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DNA damage and autophagy

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Abstract

Both exogenous and endogenous agents are a threat to DNA integrity. Exogenous environmental agents such as the ultraviolet (UV), ionizing radiation, genotoxic chemicals and endogenous byproducts of metabolism including reactive oxygen species can cause alterations in DNA structure (DNA damage). Unrepaired DNA damage has been linked to a variety of human disorders including cancer and neurodegenerative disease. Thus, efficient mechanisms to detect DNA lesions, signal their presence and promote their repair have been evolved in cells. If DNA is effectively repaired, DNA damage response is inactivated and normal cell functioning resumes. In contrast, when DNA lesions cannot be removed, chronic DNA damage triggers specific cell responses such as cell death and senescence. Recently, DNA damage has been shown to induce autophagy, a cellular catabolic process that maintains a balance between synthesis, degradation, and recycling of cellular components. But the exact mechanisms by which DNA damage triggers autophagy are unclear. More importantly, the role of autophagy in the DNA damage response and cellular fate is unknown. In this review we analyze evidence that supports a role for autophagy as an integral part of the DNA damage response.

Keywords

Autophagic cell death; genotoxic stress; p53; ATM; cancer

1. DNA damage

The survival of organisms depends on the protection and transmission of genetic information from one cell to its descendants. Such transmission requires not only extreme accuracy in replication of DNA, but also the ability to survive spontaneous and induced DNA damage while minimizing the number of heritable mutations [1]. The DNA structure does not guarantee stability or proper function. There are a number of complex and diverse factors that can damage the DNA from two main sources. First, environmental agents such

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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as the ultraviolet (UV) component of sunlight, ionizing radiation and numerous genotoxic chemicals cause alterations in DNA structure. Second, byproducts of regular cell metabolism are a constant menace to DNA integrity. Among them are included reactive oxygen species (such as superoxide anion, hydroxyl radical and hydrogen peroxide) coming from oxidative respiration and products of lipid peroxidation or through redox-cycling events involving environmental toxins and heavy metals. These DNA lesions if left unrepaired, may lead to the development of human diseases such as cancer and neurodegenerative disorders [2]. In response to DNA damage, cells trigger a complex array of processes that include: (a) repair or removal of DNA damage and restoration of the DNA integrity; (b) activation of DNA damage checkpoints to arrest cell cycle progression and allow the repair and/or prevention of the transmission of DNA lesions; (c) activation of a transcriptional response leading to alterations in the transcription profile; and (d) activation of cell death pathways in order to eliminate damaged or deregulated cells.

1.1. Types of DNA damage and repair systems

Cells are constantly exposed to DNA-damaging conditions which can block genome replication and transcription leading to mutations or wider-scale genome aberrations threatening normal cellular homeostasis. DNA lesions, such DNA mismatches and DNA strand breaks, arise via physiological processes, due to their sporadic insertion during DNA replication and abortive topoisomerase I and topoisomerase II activity. Hydrolytic reactions and non-enzymatic methylations also generate thousands of DNA-base lesions. DNA damage can also be generated by reactive species that attack DNA leading to adducts that impair base-pairing and/or block DNA replication and transcription, base loss, or DNA single-strand breaks (SSBs). When two SSBs arise in close proximity, or the DNA-replication apparatus encounters a SSB or certain other lesions, double-strand breaks (DSBs) are formed [3, 4]. DNA DSBs (potently induced by ionizing radiation and radiomimetic drugs), are the highest lethal DNA lesions, and failure of repair mechanisms can cause genomic instability, leading to cellular transformation [2, 5, 6]. If not repaired, chromosomal SSBs and DSBs pose a serious threat to DNA stability and cell survival. DNA damage caused by reactive oxygen species (ROS) generates a large variety of lesions spanning from base and sugar damage to DNA breaks and DNA-protein cross-links [7, 8]. So far, over a 100 oxidative modifications have been identified in DNA [9].

