3 Cross-Talk Between DNA Damage and Autophagy and Its Implication in Cancer Therapy

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Abstract

Chemotherapy and radiotherapy regimens are designed primarily to induce DNA damage to kill cancer cells. DNA damage response (DDR) proteins recognize and repair a variety of DNA damages. In response to DNA damage, a well-orchestrated autophagy program, comprising of than 30 autophagy-related genes (ATG), are triggered to degrade and recycle damaged proteins and cellular components for aiding DNA repair process. Recently, several interesting reports have showed the pivotal role of DDR proteins in regulating dozens of autophagy proteins and vice versa. Cross-talk between these two functionally different cellular processes may immensely contribute towards the understanding of resistance or sensitization of cancer cells in response to chemotherapy and radiotherapy. Nevertheless, the precise molecular link between DDR and autophagy still remains obscure and elusive. In the current review, we provide comprehensive insights into the underlying mechanisms involved in the molecular crosstalk between DDR and autophagy, which differentially regulate cancer cell fate in response to DNA damaging chemotherapeutics and radiotherapeutics or chemotherapy and radiotherapy.

Keywords

Autophagy · DNA repair · DNA damage response (DDR) · Chemotherapy · Radiotherapy · Cisplatin · Radiosensitization · DNA damage response · Cancer

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Abbreviations

AMPK	Adenosine monophosphate Kinase
ATG	Autophagy related gene
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasia and rad3-related Protein
BAK	Bcl-2 homologous antagonist killer
BAX	Bcl-2-associated X protein
CMA	Chaperone mediated autophagy
DDR	DNA damage response
HR	Homologous recombination
HSP	Heat shock protein
LAMP2A	Lysosome associated membrane protein 2A
LC3/MAP1LC3	Microtubule-associated protein 1 light chain 3
LKB1	Liver kinase B1, also known as serine/threonine kinase 11 (STK11)
MMR	Mismatch repair
mTOR	Mechanistic target of rapamycin kinase
NER	Nucleotide excision repair
NHEJ	Nonhomologous end joining
PARP1	Poly(ADP–ribose) polymerase 1
PCD	Programmed cell death
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
ROS	Reactive oxygen species

3.1 Introduction

Cancer is one of the leading causes of death in many developing countries, including India. In recent years, advancements in the chemotherapeutic regimes, especially the development of novel drugs or a combination of drugs, and radiotherapy provide better therapeutic outcomes and enhance disease-free survival (Jemal et al. 2011). However, the development of inherent and adaptive resistance to therapeutics is the key feature of therapeutic failure in oncology (Luqmani 2005). Resistance to chemo and radio-therapeutics have been attributed to multiple factors like evading growth suppressors, avoiding immune destruction, enabling replicative immortality, tumor promoting inflammation, activating invasion and metastasis, inducing angiogenesis, genome instability, mutation, resisting cell death and deregulating cellular energetics (Hanahan and Weinberg 2011). In the recent past, several evidences have shown that cellular autophagy is yet another mode of resistance, linking to therapeutic failure (Abedin et al. 2007).

Cellular autophagy is an evolutionarily conserved process of packaging damaged or aged organelles or misfolded proteins into autophagosome and their fusion with lysosome for degradation. Subsequently, degraded materials can be recycled for

renewal (Mizushima and Komatsu 2011). Autophagy is categorized into (1) macro-autophagy, (2) micro-autophagy, and (3) chaperone-mediated autophagy (CMA). While macro-autophagy is an autophagosome mediated process, micro-autophagy is direct engulfment of cytosolic materials by lysosomes. CMA is involved in the lysosomal delivery of unfolded proteins through multimerization of lysosomal membrane-associated protein (LAMP2A) and heat shock protein 70 (HSP70) complex. Autophagy can behave dichotomously by inducing the pro-survival or death process in a context-dependent manner (Buszczak and Kramer 2019). Controlled induction of autophagy plays a vital role in cell survival, while the hyperactivation of autophagy is linked to autophagic cell death (Nyfeler and Eng 2016). Many chemotherapeutics and radiotherapy treatment kill cancer cells by primarily inducing DNA damage and additional genomic instability. Cancer resistance to DNA damaging therapeutics might also stem from processing additional sources of genomic instability, including micronuclei (Bartsch et al. 2017), chromatin fragments (Ivanov et al. 2013), and endogenous retrotransposons (Guo et al. 2014). Although the mechanism of DNA repair in cancer resistance is well established, autophagy inhibition was also shown to abolish resistance in cancer cells in response to chemotherapeutics and radiation therapy. Therefore, a better understanding of the crosstalk between DNA damage/repair and autophagy in the context of chemotherapeutics and radiotherapy is required. This article is focused on reviewing several such findings to shed light on how key players of the DNA repair process are involved in autophagy regulation and vice versa in response to DNA damaging therapeutics.

