Chapter 6

The Cross Talk among Autophagy, Ubiquitination, and DNA Repair: An Overview

Francesca Nazio, Emiliano Maiani and Francesco Cecconi

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71404

Abstract

Cellular plasticity is modulated by protein posttranslational modifications, which act on most intracellular pathways. Ubiquitination is a versatile posttranslational modification (PTM) that influences protein fate, controlling their degradation or modulating their activity and subcellular localization. The ubiquitin proteasome system, UPS, and the autophagic pathway are the main degradative intracellular machineries, which rely on ubiquitination for their activation and/or the selective recycling of proteins and organelles. Recent findings indicate that the cross talk between UPS and autophagy plays a key role in controlling DNA repair pathways. Even being a cytoplasmic process, it is now clear that autophagy can directly impact on the correct activation of DNA repair. Of note, defects on autophagy are related to the impairment of homologous recombination repair and to an increase of the nonhomologous end joining repair activity. These evidences give new insights into the molecular processes underlying the DNA damage response and provide further explanation for the tumorigenesis associated with autophagy impairment. Moreover, these findings introduce new examples of synthetic lethality between autophagy and DNA repair genes and lead to the possible development of target therapies for tumors with defective autophagy.

Keywords: autophagy, DNA repair, ubiquitination, ubiquitin proteasome system, p62

1. Introduction

In eukaryotic cells, protein homeostasis is essential to maintain cell survival and it occurs through two major pathways: the ubiquitin-proteasome system (UPS) and autophagy. The UPS is responsible for degradation of both cytosolic and nuclear short-lived or damaged proteins, and it is involved in the removal of 80–90% of cellular proteins. It regulates several

processes, including maintenance of cellular quality control, transcription, cell cycle progression, DNA repair, receptor-mediated endocytosis, cell stress response, and apoptosis [1]. By contrast, autophagy mediates the degradation of long-lived proteins, entire organelles (e.g., mitochondria and peroxisomes), or pathogens and aggregates *via* the lysosome. On the one hand, autophagy is related to cell growth, survival, and development; on the other hand, it is involved in cell death and it has been implicated in human pathologies such as cancer, neurodegeneration, myopathies, and heart and liver diseases [2].

The ubiquitin-proteasome system and autophagy were long viewed as independent and parallel processes. However, it becomes increasingly clear that the UPS and autophagy crosstalk to each other [3]. The need for energetic homeostasis and protein balance requires that both these degradation systems are tightly controlled and coordinated during a cell life. In particular, the balance of cellular homeostasis needs to be carefully regulated and this is made possible by protein posttranslational modifications (PTMs) such as phosphorylation, acetylation, methylation, and ubiquitination [4–6]. PTMs, indeed, due to their reversible or irreversible nature, provide the necessary flexibility in order to adapt the cells rapidly to different environmental stress.

Accumulating evidence indicates that ubiquitination regulates autophagy through at least two mechanisms [7]. One is controlling the stability of upstream autophagy-related (ATG) genes. In this context, many E3 ligase and deubiquitinase (DUBS) enzymes have been identified as crucial for autophagy induction, maturation, or termination [8–14]. The other one facilitates the recruitment of ubiquitinated substrates to the autophagy machinery [15]. In this case, ubiquitination plays an essential role in determining the selectivity of autophagy cargos.

There are different interfaces between autophagy and UPS. First, ubiquitin or ubiquitin-like proteins are common degradative tags; ubiquitin, indeed, is a very small molecule that can be attached to the substrate by several ways, generating a broad repertoire of signals. These degradative tags are then recognized by specific adaptor proteins, such as p62/sequestosome 1 (SQSTM1) or neighbor of BRCA1 gene 1 (NBR1), that are molecules capable of directing ubiquitinated target proteins to both systems [15]. They act through specific domains, such as the ubiquitin-associated domain (UBA) or the ubiquitin-binding domain (UBD), able to specifically recognize the substrate for mediating its degradation. The other point in common is the participation of these mechanisms to general cellular programs, such as the ER stress response [16] or the atrophy program [17]. Moreover, recent studies have revealed that autophagy and UPS participate together also in DNA damage response (DDR) [18, 19]. DDR is an essential mechanism to maintain genome integrity; similar to protein homeostasis, maintenance of genomic integrity is essential for an organism's survival. Although these mechanisms occur in spatially distinct cellular compartments, evidence has been accumulated about a strict cross talk among autophagy, ubiquitination, and DNA repair. When a DNA lesion occurs, chromatin undergoes a relaxed conformation through a series of histone PTMs, recruitment of DDR sensors, and additional proteins to further regulate DNA replication, cell cycle, repair, and cell survival *versus* cell death. In this context, a key role is played by p62 that has been recently found to be able to shuttle between cytoplasm and nucleus, where it is able to inhibit homologous recombination (HR) or the recruitment of DNA-binding factors [20–22]. In this chapter, we provide an

http://dx.doi.org/10.5772/intechopen.71404

overview of the current knowledge about the coordination among autophagy, ubiquitination, and DNA repair pathways, and its importance to maintain cell homeostasis and survival.

2. Cross talk between autophagy and UPS

UPS and autophagy are two crucial mechanisms that are involved in cellular catabolism in normal physiology and development, but also in human pathologies such as cancer, neurodegeneration, and aging. By these processes, cells are able to recycle proteins, aggregates, or entire organelles to obtain energy. Although these pathways differ for specificity, kinetics, and substrates, it is increasingly clear that they are cooperative and complementary to ensure cellular homeostasis and survival.

2.1. Autophagy: an overview of its main actors and functions

Autophagy is a catabolic process occurring in all eukaryotic cells to maintain cellular viability and homeostasis in basal conditions, by controlling long-lived proteins and damaged organelles. However, autophagy can also be stimulated in response to sublethal stresses, such as nutrient or growth factor deprivation, hypoxia, reactive oxygen species (ROS), or viral and pathogen invasion to maintain cell survival [23]. During autophagy, cells undergo rapid changes to adapt their metabolism and protect themselves against potential damages. Depending on the delivery route of cytoplasmic material to the lysosomal lumen, three different forms of autophagy are known: microautophagy, chaperone-mediated autophagy, and macroautophagy. In microautophagy, portions of cytosol are instantly engulfed by the lysosomal membrane. In chaperone-mediated autophagy, proteins characterized by a specific sequence signal are recognized by lysosomal receptors and then degraded by lysosomal proteases. During macroautophagy (hereafter, more simply, autophagy), cytoplasmic material (e.g., proteins, lipids, and organelles) is sequestered by a cup-shaped membrane (called isolation membrane or phagophore), which expands while becoming spherical to urn into a double-membraned vesicle, termed autophagosome; this slides along cytoskeletal structures and fuses with lysosomes, thus forming a single vesicle called the autophagolysosome, in which both autophagosome membrane and contents are degraded by lytic enzymes [24].

Taking advantages from yeast genetics, more than 35 ATG genes have been identified and characterized, with most of them being well-conserved from yeast to mammals [25]. The autophagy process is divided into mechanistically distinct steps, including induction, autophagosome formation, and autophagosome-lysosome fusion, followed by the release of the degradation products back into the cytosol. Different sets of ATG proteins are involved in these steps and constitute the core autophagic machinery.

Indeed, the core pathway of mammalian autophagy involves at least five molecular complexes including (1) the ULK1 complex, (2) the BECLIN 1/class III PI3K complex, (3) two transmembrane proteins: ATG9 and VMP1, (4) two ubiquitin-like protein (ATG12 and LC3) conjugation systems, and (5) proteins that mediate the formation of autophagolysosomes [24].