The effect of DNA damage is usually adverse, affecting the metabolism, triggering cell-cycle arrest or cell death. Eukaryotic organisms have evolved to develop effective molecular mechanisms (DNA damage response) to detect DNA lesions, signal their presence and promote their repair. These sophisticated and intricate DNA repair systems cover most of the DNA damage insults. DNA repair systems include (1) the direct protein-mediated reversal pathway, (2) the mismatch repair (MMR) pathway, (3) the nucleotide excision repair (NER) pathway, (4) the base excision repair (BER) pathway, (5) the homologous recombination (HR) pathway, and (6) the non-homologous end joining (NHEJ) pathway [10–12]. Briefly, MMR system is critical for maintaining the overall integrity of the genome and defects in this pathway are associated with an increased risk of cancer. MMR proteins also function in the activation of cell cycle checkpoints and the signaling of an apoptotic cell death response in tumors cells after exposure to chemotherapeutic drugs. Thus, MMR deficiency renders cells resistant to several classes of chemotherapy agents [13]. NER is a repair process capable of removing a large variety of DNA lesions from the genome [10, 14, 15]. NER deals with a wide class of helix-distorting lesions that interfere with base pairing and generally obstruct transcription and normal replication. BER is the primary DNA damage repair mechanism for repairing small base lesions resulting from oxidation and alkylation damage [16, 17]. The majority of NER lesions involve exogenous sources, whereas BER is mostly, but not exclusively concerned with damage of endogenous origin.

Both systems repair damages affecting just one DNA strand (SSBs) [18]. Most problematic are DSBs, as both DNA strands are affected. The two repair systems involved in DSB repair are the HR and the NHEJ pathways [19, 20]. The main difference between them is their dependence on DNA homology and accuracy of repair. In general, HR guarantees accurate repair by using the undamaged sister chromatid or homologous chromosome as a template. On the other hand, NHEJ uses no or extremely limited sequence homology to rejoin ends and is less accurate [21].

DNA damage is directly linked to human disease progression. Most carcinogens operate by generating DNA damage and causing mutations, and inherited DNA damage response defects commonly predispose to cancer. High mitochondrial respiration and its associated reactive oxygen species production in neurons have been associated with the accumulation of mitochondrial and nuclear DNA damage. Thus, accumulation of DNA lesions in neurons is associated with neurodegenerative disorders such as ataxias, Alzheimer's, Huntington's and Parkinson's diseases. [2, 22–25].

1.2. DNA damage response signaling

The response to genotoxic stress is a complex process, and starts with the detection of the DNA damage and activation of transcription factors involved in DNA repair, cell cycle arrest, and cell death. The total number of proteins involved in sensing aberrant DNA structures and initiation of the repair response is unknown. DNA damage checkpoints require the recognition of DNA damage to initiate subsequent events (Figure 1). Sensors for repair and for the checkpoint response are distinct and two groups of proteins have been identified as checkpoint-specific damage sensors including the phosphoinositide 3-kinase-like kinase family members, ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3 related), and the RFC/PCNA (clamp loader/polymerase clamp)-related Rad17-RFC/9-1-1 complex [26].

The mammalian Rad1, Rad9, Hus1 (part of the 9-1-1 complex), and Rad17 play essential roles in the activation of the DNA damage response pathway and have the potential to interact with nucleic acids. hRad1, hRad9, and hHus1 are structurally related to proliferating cell nuclear antigen (PCNA) and form a DNA damage-responsive ring-like complex [27–29]. It has been postulated that this group of proteins might function as sensors of DNA damage. Rad17 shares homology with all five subunits of replication factor C (RFC) [30–33]. The analogy between Rad17 and the RFC subunits raises the possibility that a Rad17-containing complex might be involved in recognizing certain DNA structures during the damage response. The Rad17 complex can recruit the Rad1–Rad9–Hus1/9-1-1 complex to the DNA damage site in a similar way as PCNA by RFC. Indeed, Rad1, Rad9, and Hus1 are less extractable from the nucleus after damage, implying that the Rad1–Rad9–Hus1 complex might associate with damaged DNA [34].

ATM responds primarily to double-strand breaks induced by ionizing irradiation (IR) [35, 36], while ATR also reacts to UV or stalled replication forks [37–44]. DSBs trigger activation of the ATM protein kinase, through the Mre11-Rad50-Nbs1 (MRN) complex formation, which is essential for DSBs repair and genomic stability. Cells from patients with Nijmegen breakage syndrome (NBS) or ataxia telangiectasia-like disorder (ATLD) express mutant forms of the Nbs1 or Mre11 proteins, respectively, and exhibit decreased levels of ATM substrate phosphorylation, particularly on CHK2 (1–4). MRN stimulates ATM activity *in vitro* leading to the activation of p53 and CHK2, in a kinase assay with purified recombinant components. MRN and ATM are associated through multiple protein-protein interactions, and MRN contributes to ATM kinase activity by increasing the affinity of ATM for its substrates (5).

