy leads to both an intracellular accumulation of p62 and an increase in the number of p62-bodies. In a clinical context, the dissection of the mechanisms sustaining the autophagic elimination of p62-bodies is a very important challenge in the development of new treatments to circumvent neurodegenerative disorders, such as Huntington’s chorea.

Huntington’s chorea is characterized by strong neurologic deficits that affect muscle coordination and lead to cognitive decline, psychiatric problems and, ultimately, to the death of the patients. Huntington’s disease is caused by a mutation in the gene encoding huntingtin. Mutant huntingtin accumulates in the cytoplasm where it forms inclusions that are deleterious to neuronal cells. It has been shown that p62 can surround and associate with such inclusions and interact with the LC3-II protein to promote the degradation of these inclusions via an autophagic process [12, 29]. In agreement with this observation, deletion of the UBA domain abrogates the protective effect of p62 and increases the amount of cell death induced by the mutant huntingtin [29].

The accumulation of p62 into cytoplasmic inclu-
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...ion bodies has also been described in other neuropathies such as Alzheimer’s disease, Parkinson’s disease and amyotrophic lateral sclerosis, which is also known as Charcot disease. The evolution of Alzheimer's disease is strikingly associated with the pathological accumulation of filamentous structures called PHFs (paired helical filaments) in the cytoplasm of neuronal cells. These structures are essentially formed by the Tau protein, which belongs to the microtubule-associated protein (MAP) family and plays an important role in microtubule stabilization. The aberrant accumulation of Tau in neuronal cells leads to the apoptotic cell death that is associated with neurofibrillar degeneration. It has been reported that p62 colocalizes with these Tau-positive inclusions in neuronal cells [30, 31]. Similarly, p62 also colocalizes with α-synuclein, whose accumulation is associated with Parkinson’s disease. The functional link between Tau and p62 has been highlighted in p62-deficient mice that exhibit a pathological accumulation of Tau associated with a massive neuronal degeneration [32]. These findings, therefore, clearly reflect the preventive role of p62 in the emergence of neurodegenerative disorders.

Very interestingly, the inhibition of autophagic degradation pathways has also been associated with the genesis of Alzheimer’s disease [33, 34]. Indeed, the autophagosome-lysosome system participates to the elimination of Tau protein aggregates [35, 36]. Although this hypothesis still lacks formal evidence, it is likely that p62 is the molecular clutch necessary for the autophagic clearance of Tau or α-synuclein proteins. Thus, the inhibition of autophagy or the knockdown of p62 would lead to a toxic intracellular accumulation of these proteins. A recent study by Du et al. highlighted an interesting mechanism responsible for blocking p62 expression, which may explain the onset of Alzheimer’s disease. Staining of brain slides from p62−/− mice with 8-OHdG, an oxidative stress sensor, revealed an increased level of reactive oxygen species (ROS) production compared with that of the control mice [37]. One consequence of this exacerbated ROS production is the alteration of the p62 promoter as mice get older. This deterioration of the p62 promoter results in a blockade of p62 expression that impairs the autophagic elimination of Tau aggregates that are the cause of the neuropathy [37]. The authors next reported a negative feedback loop in which increased ROS production, resulting from the occurrence of Tau protein clusters, blocks the expression of p62 due to the alteration of its promoter. Finally, this blocked expression contributes to a further increase in the accumulation of Tau aggregates that, in turn, exacerbates intracellular ROS production [37].

Regulation of the p62/SQSTM1 intracellular level

As previously mentioned, the expression of p62 is highly regulated at the transcriptional level. However, we will also describe a second autophagy-dependent mechanism involved in the control of intracellular levels of p62 (Figure 4).