The activation of this molecular machinery is extremely complicated and it involves multiple signaling inputs. According to current knowledge, the most important sensor of cellular stress is mammalian target of rapamycin complex 1 (mTORC1). This serine-threonine kinase shuts off autophagy in cells growing in the presence of nutrients and growth factors; in basal conditions, mTORC1 negatively regulates the ULK1 complex, the early most important structural complex of the core autophagic machinery.

As a consequence of the autophagy role on cellular homeostasis, increasing evidence reveal that alteration in autophagy occurs in many human diseases, such as neurodegenerations, myopathies, infectious disease, aging, and cancer, contributing to their pathogenesis. Autophagy results to be deregulated in many neurodegenerative diseases, causing the accumulation of aggregates of mutated toxic proteins [26]. Autophagy has also been identified as a crucial process in oncogenesis and cancer progression [27, 28]. Many autophagy-related proteins are considered tumor suppressor genes and are mutated in cancer (Beclin 1, ATG5, Bif-1, ATG4C, and UVRAG), leading to an accumulation of DNA damage and genome instability [28]. Finally, the activity and recruitment of ATG proteins are important also for antigen presentation, innate immune signaling, and pathogen degradation.

2.2. UPS

Ubiquitin proteasome system (UPS) is the major pathway responsible for the degradation of cytosolic short-lived proteins and of proteins residing in the nucleus and the endoplasmic reticulum (ER) [29]. The tagging molecule is ubiquitin, a small protein of 76 amino acids that is covalently linked to thousands of different proteins by a bond between the glycine at the C-terminal end of ubiquitin and the side chains of lysine on proteins. The earmarked proteins are then degraded by the 26S proteasome, a highly conserved multicatalytic ATP-dependent protease complex. Conjugation of ubiquitin to a substrate is mediated by the action of three ubiquitin-activating enzymes called E1, E2, and E3. E1 binds ubiquitin and transfers it to the active site of E2; finally E3 enzyme transfers the ubiquitin molecule directly to the substrate. Regarding the selection of the substrates, many strategies could exist; in some cases, the E3 enzyme recognizes and binds a signal in the protein sequence [30].

In the human genome, 2 E1s, 50 E2s, and 600 E3s have been identified [31]. The classification of ubiquitin ligases is based on their biochemical and structural features. The best known domain subclasses include HECT (homologous to E6-associated protein carboxy-terminus), RING-fingers (RING, really interesting new gene), and U-box domains (a modified RING motif without the full complement of Zn2 + −binding ligands).

Ubiquitination is a reversible, specific, and adaptable PTM, similar to phosphorylation; by means of seven lysine residues in ubiquitin (at positions 6, 11, 27, 29, 33, 48, and 63) that act as acceptors of other ubiquitin molecules, this PTM is considered very versatile.

2.3. Connections between autophagy and UPS

The different molecular machinery characterizing UPS and autophagy is just one of the differences between these two processes; they are also responsible for the disposal of different

substrates. The proteasome is responsible for degradation of short-life proteins, while those with long-life, organelles and aggregates, are autophagic substrates. At variance with UPS, autophagy is restricted to the cytoplasm; moreover, the two processes differ in the time window in which they act, since autophagy is considered slower than UPS (**Figure 1**). However, several recent lines of evidence have suggested that UPS and autophagy are functionally connected [32]. Indeed, the need for energetic homeostasis and protein balance requires that both degradation systems are tightly controlled and coordinated during a cell life.

The first unifying factor linking UPS and autophagy is ubiquitin. Although autophagy was considered originally a nonspecific process, it has recently emerged as a selective mechanism that specifically removes damaged organelles, such as mitochondria, or defective proteins. This specificity may be accounted for by special proteins called autophagy receptors and adaptors that are able to recognize and bind the ubiquitinated proteins listed for degradation by the autophagy machinery. They include p62/SQSTM1, neighbor of BRCA1 gene 1(NBR1), histone deacetylase 6 (HDAC6), the BH3-only family protein BNIP3L/Nix, the ubiquitin receptor nuclear dot protein 52kd (Ndp52), and optineurin [15]. These receptors recognize ubiquitin chains (including Lys-63-poly Ub and others) through their UBA domain on one side and directly bind LC3 or other ATG8 proteins via their LC3-interacting region (LIR). This allows the incorporation of autophagy substrates into the autophagosome. Among them, p62 has been extensively studied. P62 molecules are distributed not only in the cytosol but also in the nucleus, as well as they localize with autophagosomes and lysosomes. Besides its role in macroautophagy and selective autophagy (such as mitophagy) that has been fully investigated, there are several evidence that p62 is the main actor in mediating the cross talk between autophagy and UPS. First, the proteasome is inhibited in autophagy-deficient cells due to accumulation of p62; second, pharmacological inhibition of the proteasome also increases p62 expression [33]; third, p62 silencing attenuates the accumulation of proteasome substrates [34]. One explanation is that accumulation of p62 sequesters ubiquitinated proteins that aggregate and become inaccessible to the proteasome.

Intriguingly, p62/SQSTM1 is also known as an inhibitor of proteasomal degradation of LC3 [35]. In linking proteasomal degradation and autophagy, an important role is also played by HDAC6, the enzyme that regulates the acetylation of γ-tubulin and facilitates the transport of polyubiquitinated protein aggregates to the nascent phagophore [36]. HDAC6 has been shown to be involved in both aggresome formation and the fusion of autophagosomes with lysosomes, thus making it an attractive target to regulate protein aggregation.

A second important link is that ubiquitination can affect stability and function of ATG proteins and their upstream regulators. Many ubiquitin E3 ligases have been demonstrated to regulate autophagy: for instance, RNF5, which directly modulates the stability of ATG4B, or TRAF6, Nedd4 or NEDD4L, which mediate ubiquitination of Beclin 1 and ULK1, respectively [8]. Intriguingly, a catalytic activity–independent role for ubiquitin ligases such as TRIM13 and c-Cbl in autophagy is emerging by regulating the recruitment of autophagy adaptors like LC3 and p62 [37].