ATM and ATR can also interact with many proteins that co-localize at the site of DNA damage. For example, ATM is part of a protein complex called BRCA1-associated genome surveillance complex (BASC), which is involved in the recognition and repair of DNA aberrant structures. It has been found that this complex contains several other proteins such as breast cancer gene 1 (BRCA1), mismatch-repair protein hRad50, and BLM helicase [45]. ATM also binds to histone deacetylase-1 (HDAC1) both *in vitro* and *in vivo* [46]. ATR has been found to bind to Rad17 [47] and BRCA1 [48], and is associated with components of the nucleosome remodeling and deacetylating (NRD) complex, such as chromodomainhelicase-DNA-binding protein 4 (CHD4) and histone deacetylase-2 (HDAC2) [49]. DNA damage also induces the activation of transcription factor FOXO3a that modulates the activity/expression of DNA repair genes [50]. ATM has also been shown to be regulated directly by FOXO3a in the DNA damage response [51].

2. DNA damage and Cell Death

Cell death is classified by morphological criteria as necrosis, apoptosis and autophagy. For instance, necrosis is morphologically characterized by a gain in cell volume (oncosis), swelling of organelles, plasma membrane rupture and subsequent loss of intracellular contents. For a long time, necrosis has been considered as an accidental and uncontrolled form of cell death, but there is evidence that the execution of this type of cell death can be regulated [52, 53].

On the other hand, apoptosis and autophagy are considered as cell death programs. The morphological changes for apoptosis are reduction of cellular volume (pyknosis), chromatin condensation, nuclear fragmentation (karyorrhexis), plasma membrane blebbing and maintenance of its integrity until the final stages of the process. Extrinsic and intrinsic pathways of apoptosis have been characterized. In the extrinsic pathway or “death receptor pathway”, apoptosis is triggered by an external molecule known as ligand that induces activation of death receptors at the cell surface. In the intrinsic pathway, also called “mitochondrial pathway”, apoptosis is induced by an intracellular cascade of events where mitochondrial permeabilization plays a crucial role [54].

Most cell death in mammalian cells is through the intrinsic pathway [55]. The intrinsic pathway of apoptosis is activated by a wide variety of cytotoxic stimuli. Release of pro-apoptotic proteins by mitochondrial membrane permeabilization (MMP) is thought to be an important event responsible for the activation of the mitochondrial pathway. MMP is regulated primarily through interactions between pro- and anti-apoptotic members of the B cell lymphoma 2 (BCL-2) protein family [56]. Cytochrome c (Cyt c) resides in the intermembrane space of the mitochondria where it functions as an electron shuttle in the respiratory chain [57]. Released Cyt c upon mitochondrial permeabilization dimerizes with the adaptor molecule apoptosis protease activating factor 1 (APAF-1). APAF-1 pre-exists in the cytosol as a monomer, and its activation depends on the presence of Cyt c and ATP/dATP to cleave caspase-9 and form a multiprotein complex called apoptosome [58]. The executioner caspases are subsequently cleaved and activated by the initiator caspase-9/apoptosome complex. Interestingly, MMP can induce cell death without caspases pathway activation. This “caspase-independent death” can occur because of an irreversible loss of mitochondrial membrane stability and the disrupted mitochondrial membrane release of caspase-independent death effectors, including apoptosis-inducing factor (AIF) [59, 60], endonuclease G (EndoG) [61], and others [60, 62].

