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Review

Autophagy and genomic integrity

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DNA lesions, constantly produced by endogenous and exogenous sources, activate the DNA damage response (DDR), which involves detection, signaling and repair of the damage. Autophagy, a lysosome-dependent degradation pathway that is activated by stressful situations such as starvation and oxidative stress, regulates cell fate after DNA damage and also has a pivotal role in the maintenance of nuclear and mitochondrial genomic integrity. Here, we review important evidence regarding the role played by autophagy in preventing genomic instability and tumorigenesis, as well as in micronuclei degradation. Several pathways governing autophagy activation after DNA injury and the influence of autophagy upon the processing of genomic lesions are also discussed herein. In this line, the mechanisms by which several proteins participate in both DDR and autophagy, and the importance of this crosstalk in cancer and neurodegeneration will be presented in an integrated fashion. At last, we present a hypothetical model of the role played by autophagy in dictating cell fate after genotoxic stress.
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Facts

- Autophagy can be activated by the DNA damage response (DDR) and influences the processing of genomic lesions; in some cases, autophagy may contribute to cell death after genotoxic stress.
- By degrading dysfunctional mitochondria and toxic protein aggregates, autophagy contributes to genomic stability, thereby acting as a tumor suppressor mechanism.
- Genomic stabilizing properties of autophagy can also be achieved through removal of micronuclei and damaged nuclear parts.

Open Questions

- How does autophagy influences the processing of DNA lesions?
- Do different types of DNA lesions activate autophagy through specific or shared pathways?
- What determines whether autophagy will prevent or contribute to cell death after genotoxic stress?
- Can the interplay between DDR and autophagy be exploited to improve the treatment of cancer or neurodegenerative diseases?

Cells have evolved complex mechanisms to safeguard the genome, which is constantly threatened by environmental and endogenous DNA damage-inducing agents. In the event of genomic assault, the DNA damage response (DDR) takes place, leading to the detection, signaling and repair of lesions. In the case of excessive damage, cells activate apoptosis or senescence, thereby avoiding the proliferation of potentially tumorigenic cells.[1–4]

Macroautophagy (hereafter referred to as autophagy) is a lysosome-dependent degradation pathway that promotes cell homeostasis in response to stress such as nutrient deprivation, oxidative stress or DNA damage. This mechanism is centrally controlled by the autophagy-related (*atg*) family of genes,[5] which is modulated by several kinases

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Abbreviations: Ambra1, activating molecule in Beclin1-regulated autophagy; AMPK, AMP-activated protein kinase; ATG, autophagy-related; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia mutated and Rad3 related; BER, base excision repair; Bif-1, endophilin-B1; C-EBPα, CCAAT/enhancer-binding protein alpha; Bnip3, Bcl-2/adenovirus E1B 19-kDa-interacting protein 3; DDR, DNA damage response; DNAPK, DNA-dependent protein kinase; dNTP, deoxyribonucleoside triphosphate; DRAM, damage-regulated autophagy modulator; DSB, double strand break; E2F1, E2F transcription factor 1; ERK, extracellular signal-regulated protein kinase; FIP200, focal adhesion kinase family interacting protein of 200 kDa; γ-H2AX, phosphorylated histone H2AX; HDAC, histone deacetylases; HR, homologous recombination; ISG20L1, interferon stimulated gene 20- like 1; LC3, light chain 3; LKB1, liver kinase B1; 3-MA, 3-methyladenine; MAPK, mitogen-activated protein kinase; MLH, MutL-homolog; MMP, mitochondrial membrane permeabilization; MMR, mismatch repair; MSH, MutS-homolog; mtDNA, mitochondrial DNA; mTOR, mammalian target of rapamycin; NAC, N-acetyl l-cysteine; nDNA, nuclear DNA; NER, nucleotide excision repair; NHEJ, non homologous end joining; OGG1, 8-oxoguanine DNA glycosylase 1; PALA, N-(phophonoacetyl)-L-aspartate; PARP, poly (ADP-ribose) polymerase; PCNA, proliferating cell nuclear antigen; PI3K, phosphatidylinositol 3-kinase; PINK1, PTEN-induced kinase 1; PTEN, phosphatase and tensin homolog; p70S6 kinase, p70 ribosomal protein S6 kinase; ROS, reactive oxygen species; SQSTM1, sequestosome 1; SSB, single strand break; TSC2, tuberous sclerosis complex 2; ULK-1, unc-51-like kinase 1; UVRAG, UV irradiation resistance-associated gene; VPA, valproic acid
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including mTOR,[6] PI3k/Akt,[6] AMPK[7] and MAPK.[8] The protective functions of autophagy are achieved through the recycling of damaged and/or obsolete cellular components, such as dysfunctional mitochondria and toxic protein aggregates, thereby generating metabolic precursors for vital processes such as ATP production and macromolecular synthesis.[9–11] In addition to its role in cell survival, autophagy also contributes to organism homeostasis by clearing apoptotic cells during embryonic development[12] and after certain types of DNA damage.[13,14]

In this review, we examine the roles proposed for autophagy in preventing genomic instability, as well as the connection of autophagy to DDR and cell fate after DNA damage. We also discuss the roles proposed for autophagy in the development and therapy of cancer and other human diseases.