3.1.1 Role of Autophagy in Response to Cisplatin Treatment

Cisplatin is mainly used for lung-cancer treatment. Cisplatin primarily causes DNA damages through intra-strand crosslinking. Nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR) and non-homologous end-joining (NHEJ) are involved in repairing cisplatin-induced DNA damage (Rocha et al. 2018). Interestingly, the formation of autophagosomes in response to cisplatin treatment was observed in the 1980s (Nilsson 1988). Later, autophagy was detected as early as 2–4 h after cisplatin exposure and co-treatment with an autophagy inhibitor (3-methyladenine) led to an increase in caspase activation and cell death in renal proximal tubular cells (Yang et al. 2008). In the mouse renal proximal tubular cells, cisplatin was found to induce cytoprotective autophagy in p53 (tumor suppressor protein) dependent manner as the use of p53-inhibitor (pifithrin-α) partially suppressed the autophagosome formation (Periyasamy-Thandavan et al. 2008). Induction of p53 in response to cisplatin has also been shown to activate microRNA dependent survival of mouse proximal tubular cells. In this study, pifithrin-α or specific antisense oligonucleotides for miR-32 increased cell death by reducing miR-34a induction (Bhatt et al. 2010). The DNA damage-dependent activation of p53 can have a dual effect on autophagy. It may upregulate autophagy through its transcriptional activity or downregulate through its

cytoplasmic functions (Budanov and Karin 2008; Green and Kroemer 2009). Upregulation of Beclin1 after cisplatin treatment is reported to be responsible for cisplatin-induced autophagy in human bladder cancer cells (Lin et al. 2017). Low-dose cisplatin also induced autophagy and the inhibition of autophagy using 3-methyladenine resulted in apoptosis (Yang et al. 2012). This study suggests that even a low amount of DNA damage may also induce pro-survival autophagy. ISG20L1 another regulator protein of the p53 protein family has also been identified as a regulator of autophagy after DNA damage induction by cisplatin and etoposide (Eby et al. 2010). The knockdown of ISG20L1 suppresses autophagy in response to cisplatin. In glioma and fibrosarcoma cells, inhibition of autophagic response after cisplatin treatment was found to increase the ROS production. Autophagy induction is also reported to precede adenosine monophosphate-activated protein kinase (AMPK) activation, which switches signaling AMP/ATP ratio to ATP-generating catabolic pathways and concomitant down-regulation of mammalian target of rapamycin (mTOR)-mediated phosphorylation of p70 S6 kinase (Harhaji-Trajkovic et al. 2009) (Fig. 3.1). Activated AMPK (phosphorylated at Thr-172) is known to activate TSC2 (Tuberous sclerosis complex 2) and subsequent inhibition of mTOR function (Fig. 3.1). The use of both early-stage autophagy inhibitors (wortmannin) and late-stage blockers (bafilomycin and chloroquine, CQ) augmented cell death by cisplatin, indicating a role for autophagy in suppressing cisplatin-triggered apoptotic death (Harhaji-Trajkovic et al. 2009). Recently, AMPK activation in nutrient-deficient cells has been linked to Poly(ADP-ribosyl)ation (PARylation) dependent spatial and temporal regulation leading to nuclear export followed by autophagy induction (Rodríguez-Vargas et al. 2016). Since cisplatin treatment leads to PARylation of various proteins (Prasad et al. 2017; Schaaf et al. 2016), it may be plausible that PARylated AMPK plays a role in the induction of autophagy (Fig. 3.1).

3.1.2 Role of Autophagy in Response to Topoisomerase Inhibitor Treatment

Inhibitors of topoisomerase I (topotecan, irinotecan) and topoisomerase II (VP-16 or etoposide) are extensively used for the treatment of the different types of cancers. These drugs cause stalled replication fork mediated DNA double-strand breaks. In contrast to the survival role of autophagy, the embryonic fibroblasts from BAX/BAK double knockout mice, resistant to apoptosis were found to display an autophagy-dependent non-apoptotic cell death in response to DNA-damaging agent like etoposide (Shimizu et al. 2004). Alexander et al. reported the activation of ATM/ATR in response to etoposide (Alexander et al. 2010). In this study, it has been shown a cytoplasmic function of ATM in activating a tumor suppressor, TSC2 via the LKB1/AMPK metabolic pathway to repress mTORC1 and activate autophagy (Fig. 3.1). Further, the dysregulation of mTORC1 in ATM-deficient cells was inhibited by rapamycin (Alexander et al. 2010).