DNA-repair deficiency sensitizes cells to apoptosis induced by genotoxins. Specific DNA lesions that trigger apoptosis include O6-methylguanine, base N-alkylations, bulky DNA adducts, DNA cross-links and DNA double-strand breaks (DSBs). ATM and ATR exert

three crucial functions which are: regulation and stimulation of DSBs repair, cell-cycle checkpoints and apoptosis signaling. DNA damage leads to the activation of ATM and/or ATR which phosphorylate a variety of signaling proteins such as checkpoint kinases 1 and 2 (CHK1, CHK2), and p53. p53 induces transcriptional activation of pro-apoptotic factors such as FAS, PUMA and BAX [63]. While pro-apoptotic p53 effects are mediated by its activity as transcription factor in the nucleus, p53 can also bind directly to the anti-apoptotic Bcl-2 proteins, Bcl-2 and Bcl-xl, activating the pro-apoptotic Bcl-2 proteins, Bax and Bak, to regulate the mitochondrial apoptotic pathway [64]. Bulky DNA adducts and DNA cross-linking agents impair both DNA replication and transcription which might lead to a decline in the levels of MKP1 (mitogen-activated protein kinase phosphatase) causing sustained activation of SAPKs and apoptosis. Caspase 2 has also been involved in apoptosis-induced by DNA-damaging conditions; however, its activation has not been linked to a specific DNA lesion yet [63, 65].

3. DNA damage and autophagy

Although apoptosis has been widely studied as a cellular response to DNA damage, recent reports suggest that autophagy also plays an important role in determining cell fate. Autophagy is a ubiquitous highly conserved pathway in eukaryotic cells that takes place as a response to a variety of conditions, such as nutrient deprivation [66, 67], growth factors withdrawal, energetic stress, [68] and oxidative stress [69, 70]. There are three different types of autophagic mechanisms: microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy [71, 72]. Microautophagy implicates the engulfment of cytoplasm by invagination of the lysosomal membrane [73, 74]. CMA involves the selective delivery of soluble cytoplasmic proteins by the chaperone hsc70 and co-chaperones to the lysosome in order to undergo degradation [75–77]. Macroautophagy (hereafter referred to as autophagy), mediates the degradation of intracellular components including organelles and long-lived proteins. It starts with formation of autophagosomes which are double-membrane vesicles enclosing cytoplasm and organelles. Ultimately, autophagosomes fuse with lysosomes where the bulk cytoplasmic content undergoes degradation, resulting in the liberation of amino acids and fatty acids that can be reused by cells [69, 78, 79] (Figure 2).

It is well known that cellular maintenance demands activation of autophagy in order to preserve a homeostatic environment [80]. Autophagy plays a role in multiple physiological mechanisms, including starvation, clearance of aggregated or misfolded proteins, cell growth, antiaging, and innate immunity response, whereas deregulation of autophagy is involved in diseases including cancer, cardiovascular diseases, muscular diseases, and neurodegenerative disorders [71, 81]. Some reports have suggested that autophagy plays a dual role acting as a survival mechanism or programmed cell death [82–85]. Autophagic cell death has been morphologically defined by transmission electron microscopy as a type of cell death that occurs in the absence of chromatin condensation accompanied by a massive increase of autophagic vesicles within the cell. In contrast to apoptosis, where cell clearance is ensured by engulfment of cellular debris by neighboring cells, autophagy is a self-eating mechanism [78, 86].

Autophagosome formation is regulated by PI3-kinases (PI3K) and takes place nearby the endoplasmic reticulum (ER) [87]. The mammalian class I PI3K and its product PI(3,4,5)P3 block autophagy by activating Akt/PKB, which activates mTOR (mammalian target of rapamycin) kinase, the principal negative regulator of autophagy. Under nutrient-rich conditions, mTORC1 (mTOR complex-1) is associated to the ULK1-Atg13-FIP200 complex required for the induction of autophagy and mTOR suppresses autophagy via phosphorylation of ULK1 and Atg13. Nutrient depletion or rapamycin treatment leads to ULK1 and Atg13 dephosphorylation and triggers FIP200 phosphorylation by ULK [88, 89].

However, the class III PI3K (PI3KC3) and its product PI3P are required for autophagy [90]. In mammalian cells there are two classes of PtdIns3K, class I and class III. Class III PI3K interacts with proteins containing FYVE or PX motifs, to recruit cytosolic proteins for autophagosome formation in response to amino acid depletion to a punctate compartment partially colocalized with autophagosomal proteins. Translocation is dependent on Vps34 and Beclin 1 function [87, 91–93]. The class III PI3K is associated with the proteins Vps34, p150, Beclin 1 and Atg14 [94–96]. Atg14 seems to be involved in recruitment of Atg16 and LC3 to the phagophore and stability of Vps34 and Beclin 1 [96]. Beclin 1 is an important player in autophagy, not only confers specificity to the Vps34 complex formation, it is also a protein targeted for autophagy regulation. When cellular homeostasis is balanced, autophagy is inhibited by the interaction of the anti-apoptotic protein Bcl-2 with Beclin 1 [97]. Once starvation is induced, Bcl-2 is phosphorylated by JNK1 disrupting Bcl-2 and Beclin 1 interaction and leading to activation of autophagy [98, 99].