Autophagy, Mitochondria Metabolism and Tumorigenesis

One of the first evidences linking autophagy to tumorigenesis was described by Schwarze and Seglen in 1985. They observed that the degradation of long-lived proteins during starvation was reduced in hepatocytes from carcinogen-treated rats because of reduced autophagic activity, contributing to cell survival.[15] A few years later, a surprising number of reports highlighted the role of autophagy in tumorigenesis. In 1999, Aita *et al.* reported allelic deletions in the essential autophagy gene *beclin 1 (atg6)* in a high percentage of breast carcinoma cell lines.[16] In the same year, Liang *et al.* reported that expression of beclin 1 in MCF7 cells, a metastatic human breast cancer cell line with 17q21 loss of heterozygosity, the region where the *beclin 1* locus maps, increased contact inhibition, reduced proliferation rates and decreased tumor formation *in vivo*.[17] Conversely, heterozygous disruption of *beclin 1* compromised autophagy activation and resulted in increased cellular proliferation[18] and spontaneous tumor formation in mice.[19]

These early observations of the role of *beclin 1* in tumorigenesis were extended to other autophagy genes. Expression of the UV irradiation resistance-associated gene (*UVRAG)* protein, which participates in the autophagosome-formation regulatory complex Bcl-2-Beclin1-PI(3)KC3-UVRAG, increased autophagy, reduced proliferation and suppressed tumorigenicity of HCT116 colorectal carcinoma cells in mice.[20] Moreover, lack of *Bif-1*, which also participates in autophagosome formation during starvation, increased spontaneous tumor formation in mice.[21]

Despite these evidences, it was not until 2007 that Karantza-Wadsworth *et al.* and Mathew *et al.* shed light on the mechanism behind the tumor suppressive function of autophagy. They described that under conditions of metabolic stress, *beclin 1* $+/-$ cells accumulated mitochondria with structural abnormalities, endoplasmic reticulum chaperones and p62/SQSTM1, which target organelles and proteins to the autophagosome. These cells also underwent a marked increase in reactive oxygen species (ROS) generation, causing DNA damage and increased aneuploidy. Moreover, increased resistance to *N*-(phophonoacetyl)-L-aspartate (PALA) treatment in autophagy-defective cells suggested higher gene amplification rates, evidence that loss of autophagy increased genomic instability, a driving force behind tumorigenesis.[22–24] Accordingly, a significant association between loss of *beclin 1* and amplification of the *HER2/NEU* oncogene was described in breast carcinoma.[25] Quenching ROS with *N*-acetyl l-cysteine (NAC) delayed the promotion of aneuploidy and improved survival of *beclin 1* $+/-$ cells, revealing that ROS contributes to genomic instability in these cells.[23,24] Interestingly, expression of p62 increased ROS and DNA damage in autophagy-defective cells under metabolic stress, thereby revealing that p62 accumulation may potentiate generation of ROS due to dysfunctional mitochondria.[26] These evidences suggest that autophagy is an important tumor suppressor mechanism involved in different steps of carcinogenesis (Figure 1).

The mitochondria is central to the model linking autophagy, ROS and DNA (Figure 2). Normal mitochondrial activity inevitably generates ROS as by-products, which may cause damage to cell components, including the DNA. Direct ROS-mediated damage to the mitochondria may result in mitochondrial DNA (mtDNA) damage, alterations in the mitochondrial membrane permeability (MMP) and uncoupling of the respiratory chain, resulting in even more ROS generation in a vicious cycle (Figure 1, box i; Figure 2, #1).[27,28] Mitophagy of injured organelles has a central role in impeding this vicious cycle (Figure 2, #**6**), a process in which the protein parkin has a central role. Parkin translocates from the cytosol to the injured mitochondria, signaling for mitophagy,[29] a process that involves the BCL2/adenovirus E1B 19 kd-interacting protein (BNIP3) in cardiac myocytes (Figure 2, #3).[30] Interestingly, mtDNA deletions also trigger autophagy through the increase of oxidized proteins and a reduction of tRNA, leading to reduced levels of ATP and amino acids, triggering AMPK activation and autophagy (Figure 2, #4).[31–33]

Supporting the importance of autophagy for basal mitochondrial physiology and ROS control, deletion of *atg7* in the mouse hematopoietic system resulted in accumulation of mitochondria with high membrane potential, superoxide production, DNA damage and death of hematopoietic stem cells. Atypical myeloid infiltrates were detected in several organs of these animals, showing that loss of autophagy contributes to development of myeloproliferative disorders.[34,35] Further, hepatocytes from *atg5* mosaically deleted mice accumulated swollen mitochondria and oxidatively generated DNA damage, in addition to displaying an increase in glutathione-*S*-transferase in tumor areas as a result of oxidative stress.[36] Mice lacking MAP1S, which is involved in autophagosome biogenesis, treated with the hepatocarcinogenesis initiator diethylnitrosamine, displayed similar features.[37]

ROS can result in genomic instability (Figure 2, #2) through direct damage to the DNA and/or compromising spindle checkpoint maintenance. In contact with DNA, ROS generates base damage, such as 7,8-dihydro-8-oxo-guanine (8-oxo-G), which, if not repaired, increases the chance of mispairing adenine opposite the lesion.[38] ROS may also lead to breaks in the phosphodiester chain of DNA, including double-strand breaks (DSBs) that are normally detected by γ-H2AX.[39] These extremely toxic and deleterious lesions may cause chromosome alterations or even cell death.