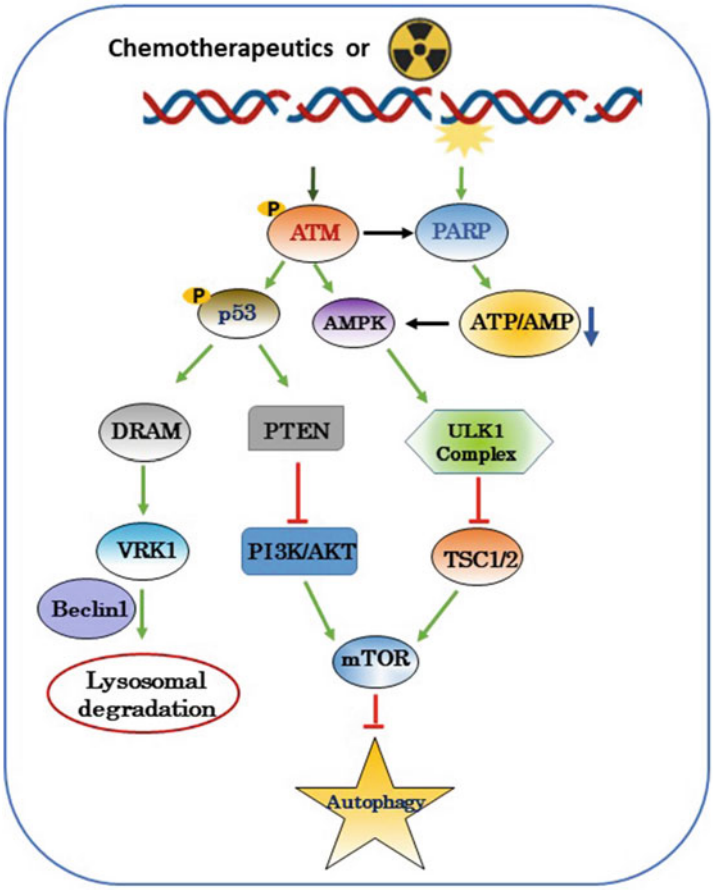

Fig. 3.1 Crosstalk between DNA damage and autophagy. Autophagy in response to DNA damaging agents (chemotherapeutics and radiation) mostly protects cancer cells from death. Key role of various DDR proteins, in the activation of autophagy, is shown in the above illustration

Another mode of autophagy was observed in ATG5 or ATG7 knockout cells. Although LC3 puncta formation, which requires lipid modification, was not observed, the autophagosome associated membranes were seen in ATG5/ATG7 deficient cells under few conditions (Nishida et al. 2009). Interestingly, etoposide induced the formation of autophagic vacuoles in ATG5 knockout mouse embryonic fibroblasts cells while the same was abrogated in ATG5-p53 double knock out cells in response to etoposide. This suggested a role of p53 in alternate autophagy. Later DRAM1, a downstream protein of p53 was found to be both necessary and sufficient to induce alternative autophagy (Nagata et al. 2018) (Fig. 3.1). DRAM1 was also found to co-localizes at the LC3-positive puncta indicating its role in conventional autophagy too. In hepatoma cell (HepG2), inhibition of AMPK also triggered apoptosis through suppression of autophagy. In contrast, augmentation of autophagy

was observed after p53 inactivation leading to cell survival (Xie et al. 2011). Recently, a Ser/Thr protein phosphatase Mg^{2+}/Mn^{2+} dependent 1D (PPM1D), which is transactivated by p53, was identified as a factor that dephosphorylates serine-637 of ULK1 (unc-51 like autophagy activating kinase) (Torii et al. 2016). ULK1 is a subunit of the ATG1-complex that functions at the most upstream position in ATG signaling and the dephosphorylation of this complex is well-known to be essential for the induction of autophagy during starvation. This study links the possibility of ULK1 dephosphorylation in response to p53 activation as a trigger for the initiation of autophagosome formation in response to genotoxic stress (Fig. 3.1). Topoisomerase I inhibitor, topotecan, the treatment also leads to autophagy induction in terms of LC3 puncta formation, LC3 I/II conversions, and p62 degradation in colon carcinoma cells (Li et al. 2012). Topotecan induces DNA damage-dependent cytoprotective autophagy in p53 positive colon cancer cells while autophagic death was observed in p53 knock out cells. This suggests a role of p53 in switching the fate of autophagy from death to cell survival. DNA damage-dependent activation of p53 upregulates expression of Sestrin 2, enhances phosphorylation of AMPKα, and inhibits mTORC1, leading to activation of autophagy (Li et al. 2012).