Transcriptional regulation of p62/SQSTM1

Transcriptional regulation dependent on the transcription factor NRF2: One of the main modes of transcriptional regulation of p62 is dependent on the NRF2 transcription factor, which belongs to the basic leucine zipper (bZIP) family of transcription factors. In response to an oxidative stress such as H2O2, NRF2 specifically binds to the antioxidant-responsive element (ARE motif) located in the p62 promoter to promote the expression of p62 mRNA [38]. In the absence of oxidative stress, NRF2 is maintained as an inactive protein in the cytoplasm through its interaction with the E3 ubiquitin ligase KEAP1 (Kelch-like ECH-associated protein 1) [39]. In addition to its specific regulation by NRF2, p62 can interact directly with KEAP1 via its KIR domain that is juxtaposed to the LC3-interacting (LIR) domain. Hence, p62 is able to dissociate NRF2 from KEAP1, thus promoting the activation and nuclear translocation of NRF2. This process contributes to the induction of a positive feedback loop in which p62 activates NRF2 to drive its own transcription [38]. This mechanism provides a pertinent explanation for the increased ROS production observed in Tau-positive neuronal cells of p62−/− mice [37]. Although p62 deficiency is sufficient to impair the autophagic degradation pathway and result in an oxidative burst, the sustained inactivation of NRF2 by KEAP1 in p62−/− mice could also participate to amplify this deleterious process.

In contrast, given that p62 is degraded during the autophagic process [12], one can assume that a defect in autophagy may cause an in-
crease in NRF2 transcriptional activity. This non-canonical mechanism for NRF2 activation would strictly rely on p62 degradation during autophagy [41]. These findings are corroborated in vivo by the specific invalidation of Atg7 in mouse livers in which the inhibition of autophagy triggers the formation of adenomas containing p62-positive aggregates, which are also found in 25% of hepatocellular carcinoma cases [42]. In correlation with the presence of p62-positive aggregates, NRF2 activation is also detected in these hepatocellular carcinoma samples. Based on these reports, we conclude that there may be an intriguing link between the variation in the activity of the autophagic machinery and the p62-dependent oxidative stress response during carcinogenesis.

This striking interrelation between NRF2 and p62 is also involved in the regulation of other signaling pathways. Thus, following the non-canonical p62-mediated activation of the NRF2 pathway, NQO1, the transcriptional target of NRF2, would stabilize the tumor suppressor p53 [43]. The p62/NRF2/NQO1 complex also plays a role in the maintenance of mitochondrial stability during cell aging. The ROS accumulation linked to aging alters mitochondrial functions. Hence, whereas the tissues of p62-deficient mice display an accelerated aging phenotype, the overexpression of NQO1 in this p62-dependent context maintains the integrity of the mitochondrial membrane potential [44]. Another study demonstrated that the NRF2/p62 pathway is necessary for signaling through the TLR4/Myd88 receptor [45, 46]. Following the engagement of the TLR4 receptor by LPS or E Coli, the p38MAPK pathway is activated, leading to the nuclear translocation of NRF2 and the increased expression of p62.

Transcriptional regulation independent of the transcription factor NRF2: p62 expression is not only dependent on NRF2 activity. Indeed, other stimuli including phorbol 12-myristate 13-acetate (PMA), calcium and IL-3, which act independently of NRF2, drastically increase the expression of p62 mRNA in a very short time (from 30 minutes to 2 hours) [47]. In agreement, an analysis of the 5'-flanking region of the p62 promoter revealed the presence of binding sites for several transcription factors, such as SP-1, AP-1, NF-κB and Ets-1 [48]. Importantly, Duran et al. show that Ras-transformed fibroblasts exhibit a high level of p62 mRNA, which is not observed after invalidation of the AP-1 binding site located upstream on the p62 promoter [49]. Thus, these findings strongly suggest that the constitutive activity of the Ras/MEK/ERK1/2 pathway regulates p62 transcription via the AP-
the blockade of autophagy is linked to p62 ac-
decrease its intracellular levels [55]. In contrast,
induce p62 degradation and, subsequently,
hypoxia, amino acid deprivation or treatment
Hence, several pro-autophagic stimuli such as
also a substrate for lysosomal proteases.
graded. Due to its association with LC3-II, p62 is
delled into the cytosol, but some proteins present
membrane topology and therefore remains in
insensitive to Atg4 deconjugation due to its
phosphatidylethanolamine fraction (LC3-II) is
alyzed during autophagy. The first approach
its promoter.
Our own results have implicated the JNK/c-Jun
pathway in the regulation of p62 synthesis. In
chronic myelogenous leukemia (CML) cells, the
phytoalexin Resveratrol increases the expres-
sion of p62 via the activation of the JNK path-
way [50, 51]. In this context, treatment of CML
cells with SP600125, a JNK inhibitor, abrogates
the effect of Resveratrol on p62 expression.
Similarly, hydroxytyrosol a natural compound
found in olive oil, also activates the JNK/p62
pathway [52]. Collectively, these findings are in
good agreement with the observation that Pur-
kinje cells deficient in JNK1/2/3 have undetect-
able levels of p62 relative to their control coun-
terparts [53]. It remains, however, to decipher
the exact molecular mechanisms by which JNK
increases p62 expression. A recent study also
reported an indirect transcriptional control of
p62 via AP1 activation. KrasG12D-induced AP1
activation stimulates the transcription of IL-1α,
which, in turn, stimulates the synthesis of p62
through NFκB activation [54]. Finally, a positive
feedback loop in which newly synthesized p62
stimulates IKK2/β results in a constitutively
activated NFκB pathway.