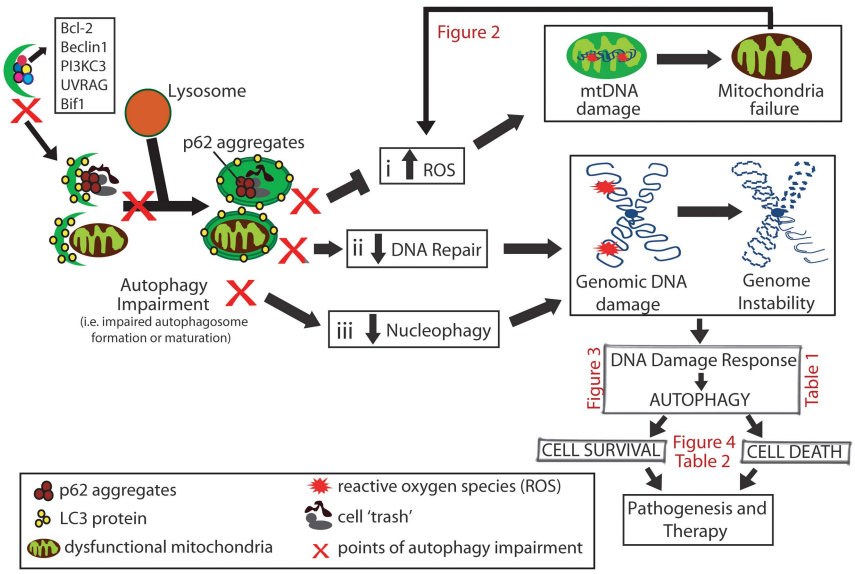

Figure 1 Overview of the genomic instability caused by autophagy impairment. Autophagy impairment leads to the accumulation of hazardous cellular components, such as dysfunctional mitochondria and toxic protein aggregates, which leads to an increase in ROS production (**box i**), cell cycle dynamic alterations, DNA damage and, consequently, genomic instability. Autophagy impairment also interferes with DNA repair (**box ii**) and removal of micronuclei (here referred to as nucleophagy (**box iii**), contributing to genomic instability. The molecular and cellular mechanisms involved in the role of mitophagy in the context of DNA damage are shown in Figure 2. Pathways that are involved in the crosstalk between DDR and autophagy are summarized in Figure 3 and Table 1, whereas the dual role of DDR-induced autophagy is shown on Figure 4 and Table 2

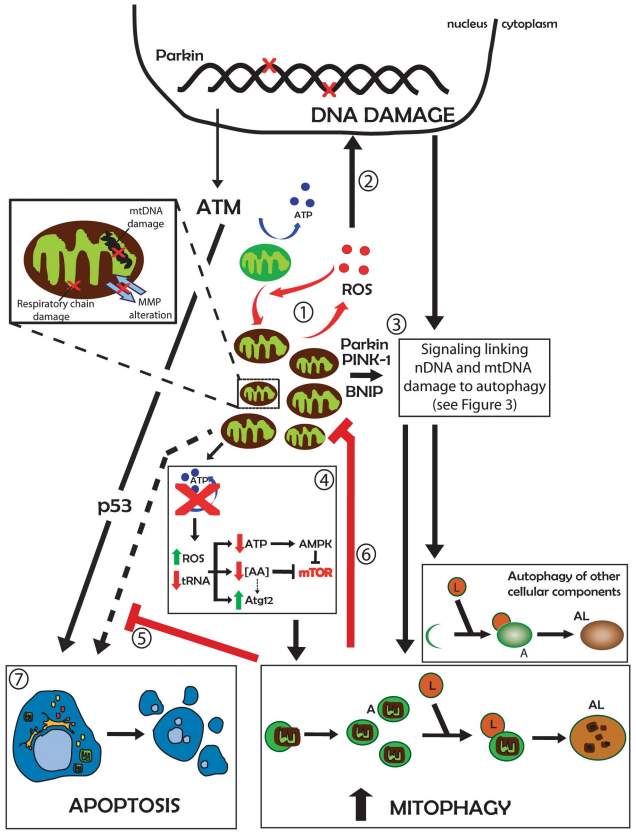

Figure 2 Mitochondria quality control by mitophagy in the context of DNA damage. Details of the processes are given in the main text. A, autophagosomes; L, lysosomes; AL, autophagolysosomes; MMP, mitochondrial membrane potential

The induction of DSBs by oxidative stress is most likely a result of the processing of other types of DNA damage, including the repair of clustered lesions, breakage during the fragile blockage of replication forks by the lesions,[40,41] or the handling of DNA–DNA and DNA–protein crosslinks induced by ROS.[42]

A second mechanism by which ROS leads to genomic instability is through the degradation of the anaphase blockers securin and cyclin B1, which impede aneuploidy by ensuring correct segregation of chromosomes during mitosis[43] and in checkpoint-arrested cells, thereby suspending the spindle checkpoint. In agreement with this, budding yeast cells activate autophagy after the induction of DSBs, accompanied by anaphase arrest. This arrest persists even when phosphorylation of the checkpoint kinase Rad53 is reduced, but is overcome when autophagy is blocked or vacuolar proteolysis is inhibited, suggesting that autophagy is fundamental for DNA damage-induced anaphase arrest, thus avoiding improper chromosome segregation.[44] Interestingly, elimination of the mid-body, which is involved in the final stages of cytokinesis, by autophagy was also shown to influence the tumorigenic potential of cancer cells.[45] Indeed, autophagy-defective cells exhibited nuclear morphometric alterations, centrosome abnormalities and increased chromosome number under normal culture conditions.[24]

Altogether, these data show that autophagy has a strong impact on genomic stability, contributing to mitochondria quality control and, as a consequence, modulating ROS levels, ATP production and cell death signaling. These mechanisms are all directly involved in the carcinogenic process and may contribute to the tumor suppressor effect attributed to autophagy.

The Role of Autophagy in DNA Repair

In addition to mitigating DNA damage by controlling ROS production, autophagy can also influence the dynamics of DNA repair by recycling key proteins involved in the processing of lesions.[46] Alternatively, autophagy may also provide metabolic precursors for the generation of ATP, which is employed in several steps of DNA repair,[47] as well as regulate the supply of dNTPs for DNA synthesis during repair.[48]

By targeting glycogen, lipids and proteins to lysosomes, autophagy guides the breakdown of these macromolecules, thereby producing metabolic precursors that can sustain oxidative phosphorylation and glycolysis.[49,50] In cancer cells from solid undernourished tumors, response to radiotherapy or DNA-damaging chemotherapy triggers ATP production by autophagy, which may have an essential role in DNA repair (Figure 1, box ii). Supporting this hypothesis, the inhibition of autophagy suppressed ATP generation and increased mitotic catastrophe in glioma cells treated with temozolomide (TMZ). Addition of pyruvate rescued ATP levels and prevented mitotic catastrophe, suggesting that autophagy-sustained ATP generation could be employed by mechanisms that promote genomic integrity, such as DNA repair processes.[47] In fact, DNA repair requires ATP at several steps, including DNA unwinding by helicases during nucleotide-excision repair (NER),[51] ATP-dependent chromatin remodeling complexes in DSB repair[52] and PARP activity, which consumes NAD + and can cause energy collapse in DNA-damaged cells.[53,54] However, direct evidence to corroborate this hypothesis is still lacking.