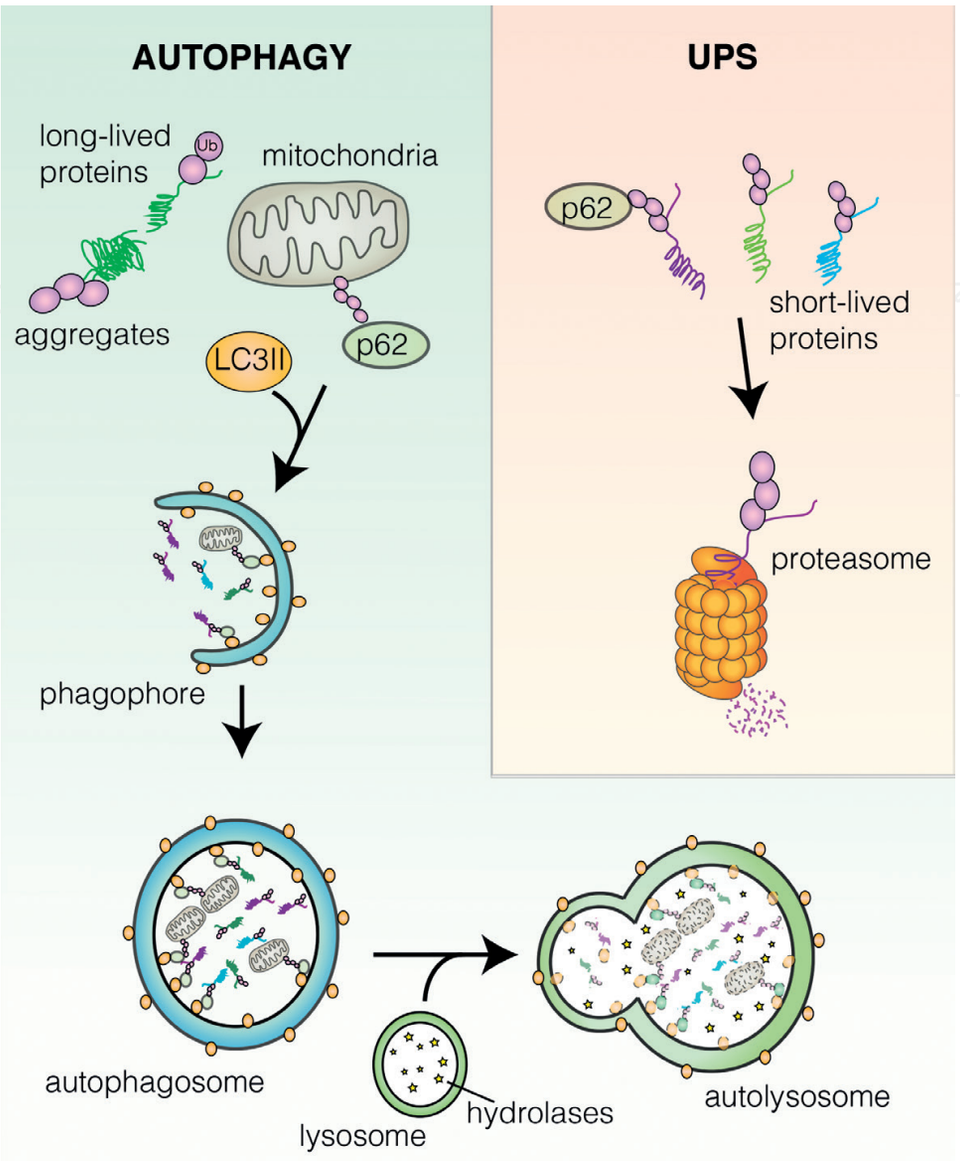

Figure 1. Overview of autophagy and the ubiquitin proteasome system (UPS). Autophagy and UPS are the main intracellular recycling processes. While autophagy degrades long-lived proteins, protein aggregates, and whole organelles (e.g. mitochondria), UPS is involved in degrading short-lived proteins. Proteins and organelles that need to be degraded are labeled by ubiquitin. Ubiquitin chains can be recognized by adapters, such as p62, that mediate the binding of the target with the proteasome (UPS) or with the protein LC3II (autophagy). Autophagy begins with the formation of the phagophore that embeds the material to be recycled and maturates into the autophagosome. The autolysosome is then formed through fusion with the lysosome, and hydrolases are responsible for the content degradation.

3. Autophagy and DNA repair

Genome integrity is preserved by an evolutionary conserved machinery named DNA damage response (DDR). Upon DNA damage, molecular key players of DNA repair pathways induce arrest of cell cycle progression and enhance activation of DNA repair pathways [38]. Programmed cell death mechanisms are then activated if the DNA lesions are not repaired and, so far, defects in DNA repair and death processes are considered the major source of

genomic instability and malignant transformation [39]. Although autophagy is a cytoplasmic process, autophagy-deficient cells display genomic instability and accumulation of DNA damage [28]. To date, a range of mechanisms have been found to be involved in linking autophagy and DNA repair, this opening important questions that need to be addressed.

3.1. DNA damage response (DDR): an overview

The DDR is comprehensively a set of intracellular pathways and specialized molecules that are activated in response to different types of damage to facilitate repair and prevent cell transformation and death. This process has been widely investigated and well-reviewed elsewhere [40–43]. We here provide only a brief overview of its function and components that is relevant to understand the cross talk between DNA repair pathways and autophagy.

DNA damage can be caused by several exogenous (e.g. ultraviolet light or ionizing radiation) or endogenous agents (e.g. reactive oxygen species (ROS)). The most common types of lesion can be single-strand breaks (SSBs), double-strand breaks (DSBs), and interstrand cross-links (ICLs). Sensing DNA damage results in the initiation of some programs, including cell cycle arrest, checkpoint activation, and DNA damage repair [38–43]. When a DNA lesion occurs, histones undergo PTMs, such as phosphorylation and acetylation, that lead to chromatin relaxation. This provides access to DDR sensors that bind DNA lesions. Initially, DSBs are bound by the Mre11 complex (MRN), including Mre11/Rad50-Nbs1, that recruits the ataxia telangiectasia mutated protein kinase ATM [44]. ATM activation is then induced by a series of PTMs that trigger the recruitment of additional proteins, including checkpoint kinase 2 (Chk2), involved in cell cycle control, the tumor suppressor protein p53 that controls cell survival, and HDAC1 and HDAC2 that regulate chromatin remodeling to further orchestrate and amplify the DSB response. In the case of DSBs, the ATM-DNAPK pathway induces phosphorylation of the histone variant γ-H2AX that flanks DBS sites.

SSBs, instead, favor the activation of ataxia telangiectasia and Rad3-related (ATR) kinase that is recruited by the replication protein A (RPA) complex [45]. ATR activity is, in turn, amplified by the recruitment of several factors, leading to the spread of SSB signal.

There are five main DNA repair mechanisms: mismatch repair (MMR), base excision repair (BER), nucleotide excision repair (NER), nonhomologous end joining (NHEJ), and homologous recombination (HR). MMR, BER, and NER are used for different types of base-associated lesions that require a single strand incision. NHEJ and HR repair mechanisms are involved in DSB repair [38, 39].

DNA repair is carried out by a large number of enzymes that chemically modify DNA to repair the damage, including nucleases, helicases, polymerases, topoisomerases, recombinases, ligases, glycosylases, demethylases, kinases, and phosphatases. PTMs of these proteins by ubiquitin and ubiquitin-like modifiers (UBLs) are essential in regulating the enzymes that reestablish genome integrity after damage.

Mutations and dysfunctions in genes involved in DNA repair pathways have been implicated in many human diseases, such as neurological and immunological defects, aging, and cancer [38]. In the nervous system, indeed, neurons exhibit high oxygen consumption by

mitochondrial respiration, which can result in oxidative stress and subsequent DNA damage; given that neurons display limited capacity of replacement, DNA repair pathways play an essential role to maintain their homeostasis [39]. Deficiency in multiple DNA repair pathways, including NER, BER, and DSB repair, has been linked to premature aging. Finally, the maintenance of genomic integrity by DNA repair pathways is critical to prevent tumorigenesis, as indicated by the cancer predisposition of several DDR syndromes [46].

3.2. Connections between autophagy and DNA repair

The first evidence that linked autophagy to DNA damage came out to understand why defects in autophagy rendered cells susceptible to metabolic stress promoting tumorigenesis. In 2007, Mathew and colleagues reported for the first time that autophagy could function to protect the genome [28]. Knockdown of autophagy genes such as ATG5 and Beclin 1 results in gene amplification, chromosomal instability, and aneuploidy, facilitating tumor progression. In detail, in autophagy-deficient cells, they found an increase in the levels of γ-H2AX and other DNA damage responses, suggesting that constitutive and stress-induced autophagy is important to prevent DNA damage and maintain the integrity of the genome [28, 47–51].

Interestingly, it has been reported that in murine embryonic fibroblasts (MEFs), knockout for the 200 kDa FAK-family interacting protein (FIP200), there is a significant decrease in DNA damage repair in response to ionizing radiation as well as to chemotherapeutic agents [52]. FIP200 is a component of the ULK1 complex and is essential for activation of autophagy. In this study, at variance with its potential tumor suppression function, inactivation of FIP200 and subsequent deficiency in autophagy sensitize cells to apoptosis-inducing agents probably due to the defective DNA damage repair.