Post-translational regulation of p62/SQSTM1

As mentioned above, p62 is also regulated at
the protein level by the autophagic machinery.
Following the fusion of lysosomes with auto-
phagosomes, most of the Atg proteins are recy-
cled into the cytosol, but some proteins present
in the lumen of the autophagosomes that are
necessary for the autophagic process are de-
graded by lysosomal proteases, including
cathepsins. For example, the LC3-
phosphatidylethanolamine fraction (LC3-II) is
insensitive to Atg4 deconjugation due to its
membrane topology and therefore remains in
the autophagosomal membrane where it is de-
graded. Due to its association with LC3-II, p62 is
also a substrate for lysosomal proteases.
Hence, several pro-autophagic stimuli such as
hypoxia, amino acid deprivation or treatment
with autophagy inducers have been shown to
induce p62 degradation and, subsequently,
decrease its intracellular levels [55]. In contrast,
the blockade of autophagy is linked to p62 ac-
cumulation. Several pharmacological ap-
proaches have been used to prevent p62 degra-
dation during autophagy. The first approach
consists of using Bafilomycin A1 to block the
membrane-bound lysosomal vacuolar ATPase (V
-ATPase) to prevent the lysosomal lumen acidifi-
cation responsible for cathepsin activation. The
second approach consists of the use of a cock-
tail of protease inhibitors (mainly Pepstatin A
and E-64d) to inhibit the activation of lysosomal
proteases [55] (Figure 4).

Intracellular level of p62/SQSTM1 as a marker
of autophagic flux

Considering the fact that p62 is rapidly de-
graded during autophagy, the analysis of its
intracellular level by western blotting is used
routinely to measure the autophagic flux in re-
response to pro-autophagic stimuli. In a recent
report, Larsen et al. described a reporter cell
system assay based on the tetracyclin-inducible
expression of GFP-p62 [56]. In this assay, cells
transduced with this vector are treated for 24 h
with tetracyclin to highly express the GFP-p62
fusion protein. Autophagy is then induced in
these cells, and the decrease in fluorescence,
which reflects the degradation of p62 following
the activation of autophagic flux, is analyzed
using flow cytometry [56]. According to these
results, the GFP fluorescence emitted by the
cells treated with the V-ATPase inhibitor Bafil-
omycin A1 does not change as a function of time
because p62 is not degraded via the autophagic
machinery [56]. In the future, this assay could
be a tremendous asset for the large-scale
screening of compounds or stimuli susceptible
to the modulation of the autophagic flux.