Autophagy was also implicated in regulating the dNTP pool levels, which are essential for DNA replication and repair. Upon methyl methane sulfonate (MMS) treatment, yeast trigger autophagy, thereby promoting degradation of ribonucleotide reductase 1 (Rnr1), which associates with other Rnr proteins to regulate the reduction of ribonucleotides to deoxyribonucleotides. This reduction in Rnr1 levels may favor assembly of the most catalytically active form of Rnr, Rnr1-Rnr3, instead of Rnr1-Rnr1 in the final RNR complex, resulting in optimization of RNR activity and dNTP levels, which in turn could be employed as substrates during DNA repair processes, such as mismatch repair (MMR).[48] It is also interesting to note that imbalanced levels of dNTPs can increase mutagenesis.[55] Thus, it is tempting to speculate that through the degradation of Rnr subunits autophagy may also fight mutagenesis by ensuring a balanced dNTP pool, which is fundamental to avoid stress replication and gene amplification, two characteristics frequently observed in autophagy-deficient cells.[23,24]

Besides dNTP recycling and ATP generation, autophagy also participates in the turnover of key proteins involved in the regulation/processing of genomic lesions. Recently, an intricate relationship between histone deacetylases (HDACS) – which are involved in DNA repair and apoptosis,[56,57] – DSB processing and autophagy was shown in budding yeast.[46,58] Treatment with valproic acid (VPA), an HDAC inhibitor, impaired the activation of Rad53 in response to DSBs. In the VPA-treated cells, Mre11, the first factor recruited to DSB sites, remained bound to the DSB site, accompanied by reduced levels of Sae2, which is responsible for removing Mre11 from the DSB region, a step required for the progress of lesion repair. In this context, inhibition of autophagy by the serine protease inhibitor PMSF or deletion of *atg1* increased acetylated Sae2 levels, whereas rapamycin, which activates autophagy through mTOR inhibition, decreased it, confirming that autophagy induced by VPA could impair DSB processing through degradation of acetylated Sae2. Moreover, Atg1 inhibition partially rescued sensitivity of an *hda1-rpd3* (HDACs) double mutant (which exhibits low levels of Sae2 as well as impaired DSB resection) to camptothecin. These results suggest that, in one hand, autophagy may be involved in destabilizing key factors, such as the acetylated form of Sae2, impairing DSB repair. On the other hand, clearance of Sae2 by autophagy could also help cells in the control of DSB repair pathway by counteracting extensive DSB resection that may be harmful to cells,[46] demonstrating the complex role of autophagy in the context of DNA damage and repair.

In the same line of thinking, FIP200 (a focal adhesion kinase that participates in autophagy induction)[59] KO MEFS showed persistent nuclear γ-H2AX staining after exposure to ionizing radiation (IR), indicating defective DNA damage repair.[60] Although the initial amount of DNA breaks were similar between *fip200* KO and WT MEFs immediately after IR, the DNA breaks persisted for a longer period in KO cells. Similar results were obtained in response to other DNA damage-inducing agents (camptothecin and etoposide) and also when autophagy was pharmacollogially inhibited using 3-methyladenine. Interestingly, silencing p62 in these cells improved DNA repair and cell viability in response to IR and camptothecin. Although accumulation of p62 was shown to increase oxidative stress,[26] the antioxidant NAC did not improve cell viability in response to camptothecin or etoposide, revealing that the mechanism underlying persistent DNA damage in *fip200* KO cells is ROS independent.

These data show that autophagy can influence the resolution of DNA injuries. Although several reports showed that inhibition of autophagy can undermine cells' resistance to chemo- and radiotherapy, only a few studies provide a more careful look into the effect of this approach over DNA repair dynamics.[47] In this sense, spatial and temporal tracking of DNA repair enzymes may provide important clues about the influence of autophagy on the resolution of genomic injuries. In this sense, yeast models can be of great value to create a library of strains in which recruitment of specific DNA repair proteins can be followed. For instance, yeast strains expressing homologous recombination enzymes tagged with fluorescent proteins allowed spatial and temporal localization of these enzymes upon DSB repair activation.[61] Thus, by using such approaches, important clues may be revealed that significantly improve our understanding of this exciting yet obscure role of autophagy in DNA repair.

Nucleophagy as a Way to Eliminate Injured DNA

Autophagic removal of whole nuclei is not as common as removal of other organelles because it may cause deleterious loss of genetic information. However, in multinucleated cells of the filamentous fungus *Aspergillus oryzae,* nucleophagy of entire nuclei contributes to cell maintenance during nutrient

deprivation.[62] Similarly, removal of nuclei from intestinal epithelial cells of *Caernohabiditis elegans* also occurs through autophagy.[63]

Autophagy of nuclear components in eukaryotes, or piecemeal microautophagy of the nucleus (PMN), was initially described in *Saccharomyces cerevisae*. It is triggered by nutrient deprivation and occurs through the release of nuclear portions into the vacuole,[64] followed by digestion by hydrolases.[65] Nuclear components targeted for PMN include granular nucleolus enriched in pre-ribosomes and nuclear envelope, nuclear pore complexes or spindle pole bodies.[66,67] PMN involves the core Atg proteins involved in macroautophagy and microautophagy, such as Atg4, 5, 7 and 12, as well as macroautophagy-specific proteins such as Atg17, 29 and 31.[64]