From then onwards, several studies have demonstrated that autophagy participates, directly or indirectly, in DNA repair pathways. Indeed, it is now accepted that autophagy, in particular mitophagy—the selective removal of damaged mitochondria, can prevent genomic instability by removing ROS-producing mitochondria, since ROS are one of the major sources of DNA damage as they could directly modify the DNA or indirectly generate different lesions, both affecting cell viability [53]. Moreover, autophagy is also necessary for providing energy and metabolites required for an efficient DNA repair. In fact, many evidence show that, by sustaining the energy demand required to support DNA repair processes, autophagy can help the development of chemoresistance mechanisms in cancer cells, delaying apoptotic cell death upon DNA damage [54, 55]. Besides that, it is now clear that autophagy can be activated by DNA damage at multiple levels. The use of the DNA-damaging agents such as camptothecin, etoposide and temozolomide, p-anilioaniline, and ionizing radiation (IR), in addition to initiate cell cycle arrest, also initiates autophagy [54, 56, 57].

As described above, ATM is a central regulator of the DDR response. In response to DNA damage, the transcription factor FOXO3a binds ATM, thus leading to its activation and promoting repair. Both ATM and FOXO3a have been linked to autophagy. ATM induces the activation of the energy sensor AMP-activated protein kinase (AMPK), leading to autophagy progression [58, 59]. On the one hand, AMPK interacts with the main negative regulator of autophagy, the mTORC1 complex *via* a pathway involving tuberous sclerosis complex 1 and

2 (TSC1/2); on the other hand, AMPK directly phosphorylates one of the key protein kinases that initiate autophagy, ULK1 [60, 61]. ATM also mediates the activation of Che-1, a RNA polymerase II-binding protein that regulates the transcription of two mTOR inhibitors: Redd1 and Deptor [62]. Otherwise, FOXO3a controls the transcription of autophagy-related genes, such as LC3 and Bnip3 [63–65]. Another DDR protein involved in autophagy is poly[ADP-ribose] polymerase 1 (PARP1). After a DNA lesion, PARP1 synthesizes poly(ADP-ribose) chains that recruit the DNA damage repair proteins. Recently, it has been demonstrated that hyperactivated PARP1 causes a depletion of ATP that leads to AMPK activation and, consequently, to autophagy induction [66].

A crucial regulator of DNA repair pathways is the tumor suppressor protein p53. P53 has a dual role in autophagy [67, 68]: on one hand, p53 together with other members of its family (p63 and p73) regulates transcriptionally autophagy-related proteins; on the other hand, p53 acts directly on AMPK signaling.

Moreover, HDAC proteins represent a significant link between autophagy and DNA repair pathways. HDACs are histone deacetylases that influence DNA damage response through acetylation of key DNA repair and checkpoint proteins. Robert and colleagues found that HDACs control chromosome stability by coordinating the ATR checkpoint and DSB processing with autophagy [36]. In particular, HDAC inhibition triggers degradation of the recombination protein SAE1 (in human CtIP) by promoting autophagy that affects the DNA damage sensitivity of *HDAC* mutants.

Recent studies have suggested that another family of proteins called sirtuins could play an important role in autophagy and DNA repair pathways. Sirtuins are protein deacetylases dependent on NAD+ that are involved in autophagosome formation by deacetylating ATG5, ATG7, and ATG8. In DNA repair pathways, sirtuins regulate transcriptional activity of p53, thus affecting cell cycle and cell death under DNA damage conditions [69].

Recently, an interesting connection between DNA repair signaling and mitophagy has also been provided. As mentioned above, damaged mitochondria may produce elevated levels of ROS, thus inducing DNA damage. In addition, blockage of mitophagy can result in the accumulation of dysfunctional mitochondria, damaged mtDNA, and an increased rate of apoptotic cell death. Feng and colleagues found that in ataxia telangiectasia patients, characterized by ATM dysfunctions, the defect in the nuclear DNA damage repair leads to defective mitophagy [70]. This occurs through the impairment of Sirtuin1 activity that, in turn, affects the expression of the mitochondrial uncoupling protein 2 (UCP2), responsible for the import, cleavage, and removal of PINK1, a key molecule in mitophagy induction.

Intriguingly, new evidence suggests a direct role for autophagy in the function of "error proof" HR, NER, or MMR. About the involvement of autophagy in NER regulation, (an adaptable DNA repair pathway that corrects helix-distorting base lesions induced by environmental carcinogens), it has been found implicated in downregulating the transcription of XPC and impairing the recruitment of DDB2 to UV-induced lesion sites through TWIST1-mediated inhibition of EP300 [71]. MMR defects also impair autophagy induced by chemotherapeutic drugs [72]. Mispairs induced by nucleoside analogs, such as 6-thioguanine (6-TG) and 5-fluorouracil (5-FU), have been reported to induce autophagy in a p53-, mTOR-dependent manner

by upregulation of BNIP3. These studies suggest that targeted inhibition of the autophagic pathway may enhance the cytotoxicity of those anticancer agents that are recognized and processed by the MMR system.

Of note, the protein UVRAG (UV-irradiation-resistance-associated gene) plays a dual role acting both in autophagosome formation and maturation and chromosomal stability [73], independently from autophagy. In autophagy, UVRAG is responsible for the activation of PI(3) class III (PI(3)KC3) kinase through Beclin 1 interaction. During NHEJ, UVRAG interacts and helps the assembly of the upstream protein kinase of the NHEJ pathway, DNA-PK. Moreover, UVRAG is found to be associated with centrosomes by its interaction with CEP63. Affecting the UVRAG-centrosome interaction destabilizes centrosomes, resulting in extensive aneuploidy. In the same way, Beclin 1 exerts a specific role on the NHEJ pathway [74]. Conversely, the genomic instability characterizing autophagy-defective mice models underlines how autophagy-deficient cells rely on the error-prone NHEJ repair process.

Of note, one of the most important connections between autophagy and DNA repair pathways is highlighted by the mediator of autophagy on UPS, the adaptor protein p62. These very recent and fascinating discoveries will be better explained in the next paragraph.

4. Autophagy, ubiquitination, and DNA repair

As previously described, p62 is an autophagic receptor and substrate that selectively targets polyubiquitinated proteins for degradation via both proteasome and autophagy. P62 levels are impaired in many diseases, such as cancer, proteinopathies, neurodegeneration, obesity, and liver diseases [75, 76]. P62 protein levels are strongly induced by different proteotoxic stresses such as oxidants, proteasomal inhibitors, or ionophores; many p62 functions are carried out by its N-terminal PB1 and C-terminal UBA domains that are necessary for protein-protein interactions and for its polymerization. P62 is commonly found in the cytosol, together with ubiquitinated proteins or aggregates; however, by now, it is evident that p62 is able to shuttle between cytoplasm and nucleus by a specific nuclear export signal (NES) and two nuclear localization signals (NLS) [22]. In the nucleus, p62 is found associated to promyelocytic leukemia bodies (PML) that usually contain proteasomes, chaperones, and ubiquitinated proteins [77]. Its nucleocytosolic distribution is finely regulated by several mechanisms, including self-association, phosphorylation, and binding to ubiquitinated proteins. In particular, accumulation of ubiquitinated proteins is able to retain p62, resulting in its accumulation in aggregates both in the cytosol and in the nucleus. It has been recently found that nuclear p62 is able to associate with markers of DNA repair, providing the first important link among autophagy, UPS, and DNA repair [20]. More in detail, Hewitt and colleagues found that after DNA damage (X-ray irradiation), p62 was associated with DNA damage *foci* (DDF); this association decreased after autophagy induction and it was impaired in autophagy-deficient cells, thus suggesting a role for p62 in mediating the effect of autophagy on DNA repair. The molecular mechanism by which p62 carries out its functions in this context involves the proteasomal degradation of two essential DNA repair-related proteins: filamin A (FLNA) and the RAD51 recombinase. FLNA is known to mediate the recruitment of RAD51 to DSB and facilitate

HR. The p62-mediated proteasomal degradation of FLNA results in reducing RAD51 protein levels and slower DNA repair. Therefore, autophagy is able to control HR by reducing the levels of its substrate p62. Overall, these findings explain how autophagy impairment leads to an increase in DNA damage and consequently to genomic instability. This is particularly relevant during aging, when nuclear levels and co-localization of p62 with DNA damage *foci* have been reported to increase and when autophagy is gradually impaired, underlining the important role of this pathway to age-related diseases.