Between the autophagy-related proteins, Atg8/LC3 is the most conserved. There are two forms of LC3 in mammalian cells. One is the cytoplasmic form termed LC3-I and its processed form LC3-II, which is tightly associated with the autophagosome membrane [100]. Atg8/LC3-I is cross-linked to phosphatidylethanolamine (PE) to form LC3-II. First, Atg4 cleaves Atg8/LC3-I and exposes a glycine residue at the C-terminus. Then Atg7 activates Atg8/LC3-I and transfers it to Atg3 (E2). Eventually, Atg12-Atg5 mediates Atg8/LC3-I lipidation through an amide bond [101, 102]. Autophagosome and lysosome fusion is modulated by the internal pH in the organelles. Acidification of the organelle compartments promotes fusion, while increasing pH inhibits the process [103]. Finally, cytoplasmic content undergoes degradation mediated by lysosomal hydrolases. Usually, breakdown of cytoplasmic material generates macromolecules such as amino acids and fatty acids for further reuse under nutrient stress conditions to either synthesize proteins or as an energy supply source. In addition, defective or damaged organelles and proteins and its accumulation might lead to their degradation as a protective response mechanism. In contrast, upregulation of autophagy has also been shown to lead to cell death in order to eliminate abnormal or damaged cells [78, 104, 105].

DNA damage induces autophagy [69, 70, 106], but its role in the DNA damage response is still unclear [107] (Figure 3). Recent reports using the DNA-damaging agents camptothecin [108], etoposide and tomozolomide [109], p-Anilinoaniline [110], and ionizing radiation (IR) [107] demonstrate that cells, in addition to initiate cell cycle arrest, also initiate autophagy. Autophagy delays apoptotic cell death induced by the DNA-damaging agent camptothecin and this is associated with mitophagy [108]. It is well known that autophagy is inhibited by the interaction of the anti-apoptotic proteins Bcl-2/Bcl-xl with Beclin 1 [111]. DNA damage-induced apoptosis is regulated by alterations in pro-apoptotic and anti-apoptotic Bcl-2 protein levels suggesting a crosstalk between autophagy and apoptosis signaling by regulation of Bcl-2 family members [112]. Rieber et al. showed that the radiation sensitizer bromodeoxyuridine enhanced UV-induced autophagosome accumulation by downregulation of Bcl-2 [107]. However, autophagy and apoptosis induced by DNA damage might also be independent phenomena regulated by distinct signaling mechanisms. For example, autophagy induced by the DNA-damaging agent etoposide is regulated independently from apoptosis by Bcl-xl [113].

The nuclear enzyme PARP-1 (poly [ADP-ribose] polymerase 1) converts β -nicotinamide adenine dinucleotide (NAD⁺) into polymers of poly(ADP ribose) (PAR), which participate in regulating nuclear homeostasis [114]. Many DNA damage insults have been shown to be able to activate PARP-1. Once hyperactivated by genotoxic stress, PARP-1 causes NAD⁺ and ATP depletion, eventually leading to irreversible cellular energy failure and cell death [115]. DNA damage-induced by doxorubicin has been recently shown to be paralleled by

autophagy. Interestingly, doxorubicin-induced autophagy was observed to depend on PARP-1. In this work, inhibition of autophagy potentiated doxorubicin-induced cell death suggesting that autophagy acts as a protective mechanism against DNA damage-induced apoptosis [116, 117].