In mammalian cells, autophagy can degrade nuclear components, thereby contributing to the maintenance of nuclear function and integrity.[68] The initial observation of the engulfment of nuclear components points to the presence of perinuclear vacuoles in skeletal and/or cardiac muscle cells from patients or mice with envelopathies,[68] disorders caused by mutations in nuclear envelope components.[69,70] Mutated cells presented higher levels of autophagic flow and the vacuoles were positive for Atg5, Atg16L and Atg9. Cells presented giant autophagosomes and autophagolysosomes containing LC3 and DNA, with the presence of histone H1 and γ-H2AX, but not the markers of nuclear envelope, lamin A and B, confirming that the DNA contained in autophagosomes was extranuclear and damaged. In this case, the contribution of autophagy to nuclear stability was clear since its inhibition increased the incidence of nuclear abnormalities, accompanied by a reduction in cell viability.[68] Autophagy was also shown to degrade micronuclei generated by treatment with cell cycle blockers. Interestingly, 'autophagic micronuclei' co-localized with p62, besides presenting reduced chromatin content and γ-H2AX foci, a DNA damage marker. However, non-autophagic micronuclei appeared p62-negative, suggesting that the presence of DNA damage directly or indirectly signaled for autophagic engulfment (Figure 1, box iii).[71]

Thus, evolutionarily, the process of nucleophagy may represent a physiological mechanism for the removal of damaged nuclear components and micronuclei, thus contributing to genomic stability. In multinuclear eukaryote cells, it is plausible that autophagy of nuclear components can be triggered under metabolically stressful situations or DNA damage, contributing to genomic stability and cellular homeostasis (Figure 1; box iii).

Pathways Connecting DDR to Autophagy

DDR comprises an array of processes triggered by DNA lesions that allows cells to cope with these insults, aiming at safeguarding the integrity of the genome and avoiding propagation of mutated cells.[4,17] The kinases ATM and ATR have key roles sensing DNA breaks and activating downstream components of DDR, such as Chk1, Chk2 and p53.[72,73] p53 is an important protein in DDR, inducing the transcription of key genes involved in cell cycle arrest, DNA repair, apoptosis[74,75] and, more recently described, in autophagy.[76]

Two central components, p53 and mTOR, link DDR to autophagy (Figure 3 and Table 1). p53 can activate autophagy after DNA damage through transcriptional induction of several genes, including damage-regulated autophagy modulator *(dram)*, UNC-51-like kinase 1/2, *(ulk1/2), sestrin1/2*, *isg20L1* and *bnip3*, among others.[77] p53 targets can regulate autophagy directly, as is the case with the lysosomal proteins DRAM[14] and ULK1/2, which interact with Atg13 and FIP200 to induce autophagy,[13] or indirectly through Sestrin 1 and 2, which activate AMPK and the TSC1/2 complex, leading to inactivation of mTORC1 and autophagy induction.[78] Additionally, ATM was shown to activate AMPK in a p53-independent manner through direct activation of the AMP kinase LKB1.[79] Interestingly, cytoplasmic p53 is able to repress autophagy, and deletion or pharmacological inhibition of p53 induces, rather than inhibits, autophagy. Accordingly, induction of autophagy by starvation requires destruction of cytoplasmic p53,[80,81] thereby revealing a complex role for p53 in the regulation of autophagy.

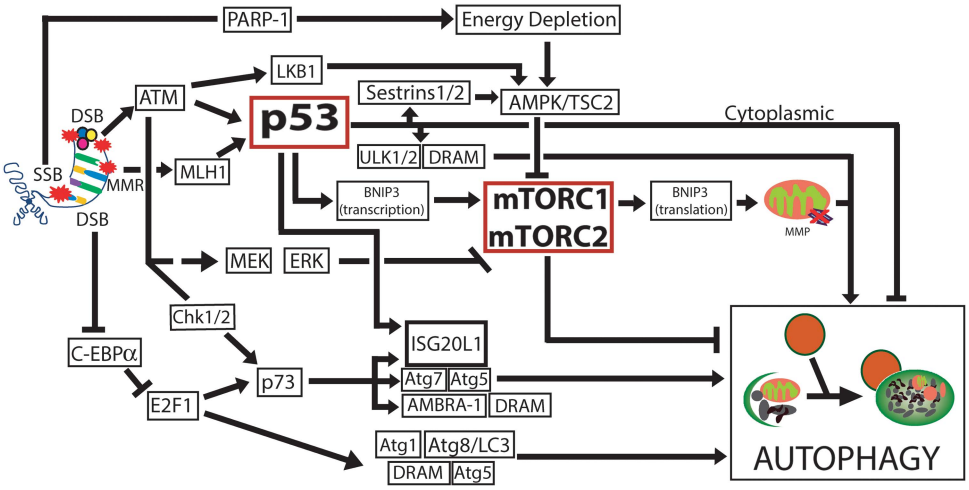

Figure 3 Autophagy modulation in response to DNA damage response (DDR). DDR-activated signaling can result in autophagy modulation. The autophagy box represents the central autophagy regulating genes. DSB, double-strand brake; SSB, single-strand break; MMR, mismatch repair