Novel important mechanistic insights into the connection among autophagy, ubiquitination, and DNA damage response have been presented in the same year, and also in those cases, a main role was played by p62. Wang and colleagues identified the E3 ligase RNF168 as a novel p62-binding protein [21]. RNF168 is an ubiquitin E3 ligase that binds ubiquitinated histones H1 and H2A, thus propagating the H2A ubiquitination at sites of DNA damage [78]. RNF168 catalyzes Ub-K63 chains on Lys13-15 of H2A and H2AX [79–81]. The RNF168 pathway has an important function in regulating the DSB repair pathway choice, by promoting the recruitment of the key repair factors for both NHEJ and HR at chromatin areas near DSBs. Wang and colleagues discovered that p62 inhibits RNF168 E3 ligase activity, leading to a decrease in RNF168-dependent polyubiquitination of histone H2A [21]. It has been reported that the LB domain of p62 is responsible for the binding and repression of RNF168. After DNA damage, p62 dissociates from RNF168, presumably because p62 is degraded by autophagy. In autophagy-deficient cells, indeed, p62 accumulates at DNA damage sites and impairs chromatin ubiquitination. When histone ubiquitination decreases, the recruitment of DNA repair proteins such as BRCA1, RAD51, and RAP80 to sites of DSBs is compromised and consequently also the repair of radiation-induced DNA damage.

Besides the direct role of p62 in DNA repair, some key factors of DNA damage response have also been found to be degraded by autophagy. The heterochromatin component HP1a is necessary to maintain chromatin in a condensed state, and this hides the RAD51 binding site at DSBs. A recent study showed that, after X-ray irradiation, the E2 ligase RAD6 interacts with HP1a, leading to its ubiquitination and degradation *via* autophagy. HP1a autophagy-mediated degradation makes chromatin more permissive for the catalysis of HR [82]. In addition, these findings are supported by another work showing that RAD6 is important for Parkin-dependent mitophagy [83]. Interestingly, checkpoint kinase 1 (Chk1), a regulator of DNA damage repair by HR, is another target of autophagy. A recent paper shows that loss of autophagy results in decreased levels of total and phospho-Chk1; the authors propose that decreased levels of Chk1, in the absence of autophagy, are due to increased proteasomal activity and this, in turn, impairs both DNA damage repair by HR (but not NHEJ) and genomic integrity [84]. Another study identified Chk1 as a target of chaperone-mediated autophagy (CMA) [85]. Park and colleagues found that CMA is upregulated by DNA damage after both irradiation and chemotherapy, thus inducing degradation of p-Chk1 [86]. Interestingly, CMA is able to degrade Chk1 only after its phosphorylation on Ser345; by contrast, Ser317-phosphorylated Chk1 is the preferred substrate of the proteasome. When CMA is defective, Chk1 accumulates in the nucleus and leads to destabilization of the MRN complex involved in the initial processing of DSBs prior to DNA repair by HR, thus facilitating genomic instability. A schematic representation of the cross talk among autophagy, ubiquitination, and DNA repair machinery is reported in **Figure 2**.

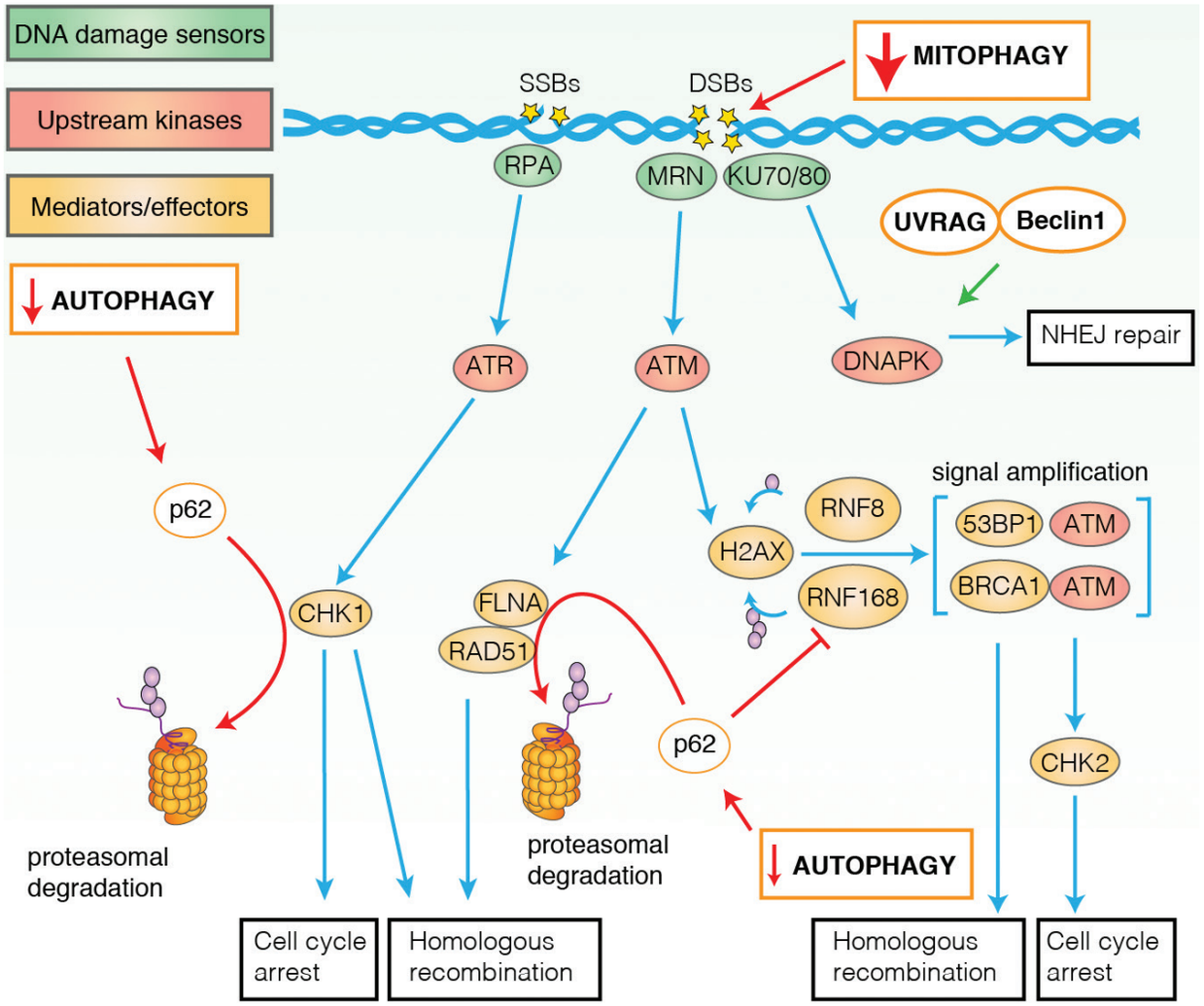

Figure 2. Model of autophagy in the DNA damage response. Autophagy impairment is directly associated with the modulation of different DNA repair pathways and with the formation of DNA double strand breaks. Mitophagy defects lead to the accumulation of malfunctioning mitochondria and to the increase of reactive oxygen species (ROS) that cause the formation of DNA double strand breaks. Upon DNA damage, different DNA repair pathways are induced, depending on the type of DNA lesion and on the phase of cell cycle. It has been demonstrated that impairment on autophagy decreases the functionality of homologous recombination (HR). P62 levels increase upon autophagy downregulation, thus inducing the proteasome-dependent degradation of CHK1 or Rad51/FLNA proteins. Moreover, p62 affects HR repair by directly binding and inhibiting the histone ubiquitin ligase RNF168. On the other hand, autophagy-related proteins, such as Beclin 1 and UVRAG, can shuttle into the nucleus and promote the nonhomologous end joining pathway of DNA repair.