3.1.3 Role of Autophagy in Response to Doxorubicin

Doxorubicin is a DNA intercalating drug and used for the treatment of breast cancer, bladder cancer, Kaposi's sarcoma, lymphoma, and acute lymphocytic leukemia. Doxorubicin is known to activate genotoxin stress-induced autophagy (GTA), which involves ATM-p53-mTOR signaling axis. The role of p53, a protein known to get induced during DNA damage in autophagy was determined through high-throughput sequencing via analyzing global p53 transcriptional networks in primary mouse embryo fibroblasts (Kenzelmann Broz et al. 2013). This study demonstrated that p53 is activated in an ATM/ATR-dependent manner and can bind the promoters of various autophagy genes leading to their transcriptional upregulation (Fig. 3.1). This p53-mediated transcriptional upregulation was found to be important for GTA as p53−/− cells were unable to induce autophagy after doxorubicin-induced DNA damage. Chromatin immunoprecipitation and RNA sequencing led to the identification of p53-bound and regulated genes, involved in multiple steps of autophagy, including upstream (TSC2, FOXO3a, mTOR, LKB1, and AMPK), core machinery-encoding genes (ULK1, ATG4a, ATG7, ULK2, and UVRAG) and lysosomal protein-encoding genes (*Ctsd*, *Laptm4a*, and *Vmp4*).

3.2 Linkage of Starvation-Induced Autophagy with DNA Damage

Rodríguez-Vargas et al. demonstrated DNA damage is an early event of starvation-induced autophagy. Here accumulation of both γH2AX and comet tails were found to be due to ROS generated in response to starvation. Further, ROS-induced DNA damage activates PARP-1, leading to ATP depletion and thus activation of AMPK-autophagy network (Rodríguez-Vargas et al. 2012). PARP-1 knockout cells blunted AMPK activation, leading to a delay in autophagy (pro-survival role) in starved cells. Recently, Poly-ADP-ribosylation (PARylation) of proteins was found to regulate autophagy in both spatial and temporal manner by modulating AMPK subcellular localization and activation (Rodríguez-Vargas et al. 2016). Here, the nutrient deprivation induces PARP-1 catalyzed PARylation, leading to the dissociation of the PARP-1/AMPK complex followed by the export of free PARylated nuclear AMPK to the cytoplasm to activate autophagy. DRAM (damage-regulated autophagy modulator) is a lysosomal protein essential for p53-mediated apoptosis and also reported to mediate a specific DNA damage responsive branch of the autophagy pathway (Crighton et al. 2006) p53 can activate autophagy via activation of the protein death-associated protein kinase (DAPK). The activated form of DAPK triggers autophagy in a Beclin-1-dependent manner. DAPK phosphorylates Beclin 1 on Thr 119 located at a crucial position within its BH3 domain, and thus promotes the dissociation of Beclin 1 from BCL-XL and the induction of autophagy (Zalckvar et al. 2009). Another DNA damage response protein p73 belongs to the p53 family of transcription factors, is known to regulate DRAM and autophagy during starvation. However, further studies revealed that p73-mediated autophagy is DRAM-independent (Crighton et al. 2007). Interestingly, p73 also modulates many mTOR regulated autophagy-associated genes. Besides, endogenous p73 binds to the regulatory regions of several autophagy genes such as ATG5, ATG7, and UVRAG and is an important regulator of autophagy (Rosenbluth and Pietenpol 2009).

3.3 Role of Autophagy in Response to Radiation Treatment

Ionizing radiation can damage DNA directly and indirectly by ROS generation, resulting into single-strand breaks (SSBs), base oxidation, apurinic, or apyrimidinic (AP) sites, and particularly, double-strand breaks (DSBs). Radiotherapy is one of the major treatment modality for cancer therapy but often fails to control tumor growth due to the development of resistance and dose-limiting side effects. It is reported that apoptotic death comprises less than 20% of radiation-induced cell death. So, it is imperative to explore other pathways of cell death to gain the therapeutic index by radiation. Radiation-induced activation of autophagy is well known in both cancer and normal cells (Zois and Koukourakis 2009). In response to radiation treatment, autophagy plays a dual role in promoting resistance or sensitization, depending upon severity and duration of stress, also the type and stage of tumor.