The fact that the intracellular expression of p62
is regulated at both the transcriptional and post-
translational levels is a source of concern for
the use of p62 as a general index to monitor
autophagic flux. Indeed, if the autophagy in-
ducer activates p62 transcription, the increased
autophagic flux may not be sufficient enough to
fully clear intracellular level of p62. Several re-
cent studies using three compounds that are
able to trigger autophagy, i.e., Resveratrol, PMA
and AICAR, nicely illustrate this notion [51, 57,
58]. Whereas each of these three compounds
increased the autophagic flux in CML cells, the
intracellular level of p62 remained unchanged.
In the case of Resveratrol, activation of the
JNK/c-Jun pathway drastically increased p62
expression, an event crucial for triggering Res-
veratrol-dependent autophagy. In this context,
the intracellular level of p62 remained stable
mainly due to the increase of its synthesis,
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which compensates for its autophagic degradation (Figure 4). The full validation of p62 as a hallmark of autophagic flux would therefore necessitate measuring the flux by combining the pro-autophagic stimuli with a transcriptional inhibitor such as Actinomycin D to ensure that a potential increase in p62 transcription would not counteract the degradation of p62 via autophagy.

An emerging role of p62/SQSTM1 in cancer

As previously mentioned, p62 is at the crossroads of several signaling pathways including Ras/Raf/MAPK and NFκB, whose functions are often modified during tumoral transformation to promote the proliferation, migration and invasion of malignant cells. Importantly, p62 also plays a crucial role in the regulation of mTOR activity [15] and in the regulation of autophagy. The implication of p62 in carcinogenesis is still controversial and depends on whether the protein plays a role in pro- or anti-tumoral autophagy or in the oncogenic signaling pathways. Modulation of autophagy during tumoral progression

During transformation, cells need to reprogram their metabolic machinery to escape cell death, to become autonomous and to acquire new invasive properties. It has been well established that autophagy is deregulated during carcinogenesis [59, 60]. However, in cancer cells, the molecular mechanisms underlying the tumor-promoting or, conversely, the tumor-suppressing roles of autophagy are still poorly understood.

Some findings are in agreement with the requirement of autophagy during the early stages of oncogenesis, particularly at the stage of tumor dormancy (Figure 5). This clinically undetectable phase, which precedes the period of tumor growth, is characterized by a quiescent state. In the context of residual metabolic activity, autophagy helps cells produce enough energy to address stressful conditions, such as
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during a treatment with chemotherapeutic agents, until the environment becomes more favorable [61]. It thus appears that in the initial phases of malignant transformation, autophagy is beneficial for the adaptation of tumoral cells to their microenvironment (Figure 5). The supply of nutrients and oxygen in the tumoral microenvironment is relatively poor, and in this context, autophagy would be beneficial for tumor cell development [62]. Similarly, lysosomes that are involved in autophagic degradation also play a very important role during tumorigenesis, notably through the activation of cathepsins [63].

An allograft model using apoptotic and/or autophagy-deficient mammary cells generated by overexpressing Bcl-2 (an anti-apoptotic protein) in cells monoallelically depleted of Beclin 1 (beclin 1+/− cells) greatly contributed to the understanding of the role of autophagy in the steps following this dormancy, especially during the dissemination of cancer cells [64, 65]. In this model, the autophagy process reduces the genomic instability of cancer cells. This genomic instability promotes the acquisition of the secondary mutations necessary for the growth and dissemination of these cells [66]. Consequently, these Beclin 1+/− cancer cells accumulate damaged organelles and intracellular ROS, which both contribute to increase the frequency of DNA mutations. These autophagy-deficient cells then accumulate high intracellular levels of p62 (that is not degraded), which contributes to the amplification of oxidative stress linked to ROS production and leads to NFκB activation, two events involved in tumoral transformation [67].

Other evidence argues that autophagy is capable of reducing the rate of tumor growth. Thus, the inactivation of the pro-autophagic genes TSC1/2, Atg4c, LKB1 or Beclin 1 in mice favors tumor progression [61]. In addition, in numerous types of cancer cells, the pro-autophagic genes (LKB1, PTEN, p53, TSC1, TCS2, Beclin 1, UVRAG) are often inactivated, whereas the anti-autophagic genes (PI3K, Akt, Ras, Bcl-2) are overactivated, suggesting that autophagy represents a barrier for cancer development [62, 68]. However, it should also be noted that the phenotypes associated with the mutation of all or some of these genes could be independent of the pro-autophagic activity of the genes.