Table 1 Proteins that have functions in both DNA damage response and autophagy

Protein	Functions in DNA damage response (DDR)	Functions in autophagy and/or interplay with DDR
p53	Regulates cell cycle arrest, DNA repair and apoptosis in response to DNA damage.[74,75,77]	Induces autophagy in response to DNA damage through transcription of ULK1, ULK2, DRAM, Sestrins 1/2 and ISG20L1.[13,14,78,88] In the cytoplasm, inhibits autophagy through AMPK inhibition.[81,127]
p73	Promotes apoptosis in response to chemotherapeutic-induced DNA damage.[90] Induces transcription of glycosilases (associated to BER) in response to bile acid-induced DNA damage.[91]	Induces autophagy in a DRAM-independent manner.[89] Binds to genomic sites near to the autophagy-related genes *atg5, atg7, ambra1.*[128]
UVRAG	Was shown to partially complement sensitivity of XPC transformed cells to UVC.[129] Binds to and activates DNAPK complex, thereby promoting repair of DNA DSB through NHEJ.[124]	Participates in the multiprotein complex Bcl-2-Beclin1-PI(3) KC3-UVRAG that regulates autophagosome formation.[20]
E2F1	Promotes DNA repair and survival or apoptosis in response to DNA damage. Recruits NER factors to sites of UV-induced DNA damage to augment repair activity.[130,131]	Upregulates transcription of *atg1*, *lc3*, *atg5* and *dram*. Activates autophagy in response to Etoposide.[87]
Parkin	Was found to associate with PCNA in the nucleus and enhance NER-mediated resolution of UV-induced lesions and BER-mediated resolution of H_2O_2-induced lesion.[115,116]	Recruited to damaged mitochondria (by PINK1) to promote their degradation through mitophagy.[29]
ATM	Senses and responds to DNA double strand breaks, thus regulating cell cycle arrest, DNA repair and apoptosis.[72,73]	Induces autophagy through of activation of TSC2 and inhibition of mTORC1 in response to ROS.[79] Involved in autophagy activation in response to the N-mustard derivative BO-1051-induced DNA damage.[132]
HDAC	May influence repair of damaged DNA by regulating accessibility of DNA repair enzymes at sites of lesions. Downregulates expression of apoptotic genes.[56,57]	Impairs autophagy activation.[58] Inhibition of HDAC by valproic acid was shown to promote autophagic degradation of acetylated Sae2 and further reduction of DNA double strand break repair in yeast.[46]
PARP	Recruits BER proteins to sites of DNA containing single strand breaks through poly ADP-ribosylation.[133,134]	PARP activation consumes NAD^+, which results in ATP depletion, AMPK activation and further autophagy induction.[53,54]

The other protein placed in the core of DDR to autophagy signaling, mTOR, is an important repressor of autophagy, and inactivation of the mTOR complex 1 (mTORC1) by AMPK-TSC1/2 has an important role in autophagy induction upon starvation. Interestingly, DDR was also shown to participate in autophagy induced by starvation,[53] which may increase mitochondria-dependent ROS generation, causing DNA damage, PARP-1 activation and ATP depletion. As a consequence, AMPK is activated, thereby inhibiting mTOR and inducing autophagy.[53,54] It is worth noting that autophagy activation was shown to precede phosphorylation of ATM and p53 and activation of DNA repair proteins in response to capsaicin treatment, revealing an intricate pathway in which autophagy acts upstream, and not just as a consequence, of DDR activation.[82]

BNIP3 is a Bcl-2 homologous protein and is activated after conditions of stress such as hypoxia. MMR induced by 6-thioguanine activates autophagy through MLH1, p53 activation and transcription of *bnip3*. Additionally, the TORC1 target p70S6 kinase 1 promotes translation of BNIP3, which induces loss of mitochondrial outer membrane potential and further autophagy activation, possibly through ROS generation, thus triggering mitophagy and preventing apoptosis.[83,84] It is worth noting that in this case mTOR activation, rather than inhibition, activates autophagy.

E2F1 transcriptional activity is activated after DNA damage, most likely due to the removal of C-EBPα repression[85,86] and activates autophagy by directly inducing the transcription of *atg1*, *atg6, atg5* and *dram,*[87] as well as by inducing p73, which is a transcriptional activator of *atg5, atg7, ambra, dram* and *isg20l1.*[88,89] Several chemo therapeutic agents induce TA-p73α and TA-p73β expression,[90] which are sufficient to activate autophagy through direct transcriptional regulation of the above-mentioned genes. Moreover, p73 induces the expression of several DNA repair genes,[91] thus positioning p73 in the interface between DNA repair and autophagy. In strong contrast to p53, p73 is rarely mutated in primary tumors.[92] Thus, it is plausible that p73-induced autophagy has an important role in the resistance of p53-compromised cells, making p73 inhibition a good target for chemotherapy sensitization.

As seen in Figure 3, the signaling that links DNA damage to autophagy is complex and redundant, as is the case for signaling pathways fundamental for life. It is unlikely that all these pathways are activated in a given cell by one type of DNA damage. However, the relative contribution of these pathways to the cellular response to different types of damage is not clear and may be an important part to understand the link between DNA damage and cell fate.

The Dual role of Autophagy in the Context of DNA Damage

As previously discussed, autophagy can either contribute to or prevent cell death in response to DNA damage (Figure 4). As summarized in Table 2, the majority of studies showed that inhibition of autophagy in cells treated with DNA damaging agents leads to increased cell death, supporting a protective