3.3.1 Radioresistance Due to Autophagy

Ionizing radiation-induced DNA damage sites are recognized by PARP1 leading to PARylation of various DDR proteins and recruitment to DNA sites. However, PARP mediated PARylation of proteins occurs at the expense of its substrate NAD^+ leading to ATP depletion (Aguilar-Quesada et al. 2007). At DSB sites, ATM is activated and PARylated by PARP1 which further leads to activation of the energy sensor AMP-activated protein kinase (AMPK), leading to autophagy progression by inhibiting the mTORC1 complex (Fig. 3.1). Thus, the activation of autophagy provides sustained energy required for DNA repair processes that lead to radioresistance and delayed apoptotic cell death. This may be the reason for the accumulation of DNA damage and genomic instability in autophagy-deficient cells. For instance, radioresistant breast tumor cells show a strong post-irradiation induction of autophagy, which thus serves as a protective and pro-survival mechanism (Chaachouay et al. 2011). In addition to this, ATM binds to FOXO3a (transcription factor), which regulates the expression of autophagy-related genes like LC3 and BNIP3 and upregulates autophagy (Nazio et al. 2017). Normal tissues, which are late responding, are benefited more from prolonged fraction delivery time (FDTs) than acute-responding tissues because of ATM-AMPK mediated autophagy process (Yao et al. 2015). In response to IR, ATM is also known to activate autophagy through three pathways: the MAPK14 pathway, mTOR pathway, and Beclin1/PI3KIII complexes and modulate radiosensitivity (Liang et al. 2019).

Similarly, autophagy also induces cell survival in esophageal squamous cell carcinoma and bladder cancer, which was abrogated by autophagy inhibitor (CQ) in response to radiation treatment (Chen et al. 2015; Wang et al. 2018). A recent study demonstrated the protective mechanism of radiation-induced autophagy in hematopoietic cells by activation of STAT3 signaling, which upregulated the expression of BRCA1 via ATG–KAP1–STAT3–BRCA1 pathways and increases DNA repair ability (Xu et al. 2017b). In thyroid cancer, radiotherapy induces autophagy by increasing expression of autophagy-associated proteins Beclin-1 and LC3, which is blocked by either 3-methyladenine or Beclin-1 siRNA, leading to upregulation p53 and then apoptosis (Gao et al. 2019). This shows that p53 acts as a switch between protective autophagy and apoptosis in thyroid cancer in response to radiotherapy.

Further, it is known that due to poor vascularization, a certain population of tumor cells (known as hypoxic cells) is deprived of oxygen, nutrient supply, and waste removal caused to stimulate autophagy and inhibit apoptosis (He et al. 2012). A previous study has demonstrated that the induction of BNIP3, a downstream target of HIF-1α, in hypoxic cells disrupts the Beclin1-BCL2 complex and releases Beclin1. This in turn induces autophagy as an adaptive survival mechanism during prolonged hypoxia in different cell lines like MEF, MCF, PC3, and LS174 (Bellot et al. 2009). In a similar context, radioresistance was observed in osteosarcoma cancer cells overexpressing HIF-1α which induces protective autophagy (Feng et al. 2016). Hypoxia leads to an increase in ROS production due to its effect on ETC of mitochondria. This ROS production by hypoxia causes DNA damage which can also

stimulate autophagy by mitochondrial production and providing energy for cell survival (Zhang et al. 2008). Nevertheless, hypoxia-induced autophagy leads to a marked accumulation of autophagosomes along with RNA induction of autophagy-related genes such as Beclin-1, ATG5, and ATG12, leading to radioresistance (He et al. 2012). Thus, tumor cells create a more protective intracellular environment by glycolytic reprogramming, and the presence of mitochondrial defects, accompanied by the adaptation to hypoxic conditions, provide radioresistant properties, as well as survival and growth benefits. Apart from this, radiation also causes an increased formation of the acidic vesicle, which will induce autophagy to protect the damage, although the detailed mechanism of autophagy induced radioresistance is yet to be characterized for different tumor type and stage.