As mentioned above, p53 is a central regulator of apoptosis induced by DNA damage. Interestingly, p53 is a bidirectional regulator of autophagy [118]. Impairment of autophagic flux induces p53-dependent cell death independent from caspase activation, but whether this is associated with DNA damage has not been studied [119–121]. Inhibition of p53 enhances autophagy improving the survival of p53 defective cells by maintaining high levels of ATP [122–124]. AMPK kinase has been shown to activate autophagy through its ability to inactivate mTORC1 via a pathway involving tuberous sclerosis complex 1 and 2 (TSC1/2) and by direct phosphorylation of the protein kinase that initiate autophagy, ULK1 [125, 126]. One mechanism for p53-induced autophagy is through activation of energy sensor AMP-activated protein kinase (AMPK), with subsequent activation of TSC1 and TSC2 kinases. Additionally, p53 activation also leads to upregulation of the phosphatidylinositol phosphatase PTEN (an inhibitor of the PI3K/AKT signaling pathway) and TSC2 at the transcriptional level, which may contribute to the long-term suppression of mTOR [106, 127–129]. Another mechanism of p53-induced autophagy is mediated by transcriptional activation of DRAM (damage-regulated autophagy modulator) a lysosomal protein that induces macroautophagy [130, 131]. Thus, autophagy induced by p53 may contribute to cell cycle arrest and DNA repair by selective degradation of damaged molecules and organelles mediated by autophagy in order to provide an energy source for the DNA damage repair. Alternatively, when the DNA damage is beyond repair, autophagy may accelerate cell death in response to p53 activation [130]. The ARF tumor suppressor functions by inducing a p53-dependent cell growth arrest and apoptosis program. Recently, the ARF tumor suppressor has also been shown to induce autophagy in a p53-dependent and -independent manner [132].

One of the sensors at the forefront of the DNA damage response is ATM. In response to DNA damage, such as DNA breakage, nuclear transcription factor FOXO3a detaches from the DNA to interact with ATM in the nucleus. ATM is regulated by FOXO3a to promote the repair of damaged DNA. The conserved cysteine (C) and aspartic acid (D) residues within the carboxyl-terminal domain (amino acids 616–623) of FOXO3a bind to the FAT domain of ATM, (amino acids 1960–2566), which has been postulated as a protein-binding domain, inducing ATM autophosphorylation for its activation [51]. DNA damage-induced ATM activation has been shown to depend on its autophosphorylation at Ser 1981 contained in the FAT domain [133, 134]. Both ATM and FOXO3a have been shown to regulate autophagy. Exposing cells to genotoxic and oxidative agents induces ATM activation causing repression of mTORC1 signaling through AMPK metabolic pathway [135, 136], and mTORC1 has been shown to negatively regulate autophagy in response to DNA damage [127, 137]. In addition, FOXO3a is known to control the transcription of autophagy-related genes, such as LC3 and the (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3) Bnip3 [138–140].

Several studies have highlighted the relevance of autophagy in cancer and neurodegenerative diseases which are associated with increased DNA damage. Autophagic delivery to lysosomes is the major degradative pathway for mitochondrial turnover (mitophagy). Reactive oxygen species produced by damaged mitochondria might induce mitophagy, which in turn eliminates the damaged organelles. Thus, autophagy malfunctioning might induce DNA damage accumulation by increased oxidative stress, and disturbance of homeostasis [141]. Consequently, prevention of autophagy might lead to protein and free radicals accumulation increasing the mutation rate and growth of tumor cells [142]. Defects in mismatch repair (MMR) are associated with an increased risk of

cancer. Interestingly, MMR defects also impair autophagy induced by chemotherapeutic drugs recognized by MMR [143]. 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 [144, 145]. DNA damage and tumorigenesis have shown to be related to autophagy downregulation. Impairment of autophagy leads to persistence in damage of organelles and DNA, and energy deficiency, which might restrict essential processes such as protein degradation, removal of defective organelles and repair of DNA damage [146–149]. Mitophagy also occurs in other pathologies associated with DNA damage. Mitophagy is observed in acute intermittent porphyria (a rare autosomal dominant metabolic disorder affecting the production of heme, the oxygen-binding prosthetic group of hemoglobin) which has been shown to be associated with mitochondrial DNA damage [150].

4. CONCLUSIONS

In response to DNA damage, cells trigger a complex response to regulate DNA repair and cell death processes. Cellular fate in response to DNA damage has been shown to be determined by the ability of DNA repair pathways to restore DNA integrity which if unrepaired would proceed to the activation of cell death processes. Until recently, apoptosis was shown to be the main cell death pathway activated in response to DNA damage to remove damaged cells. Recently, DNA damage has been shown to induce autophagy, but the exact mechanism by which DNA damage triggers autophagy and the role that autophagy plays in the DNA damage response and cellular fate are still unclear. Recent studies suggest that DNA damage signaling cascades such as p53 and ATM are important inducers of autophagy. Furthermore, autophagy has been shown to exert protective effects against apoptosis-induced by DNA-damaging agents. Interestingly, defects in DNA damage repair impair autophagy. Conversely, autophagy defects lead to protein and free radicals accumulation increasing mutation rate, which might promote human diseases such as cancer and neurodegenerative disorders associated with both increase DNA damage and altered autophagic rates. These results suggest that autophagy plays an integral part in the DNA damage response.