Recent publications reported that autophagy can also positively regulate NER, acting on the levels of NER-specific damage recognition proteins such as XPC, UVRAG, and DDB1/2. As previously mentioned, UVRAG is involved in both autophagy and DNA repair. In this work, they found that, after irradiation, UVRAG localizes to DNA lesions and associates with DDB1 to promote assembly and activity of the DDB2-DDB1-Cullin4A-Roc1 ubiquitin ligase complex, thus leading to XPC recruitment and NER [87]. Moreover, impairment of autophagy leads to both transcriptional suppression and ubiquitination of XPC, a key process for DNA damage recognition [71]. Intriguingly, the DDB1-Cul4 ubiquitin complex is also known to be

directly involved in autophagy [11]. In fact, the pro-autophagy protein AMBRA1 is degraded by Cullin-4 in a time-dependent manner during autophagy. In nutrient-rich conditions, Cullin-4 association limits AMBRA1 abundance. ULK1 activation by nutrient deprivation causes a rapid release of AMBRA1 from Cullin-4 and consequent AMBRA1 protein stabilization. Several hours later, Cullin-4 reassociates with AMBRA1 and triggers its degradation, initiating autophagy termination.

How the mechanism of autophagy termination upon starvation can be applied also to other types of stress remains unknown. Recent evidence shows that Cullin-1 is responsible for termination of autophagy after DNA damage [88]. Cullin-1, *via* binding its receptor FBXL20, mediates the proteasomal degradation of VPS34, a key component of Beclin 1 complex in autophagy. Degradation of VPS34 occurs during the mitotic arrest induced by DNA damage agents by CDK1-mediated phosphorylation and after transcriptional induction of FBXL20 and p53.

5. Conclusion and perspectives

Autophagy is a central player in the regulation of DNA repair pathways and it may have evolved as a quality control system that responds to many stressful conditions, including DNA damage.

In recent years, there have been impressive advances in our understanding of the principles and mechanisms by which autophagy cross talks with the DNA damage machinery and how integration with PTMs, in particular ubiquitination, allows for optimal context-dependent DSB repair. Impairments in autophagy have been linked to increased susceptibility of the cells to genotoxic agents, and this could be important in anticancer therapy. However, it should be taken into account that this process plays a context-dependent role in cancer development.

Interestingly, defects in DNA damage repair impair autophagy. Contrarily, an impairment of autophagy causes the production of protein and free radicals increasing mutation rate, which might promote human diseases such as cancer and neurodegeneration. However, the question about the exact role of autophagy in DNA repair pathways and its implication for cancer therapy is still waiting for a complete answer.

Acknowledgements

FC lab research is supported in part by grants from AIRC (IG2016-18906), FISM (2013), the Danish Cancer Society (R146-A9364). We are also grateful to the Lundbeck Foundation (R167-2013-16100), the Novo Nordisk Foundation (7559, 22544), and the European Union (Horizon 2020 MEL-PLEX, grant agreement 642295). Further, FC lab in Copenhagen is part of the Center of Excellence in Autophagy, Recycling and Disease (CARD), funded by the Danish National Research Foundation.

Author details

Francesca Nazio[1], Emiliano Maiani[2] and Francesco Cecconi[1,2,3]*

*Address all correspondence to: cecconi@cancer.dk

1 Department of Pediatric Hematology and Oncology, IRCSS Bambino Gesù Children's Hospital, Rome, Italy

2 Danish Cancer Society Research Center, Copenhagen, Denmark

3 Department of Biology, University of Rome Tor Vergata, Rome, Italy

References

[1] Varchavsky A. The ubiquitin system. Trends in Biochemical Sciences. 1997;**22**(10):383-387

[2] Kroemer G, Mariño G, Levine B. Autophagy and the integrated stress response. Molecular Cell. 2010;**40**(2):280-293. DOI: 10.1016/j.molcel.2010.09.023

[3] Clague MJ, Urbe S. Ubiquitin: Same molecule, different degradation pathways. Cell. 2010;**143**:682-685. DOI: 10.1016/j.cell.2010.11.012

[4] Jensen ON. Modification-specific proteomics: Characterization of post-translational modifications by mass spectrometry. Current Opinion in Chemical Biology. 2004;**8**(1): 33-41. DOI: 10.1016/j.cbpa.2003.12.009

[5] Mann M, Jensen ON. Proteomic analysis of post-translational modifications. Nature Biotechnology. 2003;**21**(3):255-261. DOI: 10.1038/nbt0303-255

[6] Deribe YL, Pawson T, Dikic I. Post-translational modifications in signal integration. Nature Structural & Molecular Biology. 2010;**17**(6):666-672. DOI: 10.1038/nsmb.1842

[7] McEwan DG, Dikic I. The three musketeers of autophagy: Phosphorylation, ubiquitination and acetylation. Trends in Cell Biology. 2011;**21**:195-201. DOI: 10.1016/j.tcb.2010.12.006

[8] Kuang E, Qi J, Ronai Z. Emerging roles of E3 ubiquitin ligases in autophagy. Trends in Biochemical Sciences. 2013;**38**(9):453-460. DOI: 10.1016/j.tibs.2013.06.008

[9] Abrahamsen H, Stenmark H, Platta HW. Ubiquitination and phosphorylation of Beclin 1 and its binding partners: Tuning class III phosphatidylinositol 3-kinase activity and tumor suppression. FEBS Letters. 2012;**586**(11):1584-1591. DOI: 10.1016/j.febslet.2012.04.046

[10] Nazio F, et al. mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. Nature Cell Biology. 2013;**15**(4): 406-416. DOI: 10.1038/ncb2708

[11] Antonioli M, et al. AMBRA1 interplay with cullin E3 ubiquitin ligases regulates autophagy dynamics. Developmental Cell. 2014;**31**(6):734-746. DOI: 10.1016/j.devcel.2014.11.013

[12] Liu CC, et al. Cul3-KLHL20 ubiquitin ligase governs the turnover of ULK1 and VPS34 complexes to control autophagy termination. Molecular Cell. 2016;**61**(1):84-97. DOI: 10.1016/j.molcel.2015.11.001

[13] Nazio F, et al. Fine-tuning of ULK1 mRNA and protein levels is required for autophagy oscillation. The Journal of Cell Biology. 2016;**215**(6):841-856. DOI: 10.1083/jcb.201605089

[14] Liu J, et al. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. Cell. 2011;**147**(1):223-234. DOI: 10.1016/j.cell.2011.08.037

[15] Kim BW, Kwon do H, Song HK. Structure biology of selective autophagy receptors. BMB Reports. 2016;**49**(2):73-80. DOI: 10.5483/BMBRep.2016.49.2.265

[16] Chen X, Yin XM. Coordination of autophagy and the proteasome in resolving endoplasmic reticulum stress. Veterinary Pathology. 2011;**48**:245-253. DOI: 10.1177/0300985810385154