There are cases, where the induced autophagy exhibits neither cytoprotective nor cytotoxic functions, which we have termed as dormant autophagy. A study has shown that ATG7 and LC3 silencing lead to the sensitization of tumor cells but that is independent of autophagy (Schaaf et al. 2015). They showed that both chloroquine and knockdown of the essential autophagy genes, ATG7 and LC3b, effectively inhibit autophagy; however, only knockdown of LC3b or ATG7 but not CQ reduced survival. This indicates a radioprotective role of these autophagy-associated genes. However, the radioresistant effect is independent of autophagic degradation through lysosomes, and thus unrelated to canonical autophagy.

Ultraviolet (UVB) radiation is efficiently absorbed by DNA within the epidermis and damages DNA directly to form photoproducts. UV-induced DNA photoproducts induce the stabilization of p53. The anti-apoptotic Beclin1-binding protein BCL-2 is downregulated following UVB exposure, which may free Beclin1 to bind UVRAG (UV-irradiation-resistance-associated gene) and induce autophagy. UVRAG plays a dual role in autophagy (autophagosome formation and maturation) and DNA repair (chromosome stability); later process is autophagy-independent. In autophagy, UVRAG is responsible for the activation of PI(3) class III (PI(3)KC3) kinase through Beclin 1 interaction (Su et al. 2013). 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 (Zhao et al. 2012). Affecting the UVRAG-centrosome interaction destabilizes centrosomes, resulting in extensive aneuploidy. UVRAG is a key factor in suppressing proliferation after UVB, independent of its function in autophagy activation. For instance, a mutation of exon 8 of UVRAG reduced autophagy and promoted in colorectal and gastric cancer types (Tam et al. 2017). In response to UV or DNA alkylating agent (methyl methanesulphonate) induced DNA damage, ATR is also known to activate autophagy through ATR/Chk1/RhoB mediated lysosomal recruitment of tuberous sclerosis complex (TSC complex) and subsequent mTORC1 inhibition (Liu et al. 2018).

3.3.2 Radio-Sensitization Due to Autophagy

Recent evidence showed that autophagy regulating ATG proteins has a tumor-suppressive role because down-regulation of certain ATG proteins can promote tumorigenesis. Previous studies have confirmed that radiation-induced autophagy leads to increased radiosensitivity in BAX/BAK double knockout cells in comparison to parent cells (Kim et al. 2006). Increased radiosensitivity is due to ER stress, which is activated by unfolded protein response (UPR). Moreover, they found that PERK is essential for radiation-induced autophagy leading to increased cell death in apoptotic deficient breast cancer cells (Kim et al. 2006). Radiation-induced autophagic cell death is also mediated through the p53/DRAM signaling pathway in breast cancer cells (Cui et al. 2016) (Fig. 3.1). Various in vivo and in vitro studies have demonstrated that irradiation and rapamycin-induced autophagy lead to promote premature senescence and restrict cell proliferation in radiation-resistant glioblastoma and parotid carcinoma cells (Tam et al. 2017). In addition to this, it has been demonstrated the role of autophagy in sensitizing glioblastoma cells (SU2) by using dual PI3K/mTOR inhibitor NVP-BEZ235 (Wang et al. 2013). In similar lines, increased radiosensitivity was also observed in cisplatin-resistant NSCLC tumor cells using NVP-BEZ235 (Kim et al. 2014). Although the detailed mechanism of induced cell death is not clear yet, one recent report showed autophagy induced by ionizing radiation promotes cell death in human colorectal cancer cells in hypoxia and nutrient-depleted condition and silencing of ATG7 or Beclin1 increases the survival under oxygen and glutamine starvation (Classen et al. 2019).

3.3.3 Unfolded Protein Response (UPR) Activates Autophagy

Radiation also causes damage to protein, leading to the activation of UPR mediated ER stress. The ER membrane-associated proteins, PKR-like eIF2α kinase (PERK) and activating transcription factor-6 (ATF6) act as autophagy inducers. The PERK contributes to hypoxia tolerance by phosphorylating elF2α and stops general protein synthesis to lessen the protein load in the ER (Liang et al. 2015). However, UPR upregulates certain transcriptions factors like NF-E2-related factor 2 (NRF2), nuclear factor κB (NF-κB), and activating transcription factor 4 (ATF4) (Tam et al. 2017). NRF2 and NF-kB contribute in cytoprotective and antiapoptotic pathways and provides radioresistance, while ATF4 allows the restoration of normal ER function through the induction of CEBP homologous protein (CHOP), DNA damage-inducible protein 34 (GADD34) and lysosome-associated membrane protein 3 (LAMP3). CHOP is the pro-apoptotic component of the UPR and mediates cell death when the cell adaptation fails to withstand the ER stress (Moretti et al. 2007).