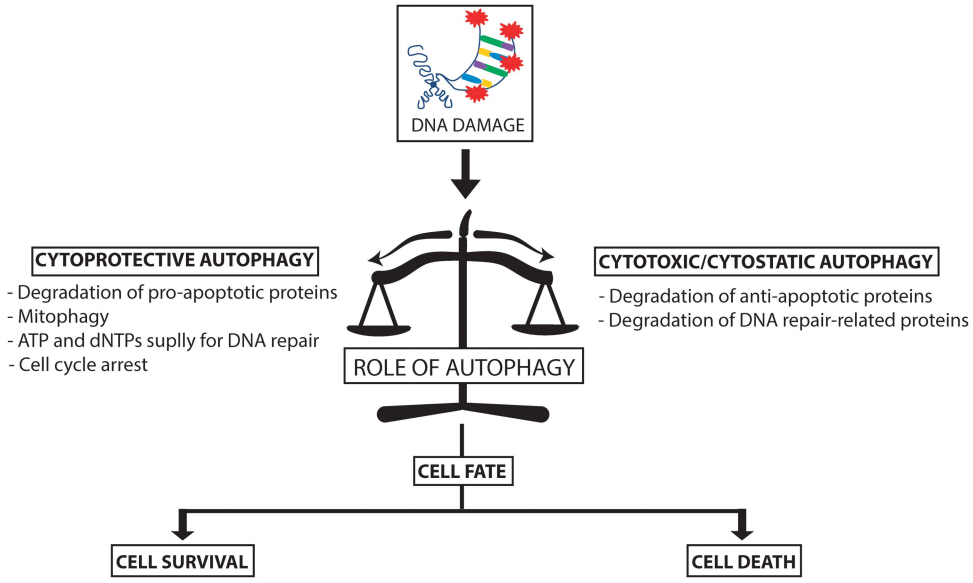

Figure 4 Roles for autophagy in regulating cell fate after DNA damage. We propose that after DNA injury, autophagy can influence cell fate, supporting or impairing cell survival. As a cytoprotective mechanism, autophagy may degrade pro-apoptotic proteins and membrane permeabilized mitochondria, enhance ATP and dNTPs generation for DNA repair and also regulate cell cycle arrest. However, autophagy may favor cell death through degradation of anti-apoptotic and DNA repair-related proteins

role for autophagy. We hypothesize that a mechanism based on the severity and/or type of genomic damage could turn on either a pro-survival or pro-death autophagic role. In this scenario, transcription factors such as p53, p73 and E2F1 would have pivotal roles, as they were not only shown to promote DNA repair, cell cycle arrest or apoptosis in response to different degrees of DNA damage, but also to control autophagy.[74,75,91,93]

Thus, we hypothesize that after low doses of DNA damage, autophagy activation by these transcription factors would result in clearance of membrane-permeabilized mitochondria,[94] generation of dNTPs and/or ATP for DNA repair activity,[47,48] degradation of pro-apoptotic proteins such as active caspase 8[95] and elimination of p62, thus preventing p38-hyperactivation.[96] Supporting the role of p53 in autophagy and cell survival, p53 mediates the transcription of parkin,[97] suggesting that p53 could regulate transcription of mitophagy genes in response to genomic damage, thus counterbalancing mitochondrial apoptotic signaling.

Conversely, autophagy can also promote degradation of anti-apoptotic proteins, thus facilitating cell death. Autophagy-mediated degradation of the inhibitor of apoptosis *dBruce* during late oogenesis in *Drosophila melanogaster*[98] and degradation of catalase in apoptosis-compromised cells resulting in increased ROS and oxidatively generated damage[99] reveal how autophagy can have an impact on cell death. As autophagy was also shown to promote degradation of acetylated Sae2 in VPA-treated yeast cells, thereby influencing, in an intricate manner, the dynamics of DNA DSB repair, it is possible that autophagy activation could contribute to perseverance of DNA damage and further enhancement of apoptotic signaling in mammalian cells by controlling turnover of certain DNA repair-related enzymes.[46] In this scenario, autophagy was shown to degrade OGG1, an enzyme that participates in 8-oxoG base-excision repair, in starved myocytes.[100]

Thus, it is tempting to speculate that, the intensity by which autophagy is activated as well as the targets to be degraded can dictate whether it is going to cooperate with or protect from cell death induced by DNA damage (Figure 4). This dual role of autophagy in cell death can be exemplified by the modulation of autophagy by the MAPK pathway. While transient or moderate activity of MEK/ERK results in mTOR inhibition, weakly beclin 1 increase and protective autophagy, sustained MEK/ERK activation results in inhibition of mTORC1 and mTORC2, stronger beclin 1 activation and toxic autophagy.[101] Thus, high levels of DNA damage could induce stronger mTORC1 inhibition, followed by stronger beclin 1 activation, thus resulting in levels of autophagy that contribute to cell death. In fact, overexpression of beclin 1, *per se*, is able to increase basal as well as induced autophagy in both normal and cancerous tissue and cells.[17,102,103]

Thus, understanding the crosstalk between DDR and autophagy may be essential to understand how autophagy has either a positive or negative role in cell death induction after activation of DDR-induced autophagy. Recent advances in the field of transcription factors and effector proteins are addressing these questions and may aid in the understanding of how cells define their fate in this context.[104,105]

Autophagy-DNA damage crosstalk in neurodegeneration, cancer and aging

All of the aforementioned findings raised a natural interest in the pharmacological modulation of autophagy, which could have a significant impact on mitigating genomic damage. This is of particular interest for human syndromes that arise from genetic deficiency in genes related to DNA repair, such as the premature aging disorder Cockayne Syndrome (CS).[106] In fact, accumulation of dysfunctional mitochondria, mtDNA mutations and oxidatively generated damage were observed in CS type B (CSB) fibroblasts,[107,108] suggesting that the

Table 2 Main factors regulating autophagy and role of autophagy in cell fate triggered by genotoxic agents