[17] Zheng Q, Wang X. Autophagy and the ubiquitin-proteasome system in cardiac dysfunction. Panminerva Medica. 2010;**52**:9-25

[18] Hewitt G, Korolchuk VI. Repair, reuse, recycle: The expanding role of autophagy in genome maintenance. Trends in Cell Biology. 2016;S0962-8924(16)30209-4. DOI: 10.1016/j.tcb.2016.11.011

[19] Eliopoulos AG, Havaki S, Gorgoulis VG. DNA damage response and autophagy: A meaningful partnership. Frontiers in Genetics. 2016;**7**:204. eCollection 2016. DOI: 10.3389/fgene.2016.00204

[20] Hewitt G et al. SQSTM1/p62 mediates crosstalk between autophagy and the UPS in DNA repair. Autophagy. 2016;**12**(10):1917-1930. DOI: 10.1080/15548627.2016.1210368

[21] Wang Y et al. Autophagy regulates chromatin ubiquitination in DNA damage response through elimination of SQSTM1/p62. Molecular Cell. 2016;**63**:34-48. DOI: 10.1016/j.molcel.2016.05.027

[22] Pankiv S et al. Nucleocytoplasmic shuttling of p62/SQSTM1 and its role in recruitment of nuclear polyubiquitinated proteins to promyelocytic leukemia bodies. The Journal of Biological Chemistry. 2010;**285**(8):5941-5953. DOI: 10.1074/jbc.M109.039925

[23] Klionsky DJ, Emr SD. Autophagy as regulated pathway of cellular degradation. Science. 2000;**290**:1717-1721. DOI: 10.1126/science.290.5497.1717

[24] Kawabata T, Yoshimori T. Beyond starvation: An update on the autophagic machinery and its functions. Journal of Molecular and Cellular Cardiology. 2016;**95**:2-10. DOI: 10.1016/j.yjmcc.2015.12.005

[25] Yang Z, Klionsky DJ. Mammalian autophagy: Core molecular machinery and signaling regulation. Current Opinion in Cell Biology. 2010;**22**:124-131. DOI: 10.1016/j.ceb.2009.11.014

[26] Richter-Landsberg C, Leyk J. Inclusion body formation, macroautophagy, and the role of HDAC6 in neurodegeneration. Acta Neuropathologica. 2013;**126**:793-807. DOI: 10.1007/s00401-013-1158-x

[27] Lin L, Baehrecke EH. Autophagy, cell death, and cancer. Molecular and Cellular Biology. 2015;**2**(3):e985913. DOI: 10.4161/23723556.2014.985913

[28] Mathew R, et al. Autophagy suppresses tumor progression by limiting chromosomal instability. Genes & Development. 2007;**21**(11):1367-1381. DOI: 10.1101/gad.1545107

[29] Coux O, Tanaka K, Goldberg AL. Structure and functions of the 20S and 26S proteasomes. Annual Review of Biochemistry. 1996;**65**:801-847. DOI: 10.1146/annurev.bi.65.070196.004101

[30] Ravid T, Hochstrasser M. Diversity of degradation signals in the ubiquitin-proteasome system. Nature Reviews. Molecular Cell Biology. 2008;**9**(9):679-690. DOI: 10.1038/nrm2468

[31] Buetow L, Huang DT. Structural insights into the catalysis and regulation of E3 ubiquitin ligases. Nature Reviews Molecular Cell Biology. 2016;**17**:626-642. DOI: 10.1038/nrm.2016.91

[32] Cohen-Kaplan V, et al. The ubiquitin-proteasome system and autophagy: Coordinated and independent activities. The International Journal of Biochemistry & Cell Biology. 2016;**79**:403-418. DOI: 10.1016/j.biocel.2016.07.019

[33] Nakaso K, Yoshimoto Y, Nakano T, et al. Transcriptional activation of p62/A170/ZIP during the formation of the aggregates: Possible mechanisms and the role in Lewy body formation in Parkinson's disease. Brain Research. 2004;**1012**:42-51. DOI: 10.1016/j.brainres.2004.03.029

[34] Korolchuk VI, Mansilla A, Menzies FM, Rubinsztein DC. Autophagy inhibition compromises degradation of ubiquitin-proteasome pathway substrates. Molecular Cell. 2009;**33**:517-527. DOI: 10.1016/j.molcel.2009.01.021

[35] Gao Z, et al. Processing of autophagic protein LC3 by the 20S proteasome. Autophagy. 2010;**6**:126-137. DOI: 10.4161/auto.6.1.10928

[36] Robert T, Vanoli F, Chiolo I, Shubassi G, Bernstein KA, Rothstein R, et al. HDACs link the DNA damage response, processing of double-strand breaks and autophagy. Nature. 2011;**471**:74-79. DOI: 10.1038/nature09803

[37] Tomar D, et al. TRIM13 regulates ER stress induced autophagy and clonogenic ability of the cells. Biochimica et Biophysica Acta. 2012;**1823**:316-326. DOI: 10.1016/j.bbamcr.2011.11.015

[38] Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;**461**(7267):1071-1078. DOI: 10.1038/nature08467

[39] Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. Science. 2008;**319**(5868):352-355. DOI: 10.1126/science.1140735

[40] Ciccia A, Elledge SJ. The DNA damage response: Making it safe to play with knives. Molecular Cell. 2010;**40**(2):179-204. DOI: 10.1016/j.molcel.2010.09.019

[41] Marechal A, Zou L. DNA damage sensing by the ATM and ATR kinases. Cold Spring Harbor Perspectives in Biology. 2013;**5**(9). DOI: 10.1101/cshperspect.a012716

[42] Ribezzo F, Shiloh Y, Schumacher B. Systemic DNA damage responses in aging and diseases. Seminars in Cancer Biology. 2016;**37-38**:26-23. DOI: 10.1016/j.semcancer.2015.12.005

[43] Filomeni G, De Zio D, Cecconi F. Oxidative stress and autophagy: The clash between damage and metabolic needs. Cell Death and Differentiation. 2015;**22**(3):377-388. DOI: 10.1038/cdd.2014.150

[44] Shiloh Y. The ATM-mediated DNA-damage response: Taking shape. Trends in Biochemical Sciences. 2006;**31**:402-410. DOI: 10.1016/j.tibs.2006.05.004

[45] Cortez D, et al. ATR and ATRIP: Partners in checkpoint signaling. Science. 2001;**294**: 1713-1716. DOI: 10.1126/science.1065521

[46] Spry M, Scott T, Pierce H, D'Orazio JA. DNA repair pathways and hereditary cancer susceptibility syndromes. Frontiers in Bioscience. 2007;**12**:4191-4207

[47] Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**(25): 15077-15082. DOI: 10.1073/pnas.2436255100

[48] Liang C, Feng P, Ku B, Dotan I, Canaani D, BH O, Jung JU. Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. Nature Cell Biology. 2006;**8**(7):688-699. DOI: 10.1038/ncb1426

[49] Karantza-Wadsworth V et al. Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. Genes & Development. 2007;**21**(13):1621-1635. DOI: 10.1101/gad.1565707

[50] White E. The role of autophagy in cancer. The Journal of Clinical Investigation. 2015; **125**(1):42-46. DOI: 10.1172/JCI73941

[51] Takamura A et al. Autophagy-deficient mice develop multiple liver tumors. Genes & Development. 2011;**25**(8):795-800. DOI: 10.1101/gad.2016211

[52] Bae H, Guan JL. Suppression of autophagy by FIP200 deletion impairs DNA damage repair and increases cell death upon treatments with anticancer agents. Molecular Cancer Research. 2011;**9**:1232-1241. DOI: 10.1158/1541-7786.MCR-11-0098