3.4 Role of Autophagy Proteins in DNA Damage Repair

Autophagy plays an important role in DNA damage repair upon genotoxic stresses and insults (Eapen et al. 2017). Although the functional significance of autophagy in DNA damage repair and response is well known, the molecular mechanisms involved are obscure. Several reports have shown that the deficiency of autophagy results in the impairment of DNA damage response and also causes replication related complexities (Liu et al. 2015; Vanzo et al. 2020; Gillespie and Ryan 2015). Cells deficient in key autophagic proteins, for example, BECN1, ATG5, ATG7 have been shown to have impaired DNA damage response (Xu et al. 2017a). The absence of these gene products and the consequential autophagic defect has also been implicated in tumorigenesis, tumor progression, and survival (Karantza-Wadsworth et al. 2007). Recently, Liu et al. have shown that loss of autophagy causes a synthetic lethal deficiency in DNA repair. It was observed that the mouse embryonic fibroblasts deficient in ATG7 showed diminished levels of phosphorylated CHK1 upon irradiation, indicating lower levels of DNA repair by HR, leading to greater dependency on error-prone NHEJ pathway (Liu et al. 2015). SQSTM1/p62, an autophagic adapter protein, plays a pivotal role in the DNA repair process (Hewitt et al. 2016; Hewitt and Korolchuk 2017). P62 protein shuttles continuously between the nucleus and the cytoplasm (Fig. 3.2). Upon exposure to ionizing radiation, it was observed that p62 accumulates in the cell, localizes to the nucleus, and binds RNF168, a ubiquitin ligase, preventing the histone ubiquitination that signals the DNA damage, hampering overall DDR (Hewitt et al. 2016). In a similar context, upon X-ray irradiation, it was observed that the p62 protein transiently associated with the DNA damage-induced foci (DDF), accumulated in the nucleus and aids in the degradation of RAD51 and Filamin A (FLNA) (Hewitt et al. 2016; Wang et al. 2016) (Fig. 3.2). This work also showed that the HR efficiency increased with p62 depletion, thus showing an inverse correlation between p62 accumulation in the nucleus and DNA damage repair. This evidence suggests that autophagic clearance of p62 is essential for the optimal and error-free repair of DNA (Fig. 3.2).

Several autophagy-independent roles of core autophagic proteins have also been reported. Beclin1, a core component of the class III phosphatidylinositol 3-kinase (PI3K-III) that aids in the formation of the autophagosomal membrane, has been found to localize in the nucleus consequent to DNA damage and promote DNA repair directly. It was found to interact with DNA topoisomerase IIβ and get recruited at the sites of double-strand breaks due to this interaction (Xu et al. 2017a) (Fig. 3.2). In the absence of BECN1, the ability of the cells treated with ionizing radiation to repair the DNA was found to be hindered (Xu et al. 2017b).

ATG5, an important protein component of the ubiquitin-like conjugation system that leads to the formation of lipidated LC3 form—LC3-II, has been shown to be induced upon DNA damage, promoting mitotic catastrophe, independent of its role in autophagy (Maskey et al. 2013) (Fig. 3.2). In response to the treatment with DNA damaging agents like cisplatin and etoposide, it was observed that ATG5 translocated to the nucleus and induced a G2/M phase arrest (Maskey et al. 2013) (Fig. 3.2). Displacement of the chromosomal passenger protein (CPC) consequent to