Genotoxic agent	Agent class	Main DNA repair pathway/enzymes involved[135]	Main factors regulating autophagy induction after DNA damage	Cell type	Autophagy inhibition employed	Effect of autophagyInhibition	Ref.
ActinomycinD	Topoisomerase and transcription inhibitor	ND	p53, DRAM	RKO Colon Carcinoma	DRAM siRNA; ATG5 siRNA	Increased clonogenic survival	14
Camptothecin	Topoisomerase inhibitor	HR, NHEJ	p53, ULK1/ULK2	U2OS Osteosarcoma	ATG13 shRNA	Increased clonogenic survival	13
Doxorubicin	Topoisomerase inhibitor	HR, NER, NHEJ	PARP-1	3T3 MEFs	Beclin 1 and ATG5 siRNA; 3-MA, CQ	Increased death (Trypan Blue)	54
6-Thioguanine	Nucleoside analog	MMR, BER	MLH1, MSH2, p53, mTOR, BNIP3	HCT116 colon carcinoma and HEC59 endometrial cancer	ATG5 shRNA	Increased apoptosis	136
BO-1051	Alkylating agent	ND	ATM	HA227/VGH Mahlavu Hepatomas	Bafilomycin A1, beclin shRNA	Reduced Viability (MTT)	132
Temozolomide	Alkylating agent	MMR, BER, MGMT	ATM, TSC2, mTORC1	LN-229 and U87 gliomas	3-MA	Increased apoptosis	137
MNNG, N-methyl-N'-nitro-N-nitrosoguanidine;	Alkylating agent	MMR, BER, MGMT	ND	Lymphomas	CQ, ATG5 shRNA	Reduced cell number	138
Cisplatin	Alkylating-like agent	MMR, NER, HR, NHEJ	ATM	HA227/VGH Mahlavu Hepatomas	Beclin 1 shRNA	Reducedviability (MTT)	132
5-methoxyflavanone	Methoxylated flavone	ND	MEK	HCT116 Colon Carcinoma	3-MA; bafilomycin A1	Reduced clonogenic survival	139
UVB	Electromagnetic radiation	NER	AMPK, ULK1	MEFs	ATG5 KO 3MA	Increased apoptosis	96

regulation of autophagy may have an impact on CS pathogenesis. Indeed, the induction of autophagy reduced mitochondrial loading and mitochondrial membrane potential in CSB cells, revealing that pharmacological modulation of this pathway is a promising approach.[109] Moreover, autophagy also has a role in stem cells maintenance,[110] suggesting that this pathway may also fight accelerated aging by maintaining the health of the stem cell population, avoiding loss of regenerative potential.[111]

Accumulation of oxidatively generated damage has been implicated in several neurodegenerative diseases such as Parkinson's and Alzheimer's diseases.[112,113] Data point to autophagy as an important factor in the context of both the pathogenesis and, consequently, therapy of these pathologies. It has become clear that parkin is necessary for p62 localization to damaged mitochondria and its consequent elimination through beclin-dependent autophagy.[29,114] Deletion of *parkin* increases ROS generation due to accumulation of dysfunctional organelles, resulting in mtDNA and nDNA damage, which is the basis for parkin deficiency-associated Parkinson's disease.[115,116] In this sense, induction of autophagy has given promising results in mouse models of Alzheimer's disease[117] and other neurodegenerative diseases.[118] Further, the increased genomic instability observed in *parkin*-deleted cells could also be explained by the observation that parkin translocates from the cytosol to the nucleus where it participates in DDR after DNA damage.[115,116] Therefore, the parkin protein is an important factor in both DDR and autophagic removal of injured mitochondria.

In cancer, heterozygous disruption of *beclin 1* compromised autophagy activation and resulted in increased cellular proliferation and increased spontaneous as well as induced tumor formation. Contrary to the normal genetic behavior of classical tumor suppressors, the remaining wild-type allele was neither mutated nor silenced in the formed tumors.[18,19] Accordingly, 40–75% of cases of human sporadic breast, ovarian and prostate cancer had monoallelic deletion of *beclin 1*.[16] Further, genes involved in autophagy are monoallelically inactivated in human cancers or occur in genes whose deletion only partially reduces autophagy. Moreover, frameshift mutations were identified in *UVRAG*, *atg2B*, *atg5* and *atg9B* in colorectal and in gastric carcinomas with microsatellite instability (MSI), but not in DNA from normal tissues of the same patients,[119–121] although the effects of these mutations on autophagic flux were not determined.

This genetic evidence points to the scenario in which reduced levels of autophagy favor tumor development, whereas the complete absence of autophagy is antitumoral.[122] However, it is important to keep in mind that several members of the classical autophagic pathway have autophagy-independent roles. For instance, ATG4C KO mice did not present altered basal or starvation-induced autophagy in several tissues, but an increased methylcholanthren-induced fibrosarcoma formation.[123] Similarly, MSI-positive colon cancer cells with monoallelic deletions of *UVRAG* or UVRAG-KD HEK cells did not show reduced autophagy. Indeed, UVRAG participates in an autophagy-independent manner in preventing centrosome overduplication and chromosome missegregation during anaphase[124] as well as

in endocytic trafficking of EGFR, whose accumulation may enhance growth factor receptor signaling, thus supporting tumor growth.[125] Therefore, an autophagy-centric interpretation must always bear in mind these other functions described for the 'autophagy' genes.[126]

Concluding Remarks

The crosstalk between autophagy and DDR, as well as its role in defining cell fate, is a hot topic that is just beginning to be explored, as can be evidenced by the new and fast-growing body of work related to this theme. Understanding this complex and intricate relationship will have profound impacts on several fields of medical interest, such as cancer, aging and neurodegeneration. Additionally, the majority of studies mentioned in this review focus on cell biology and the roles played by autophagy in response to DNA damage in DDR and survival of the cell. Much more difficult and therefore less clear is the impact of the link between DNA damage and autophagy on the physiology of the whole organism, mainly on aging and cancer, in which elimination of cellular components by high levels of mitophagy or nucleophagy may, in fact, be very beneficial. Notwithstanding, the current evidence linking DNA damage to autophagy indicates that both are involved in the normal physiology as well as in pathological processes and that modulation of the pathways linking DDR to autophagy has to be considered in therapeutic interventions for several diseases.

Conflict of Interest

The authors declare no conflict of interest.

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