[53] Scheibye-Knudsen M et al. Cockayne syndrome group B protein prevents the accumulation of damaged mitochondria by promoting mitochondrial autophagy. The Journal of Experimental Medicine. 2012;**209**:855-869. DOI: 10.1084/jem.20111721

[54] Abedin MJ, Wang D, McDonnell MA, Lehmann U, Kelekar A. Autophagy delays apoptotic death in breast cancer cells following DNA damage. Cell Death and Differentiation. 2007;**14**:500-510. DOI: 10.1038/sj.cdd.4402039

[55] Bordin DL, Lima M, Lenz G, Saffi J, Meira LB, Mesange P, et al. DNA alkylation damage and autophagy induction. Mutation Research. 2013;**753**:91-99. DOI: 10.1016/j.mrrev.2013.07.001

[56] Rieber M, Rieber MS. Sensitization to radiation-induced DNA damage accelerates loss of Bcl-2 and increases apoptosis and autophagy. Cancer Biology & Therapy. 2008; **7**:1561-1566

[57] Katayama M, Kawaguchi T, Berger MS, Pieper RO. DNA damaging agent-induced autophagy produces a cytoprotective adenosine triphosphate surge in malignant glioma cells. Cell Death and Differentiation. 2007;**14**(3):548-558. DOI: 10.1038/sj.cdd.4402030

[58] Alexander A, Kim J, Walker CL. ATM engages the TSC2/mTORC1 signaling node to regulate autophagy. Autophagy. 2010;**6**:672-673. DOI: 10.4161/auto.6.5.12509

[59] Alexander A, et al. ATM signals to TSC2 in the cytoplasm to regulate mTORC1 in response to ROS. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:4153-4158. DOI: 10.1073/pnas.0913860107

[60] Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell. 2003;**115**:577-590

[61] Zhao M, Klionsky DJ. AMPK-dependent phosphorylation of ULK1 induces autophagy. Cell Metabolism. 2011;**13**:119-120. DOI: 10.1016/j.cmet.2011.01.009

[62] Desantis A. Che-1-induced inhibition of mTOR pathway enables stress-induced autophagy. The EMBO Journal. 2015;**34**(9):1214-1230. DOI: 10.15252/embj.201489920

[63] Mammucari C, et al. FoxO3 controls autophagy in skeletal muscle in vivo. Cell Metabolism. 2007;**6**:458-471. DOI: 10.1016/j.cmet.2007.11.001

[64] Salminen A, Kaarniranta K. Regulation of the aging process by autophagy. Trends in Molecular Medicine. 2009;**15**:217-224. DOI: 10.1016/j.molmed.2009.03.004

[65] Chiacchiera F, Simone C. The AMPK-FoxO3A axis as a target for cancer treatment. Cell Cycle. 2010;**15, 9**(6). DOI: 10.4161/cc.9.6.11035

[66] Munoz-Gamez JA et al. PARP-1 is involved in autophagy induced by DNA damage. Autophagy. 2009;**5**(1):61-74

[67] Feng Z, Zhang H, Levine AJ, Jin S. The coordinate regulation of the p53 and mTOR pathways in cells. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:8204-8209. DOI: 10.1073/pnas.0502857102

[68] Valbuena A, Castro-Obregón S, Lazo PA. Down-regulation of VRK1 by p53 in response to DNA damage is mediated by the autophagic pathway. PLoS One. 2011;**6**:e17320. DOI: 10.1371/journal.pone.0017320

[69] Yi J, Luo J. SIRT1 and p53, effect on cancer, senescence and beyond. Biochimica et Biophysica Acta. 2010;**1804**:1684-1689. DOI: 10.1016/j.bbapap.2010.05.002

[70] Fang EF, et al. Defective mitophagy in XPA via PARP-1 hyperactivation and NAD(+)/SIRT1 reduction. Cell. 2014;**157**:882-896. DOI: 10.1016/j.cell.2014.03.026

[71] Qiang L. Autophagy positively regulates DNA damage recognition by nucleotide excision repair. Autophagy. 2016;**12**(2):357-368. DOI: 10.1080/15548627.2015.1110667

[72] Zeng X, Kinsella TJ. A novel role for DNA mismatch repair and the autophagic processing of chemotherapy drugs in human tumor cells. Autophagy. 2007;**3**:368-370. DOI: 10.4161/auto.4205

[73] Zhao Z, et al. A dual role for UVRAG in maintaining chromosomal stability independent of autophagy. Developmental Cell. 2012;**22**(5):1001-1016. DOI: 10.1016/j.devcel.2011.12.027

[74] Park JM. Beclin 1 and UVRAG confer protection from radiation-induced DNA damage and maintain centrosome stability in colorectal cancer cells. PLoS One. 2014;**9**(6):e100819. DOI: 10.1371/journal.pone.0100819

[75] Bitto A. P62/SQSTM1 at the interface of aging, autophagy, and disease. Age. 2014;**36**(3):9626. DOI: 10.1007/s11357-014-9626-3

[76] Nagaoka U. Increased expression of p62 in expanded polyglutamine-expressing cells and its association with polyglutamine inclusions. Journal of Neurochemistry. 2004;**91**(1):57-68. DOI: 10.1111/j.1471-4159.2004.02692.x

[77] Rockel TD, Stuhlmann D, von Mikecz A. Proteasomes degrade proteins in focal subdomains of the human cell nucleus. Journal of Cell Science. 2005;**118**(Pt 22):5231-5242. DOI: 10.1242/jcs.02642

[78] Doil C, et al. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. Cell. 2009;**136**:435-446. DOI: 10.1016/j.cell.2008.12.041

[79] Mattiroli F, et al. RNF168 ubiquitinates K13-15 on H2A/ H2AX to drive DNA damage signaling. Cell. 2012;**150**:1182-1195. DOI: 10.1016/j.cell.2012.08.005

[80] Stewart GS, et al. RIDDLE immunodeficiency syndrome is linked to defects in 53BP1-mediated DNA damage signaling. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**:16910-16915. DOI: 10.1073/pnas.0708408104

[81] Fradet-Turcotte A, et al. 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. Nature. 2013;**499**:50-54. DOI: 10.1038/nature12318

[82] Chen S, et al. RAD6 promotes homologous recombination repair by activating the autophagy- mediated degradation of heterochromatin protein HP1. Molecular and Cellular Biology. 2015;**35**:406-416. DOI: 10.1128/MCB.01044-14

[83] Haddad DM, et al. Mutations in the intellectual disability gene Ube2a cause neuronal dysfunction and impair parkin-dependent mitophagy. Molecular Cell. 2013;**50**(6):831-843. DOI: 10.1016/j.molcel.2013.04.012

[84] Liu EY, et al. Loss of autophagy causes a synthetic lethal deficiency in DNA repair. Proceedings of the National Academy of Sciences of the United States of America. 2015;**112**(3):773-778. DOI: 10.1073/pnas.1409563112

[85] Cuervo M. Chaperone-mediated autophagy: Selectivity pays off. Trends in Endocrinology and Metabolism. 2010;**21**:142-150. DOI: 10.1016/j.tem.2009.10.003

[86] Park C, Suh Y, Cuervo AM. Regulated degradation of Chk1 by chaperone-mediated autophagy in response to DNA damage. Nature Communications. 2015;**6**:6823. DOI: 10.1038/ncomms7823

[87] Yang Y, et al. Autophagic UVRAG promotes UV-induced photolesion repair by activation of the CRL4(DDB2) E3 ligase. Molecular Cell. 2016;**62**:507-519. DOI: 10.1016/j.molcel.2016.04.014

[88] Xiao J, et al. FBXL20-mediated Vps34 ubiquitination as a p53 controlled checkpoint in regulating autophagy and receptor degradation. Genes & Development. 2015;**29**:184-196. DOI: 10.1101/gad.252528.114