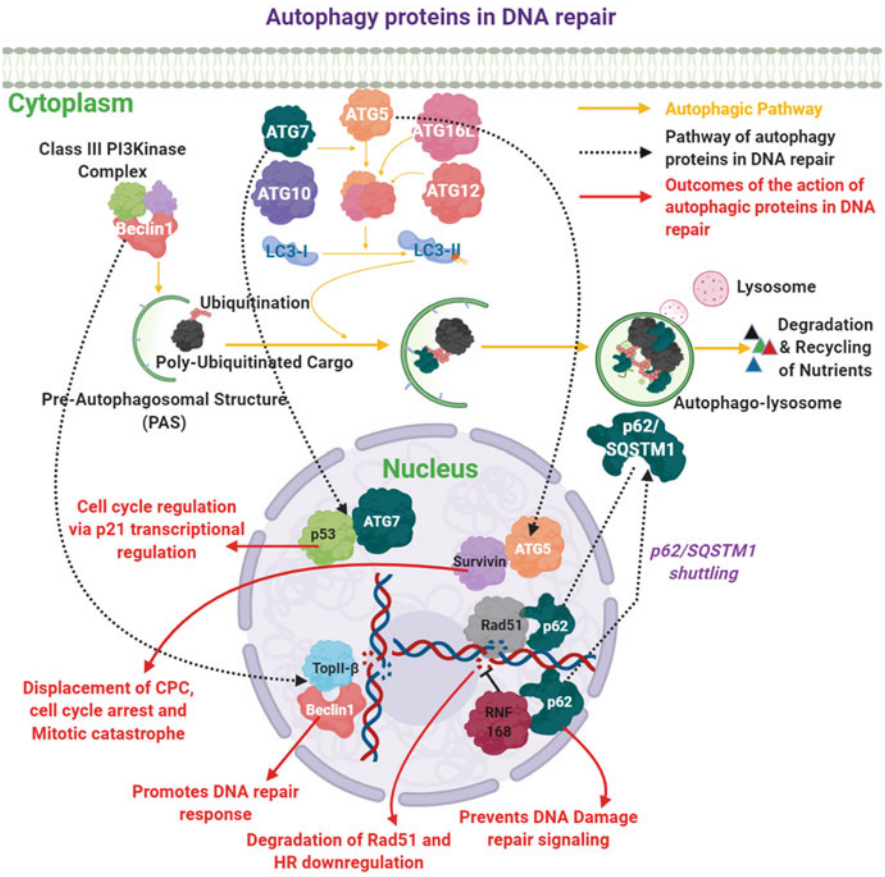

Fig. 3.2 Role of autophagy proteins in DNA repair. In response to oxidative stress, chemotherapeutic, and radiation therapy-induced DNA damage, autophagy proteins are activated and play a critical role in the DNA repair process. Above illustration was made in biorender.com

the physical interaction of ATG5 with Survivin was found to be responsible for the arrest and the ensuing mitotic catastrophe (Fig. 3.2). This activity was found to be independent of its role in autophagy and assigns two distinct functions for ATG5 based on its localization in the cell nucleus or the cytoplasm. ATG7 is another important E1 (ubiquitin-activating enzyme) like protein involved in the induction of autophagy. In one of the seminal papers, Lee et al. showed that p53 and ATG7 proteins interact with each other and aid in the arrest of cells by regulating the transcription of cell cycle inhibitor $p21^{CDKN1A}$ under starvation, in mouse embryonic fibroblasts (Lee et al. 2012) (Fig. 3.2). Withdrawal from cycling is an important response to starvation. It was also observed that the mouse embryonic fibroblasts deficient in ATG7 showed diminished levels of phosphorylated CHK1 upon irradiation, indicating lower levels of DNA repair by HR, leading to greater dependency on

error-prone NHEJ pathway (Liu et al. 2015). These works highlight the importance of autophagy and its constituent proteins in the process in the DNA damage repair and response either in the composite form of autophagy or independently as proteins (Fig. 3.2).

3.5 Conclusion and Future Perspectives

DDR and autophagy are two distinct cellular functions but they are complementing each other for protecting cells by relieving DNA damage related stress or inducing cell death under higher stress conditions. Several reports advocate that all three types of autophagy (macroautophagy, microautophagy, and CMA) are being unanticipatedly linked to DDR pathways or genes. Intriguingly, autophagy-associated proteins also seem to play an unorthodox role in DDR and DNA repair. The role of autophagy in response to chemotherapy and radiotherapy is intriguingly dichotomous; leading to cell survival or death.

However, extensive work is still required to unravel the induction of autophagy at a precise molecular level in response to different DNA damages. Considering the fact that DDR and autophagy play a crucial role in cancer resistance or sensitization in response to various DNA damaging therapy, this review article raises several concerns that ought to be addressed in the future. Whether autophagy induction is differentially regulated in cancer and normal cells in response to DNA damage? What decides the induction of pro-survival or pro-death functions of autophagy? Does different DNA damages (base damage, SSBs, DSBs, etc.) induce a common or different signaling pathways to induce autophagy? What could be the precise role of autophagy induction in DNA repair or vice versa? Whether "autophagy-dependent" and/or "autophagy-independent" role of autophagy associated proteins play a crucial role in DNA repair in response to chemotherapy or radiotherapy of cancer? The focused research in this area may further foster the development of novel cancer therapeutics.

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