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Figure 1. DNA damage response

A. Formation of a double-stranded DNA break end (DSB) leads to the recruitment of the MRN (meiotic recombination protein-11 [MRE11]–RAD50–Nijmegen breakage syndrome protein-1 [NBS1]) complex activating ataxia-telangiectasia mutated (ATM) by phosphorylation. The activated ATM phosphorylates downstream targets, including checkpoint kinase-1 (CHK2) and p53. **B.** Formation of single-stranded DNA breaks (SSB) activates two complexes: RAD9–RAD1–HUS1 (also known as 9-1-1) and one comprising the single-stranded DNA Replication Factor C (RFC), which binds to RAD17. RAD17–replication factor C (RFC) loads the 9-1-1 complex. Loading of the 9-1-1 complex brings the ATR to the damaged site leading to the phosphorylation of the downstream checkpoint kinase-1 (CHK1) and other ATR effectors that include breast cancer-1 (BRCA1). ATM- or ATR-induced phosphorylation of downstream targets leads to cell-cycle arrest, cell death pathways activation and DNA repair.

Figure 2. Autophagy (macroautophagy) pathway

Autophagy is induced by a variety of different stimuli including DNA damage. Autophagy involves the sequestration of cytosolic proteins and organelles within double-membrane structures termed autophagosomes and further degradation via lysosomal hydrolases. Atg12 ubiquitin-like system activity takes place during elongation of the phagophore mediating LC3 lipidation (LC3-II) and its localization to the autophagosome membrane. Fusion of the autophagosome and the lysosome is driven by intraorganelle acidification. Ultimately, autophagosome and lysosome fusion leads to a) the degradation of cytosolic components with ulterior generation of amino acids and fatty acids to be recycled for cellular survival; or b) the removal of potential damaging proteins and organelles. Under certain conditions autophagy might induce cell death to destroy impaired/damaged cells.

Figure 3. Autophagy as part of the DNA damage response

p53 is involved in both apoptosis and autophagy pathways in response to DNA damage. DNA damage-induced apoptosis is regulated by alterations in pro-apoptotic and anti-apoptotic Bcl-2 protein levels suggesting a crosstalk between autophagy and apoptosis signaling by regulation of Bcl-2 family members. For example, nuclear p53 induces Bax and Bak expression leading to apoptosis, and in the mitochondria, p53 can also induce apoptosis by oligomerization with Bax and Bak. In addition, p53 can bind directly to the anti-apoptotic Bcl-2 proteins, Bcl-2 and Bcl-xl. Because autophagy is inhibited by the interaction of the anti-apoptotic proteins Bcl-2/Bcl-xl with Beclin 1 p53 might promote autophagy by prevention of Bcl-2/Bcl-xl-induced Beclin 1 inhibition. Interestingly, p53 plays a dual role in autophagy regulation. Under DNA-damaging conditions, nuclear p53 activates AMPK, PTEN and/or DRAM to inhibit mTOR and induce cell survival via autophagy. Contrary, cytoplasmic p53 functions as a repressor of autophagy as well. ATM regulates autophagy in response to genotoxic and oxidative stress through signaling of AMPK and TSC2. FOXO3a transactivates ATM and mediates transcriptional regulation of Atg genes (not depicted in the figure). Mitochondrial induced reactive oxygen species (ROS) are a source for oxidative DNA damage. Mitophagy is the major degradative pathway for mitochondrial turnover which contributes to the regulation of ROS production. Thus, autophagy might protect against oxidative DNA damage by ROS. Inhibition of autophagy potentiates apoptosis induced by DNA damage agents suggesting that autophagy acts as a protective mechanism against DNA damage-induced cell death. Failure in the DNA damage response might increase toxicity and mutations with ulterior development of cancer and/or neurodegenerative diseases.