Finally, only cells that are deficient in the autophagic pathways are able to acquire the mutations required for their growth and metastatic dissemination [61, 62, 66] (Figure 5). Therefore, a failure to eliminate p62 represents an explanation of how the inhibition of autophagy can promote tumoral development. This inhibition would favor the accumulation of pro-oncogenic proteins such as p62 that would promote the development and propagation of tumors (Figure 5). If this hypothesis is correct, the use of autophagy modulators would represent a promising therapeutic strategy that would depend on the progress phase of the disease.

**Influence of autophagy on p62 tumoral functions**

Several other studies have associated the accumulation of p62 with tumoral transformation and/or progression. As previously mentioned, the defective clearance of p62 in Atg7−/− murine hepatocytes leads to the constitutive activation of the NRF2 transcription factor that is responsible for hepatoma genesis [42]. Similarly, a recent report demonstrates an increased expression of NRF2 and p62 in 34 and 37% of non-small-cell lung cancer (NSCLC) cases, respectively [69]. NSCLC patients exhibiting high p62 levels have a significantly worse prognosis than patients exhibiting a low level of p62 do [69]. Similarly, p62 is highly accumulated in breast cancer disease [70], the evolution of which (grade and formation of distant metastases) is significantly correlated with the degree of p62 expression [71].

The accumulation of p62 concomitant with autophagy blockade does not only result in the amplification of pro-tumoral signaling. Indeed, another study shows that the Wnt pathway can be targeted by p62-dependent autophagy [72]. Wnt signaling, which is essential during embryonic development and whose deregulation is involved in numerous cancers, is transduced via a key protein, Dishevelled (Dsh, Dvl), that aggregates in cytoplasmic puncta to relay the signal from the Wnt receptor to multiple downstream effectors [73] (Figure 6). During starvation, Dsh is ubiquitinated and associates with p62 before being delivered to autophagosomes to be degraded by the autophagic machinery. As a consequence, the Wnt pathway is inactivated [72, 73] (Figure 6). Although autophagy is thought to be a non-selective degradation process, this example illustrates a certain degree of plasticity and specificity of this process, which, through
actors such as p62 protein, can target major oncogenic pathways to reprogram the tumor during its development. This selectivity, which is dependent on p62, is essential for autophagy, may affect tumor development and could represent a tremendous asset for elaborating new anti-cancer strategies.

The proof-of-principle of this type of therapy was provided by studying the effect of ATRA on acute promyelocytic leukemia (APL), a common variant of acute myeloid leukemia (AML). This hematopoietic malignancy is caused by the PML-RARα nuclear translocation, which fuses the genes encoding for promyelocytic leukemia (PML) and for the retinoic acid receptor alpha (RARα). ATRA is currently the leading treatment for this pathology and has recently been shown to induce PML-RARα degradation [74]. The authors showed that this degradation proceeds by autophagy following the binding of p62 to PML-RARα [75]. By itself, the interaction between p62 and PML-RARα has no deleterious; however, only because ATRA results in an increased autophagic flux, p62 is converted to a deadly adaptor that eliminates the oncogene responsible for the disease.

**p62/SQSTM1, a protein working either as an essential participant in oncogenic signaling or as an inhibitor of tumor proliferation**

p62 is a scaffold protein, which, through its own degradation, selectively directs its targets to the autophagic machinery. However, this protein is not only a substrate allowing the autophagy to arbitrate tumor cells fate. This protein also acts intrinsically as a major pro-oncogenic regulator of several important signaling pathways. Therefore, it is possible that autophagy only intervenes as a facilitator or an inhibitor of the pro-oncogenic action of p62. Thus, the ectopic over-
expression of p62 in apoptosis- and autophagy-
deficient cells is sufficient to drive tumor forma-
tion [62].

As previously mentioned, through its interaction
with TRAF6, p62 plays an essential role in the
regulation of the NFκB pathway (Figure 6). The
p62-TRAF6 complex integrates and transduces
signals in response to the activation of IL-1 [14],
the nerve growth factor and the RANK-L recep-
tors [76, 77]. In ductal pancreatic adenocarcinoma
cells, the constitutive activation of Ras
activates the NFκB pathway to trigger increased
p62 synthesis, and this newly synthesized p62,
in turn, interacts with TRAF6 to amplify Ras sig-
aling in a positive feedback loop [15, 54]. The
same observations arise from studies on lung
in cancer in which Ras activation stimulates the
TRAF6-p62-NFκB complex to trigger an antioxi-
dant response that is necessary to inhibit dele-
terious ROS production [49]. Finally, p62 is
strongly overexpressed in the subfraction of
highly aggressive glioblastoma harboring a mes-
enchymal oncogenic signature, and this overex-
pression is concomitant with MAPK activation
[78]. In this particular context, the inhibition of
p62 drastically reduces the motility and inva-
siveness of the glioblastoma cells.

p62 also participates in the tumorigenic signal-
ing mediated by the mTOR pathway (Figure 6).
mTOR can form two distinct complexes:
mTORC1 and mTORC2. The mTORC2 complex is
relatively insensitive to nutrient deprivation and
to rapamycin and is involved in the regulation of
cell survival and actin cytoskeleton remodeling
via Akt phosphorylation. Conversely, the
mTORC1 complex, which is highly sensitive to
nutrient starvation and to rapamycin stimula-
tion, modulates autophagy [16, 79, 80] and
plays an essential role in the regulation of cell
growth and protein synthesis. mTORC1 is inhib-
it by the TSC complex (tuberous sclerosis
complex), which is composed of the TSC1 and
TSC2 proteins. TSC2, also called tuberin, con-
tains a GAP (GTPase-activating protein) domain
in its C-terminal region, which is involved in the
inactivation of the mTORC1-activating small G
protein (Ras-like GTPase) Rheb. Therefore, the
TSC complex is a tumor suppressor, and its in-
validation in mice results in the hyperactivation
of the mTOR pathway and the occurrence of
harmless tumors in several organs. A recent
study established that p62 is necessary for the
proper development of the tumors associated
with TSC2 invalidation [81]. Indeed, the survival
of mice injected with TSC2−/− mouse embryonic
fibroblasts is significantly increased when p62
expression is knocked down. According to these
results, a link between TSC2 and p62 has been
reported by Duran et al., who described an inter-
action of p62 with mTOR and Raptor that was
required for the signaling pathway that is de-
pendent on the mTORC1 complex [15] (Figure
6). Subsequently, the same authors showed
that the knockdown of p62 by shRNA inhibits
the growth of mTOR hyperactive-driven tumor
cells.

In contrast, several other studies argue for a
tumor-suppressive function of p62, especially
during mitosis. In mammalian cells, the main
protein kinases required for progression
through the cell cycle belong to the cyclin-
dependent kinase (CDK) family. CDK1 controls
the checkpoint between the S/G2 and early
mitotic phases. Importantly, p62 is phosphory-
lated by CDK1 on threonine 269 and serine 272
during the early mitotic phase, allowing the cells
to properly continue through mitosis [82]. More-
ever, these authors describe cancer cells ex-
pressing a non-phosphorylatable mutant of p62
that exhibit a better tumoral phenotype in vitro
and in vivo than cells expressing the wild-type
form of p62. Therefore, it appears that the
CDK1-dependent phosphorylation of p62 repres-
ts a constraint for tumoral transformation,
again highlighting the dual function of p62,
which can act either as an oncogene or as a
tumor suppressor depending on the cellular
circumstances.

In conclusion, p62 is a scaffold protein at the
crossroads between pro-oncogenic signaling
pathways (i.e., NFκB, MAPK, mTOR) and auto-
phagic degradative pathways. Although p62 is
by itself a significant participant in cellular
transformation and tumor propagation, it is also
regulated by autophagy, which can target some
pathways or key proteins involved in tumoral
development via its intermediate form. As a
future clinical prospective, new anti-cancer
strategies should benefit from the pivotal func-
tion of p62 between tumorigenesis and auto-
phagy to circumvent tumoral progression.

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