Crosstalk between autophagy and intracellular radiation response (Review)

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Received August 10, 2016; Accepted September 27, 2016

DOI: 10.3892/ijo.2016.3719

Abstract. Autophagy induced by radiation is critical to cell fate decision. Evidence now sheds light on the importance of autophagy induced by cancer radiotherapy. Traditional view considers radiation can directly or indirectly damage DNA which can activate DNA damage the repair signaling pathway, a large number of proteins participating in DNA damage repair signaling pathway such as p53, ATM, PARP1, FOXO3a, mTOR and SIRT1 involved in autophagy regulation. However, emerging recent evidence suggests radiation can also cause injury to extranuclear targets such as plasma membrane, mitochondria and endoplasmic reticulum (ER) and induce accumulation of ceramide, ROS, and Ca^{2+} concentration which activate many signaling pathways to modulate autophagy. Herein we review the role of autophagy in radiation therapy and the potent intracellular autophagic triggers induced by radiation. We aim to provide a more theoretical basis of radiation-induced autophagy, and provide novel targets for developing cytotoxic drugs to increase radiosensitivity.

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Abbreviations: AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; AMBRA1, activating molecule in beclin1-regulated autophagy protein 1; ARF, alternative reading frame protein; ATF6, activating transcription factor 6; ATM, ataxia telangiectasia mutated; ATP, adenosine triphosphate; BNIP3, BCL2 interacting protein 3; CAMKK2, Ca^{2+}/calmodulin-dependent protein kinase kinase 2; CHOP, C/EBP homologous protein; DAPK, death-associated protein kinase; DDR, DNA damage response; DRAM, damage-regulated autophagy modulator; DSBs, DNA double-strand breaks; FIP200, FAK family-interacting protein of 200 kDa; FOXO3a, forkhead box O3a; GTPase, guanosine triphosphatase; HDAC, histone deacetylase; HIF-1α, hypoxia-inducible factor 1α; HMGB1, high-mobility group protein 1; IGF-BP3, insulin-like growth factor-1 binding proteins 3; IRE1, inositol requirement 1; JNK, c-Jun N-terminal kinase; MAPK mitogen activated protein kinase; MDM2, murine double minute 2; mTOR, mechanistic target of rapamycin; LC3, microtubule-associated protein 1 light chain 3; NIX, BCL2/adenovirus E1B 19 kDa interacting protein 3-like; PAS, phagophore assembly site; PERK, protein kinase R-like endoplasmic reticulum kinase; PI3K, phosphatidylinositol 3 kinase; PKC, protein kinase C; PRKAA, protein kinase, AMP activated; PARP-1, poly(ADP-ribose) polymerase-1; PINK1, PTEN induced putative kinase 1; PKC, protein kinase C; PTEN, phosphatase and tensin homolog; PtdIns3K, the class 3 phosphatidylinositol 3-kinase; PI3K, class 1 phosphoinositide 3-kinase; PtdIns3P, phosphatidylinositol 3-phosphate; PUMA, p53-upregulated-modulator-of-apoptosis; RHEB, Ras homolog enriched in brain gene; RNS, reactive nitrogen species; ROS, reactive oxygen species; TSC2, tuberous sclerosis 2; SIRT1, sirtuin1; ULK1, unc-51 like autophagy activating kinase 1; UVRAG, UV radiation resistance-associated gene; VMP1, vacuole membrane protein 1

Key words: radiation, autophagy, DNA damage, mitochondrial damage, ER stress

Contents

1. Introduction

Radiotherapy is an effective strategy for the treatment of many kinds of cancer to kill or control the malignant tumor cells. However, its benefits have been limited due to radiation resistance. It is imperative to identify new targets and develop cytotoxic drugs to increase radiosensitivity. Radiosensitization has traditionally been performed with agents known to induce apoptosis. However, recent studies demonstrate autophagy induced by radiation also plays an important role in cancer

cell fate decision, particularly in solid tumors (1). Autophagy is an evolutionarily conserved catabolic process that delivers cellular constituents, including damaged or superfluous organelles and long-lived proteins to lysosomes for degradation and recycling, thereby regulating cellular homeostasis. The key steps of autophagy include initiation, phagophore nucleation and elongation, fusion and degradation. Autophagy initiation begins with the membrane distention from either the ER or Golgi complex, followed by formation of autophagosome, a cupshaped double-membrane structure which sequesters cellular components. Subsequently, autophagosome fuses with the lysosome to form an autolysosome which is the site for degradation of the sequestered cargo by the action of the lysosomal hydrolytic enzymes (2).

More than 40 autophagy-related proteins have been identified by large-scale genetic screening in yeast and various mammalian species (3). The core pathway of mammalian autophagy comprises at least four molecular components, including: i) ULK complex (comprising ULK1, ATG13, ATG101 and FIP200), and plays a key role in the induction of autophagy. Autophagy inducers cause dephosphorylation of ULK1; the ULK1 complex then dissociates from the mTORC1 complex, which leads to increasing activity of the BECN1 complex through phosphorylation of AMBRA1 and BECN1 (Beclin 1, autophagy related); ii) core PtdIns3K complex (comprising PIK3C3, PIK3R4/Vps15 and BECN1); BECN1 is a component of core PtdIns3K complex, which participates in phagophore nucleation and elongation. Phosphorylation BECN1 recruits ATG14, AMBRA1 or UVRAG to the PtdIns3K complexes to promote autophagy (4); iii) ATG9 and VMP1, two transmembrane proteins, are critical in leading to autophagosome formation. The biogenesis of the phagophore is thought to initiate at the phagophore assembly site (PAS). Most of the ATG proteins transiently reside at the PAS, although the ultrastructure of this site and the interactions among the ATG proteins during phagophore formation are not known (5). ULK1 complex assembles at the PAS where autophagy is initiated and recruits ATG9-vesicles in order to initiate the formation of autophagosomes (6). VMP1 induced by autophagy stimulus interacts with the BECN1 BH3 domain partitioning BECN1 to the autophagic pathway. This interaction promotes the recruitment of the autophagy-specific PtdIns3K complex to the PAS and activates the PtdIns3K complex, which generates the PtdIns3p. PtdIns3p is necessary to recruit the rest of the ATG machinery, leading to autophagosome formation (7); iv) ATG12 and ATG 8/LC3, are two ubiquitin-like protein conjugation systems essential for autophagosome formation. Ubiquitin-like molecule ATG12 is activated and transferred to the E2-like conjugating enzyme ATG10 by E1-like enzyme ATG7, ultimately attached to ATG5 4, 6, 7. In addition, ATG7 and the E2-like enzyme ATG3 promote the LC3 (ATG8 in yeast) is conjugated to the lipid phosphatidylethanolamine (8). Elongation of the phagophore membrane is dependent on the ATG12 and LC3 conjugation systems (9). Most of these core autophagy pathway components are directly controlled by cellular stress signals (10,11).

Autophagy induced by radiation play bi-directional effects in cell fate decision whether cells survive or die depends on severity and duration of this phenomenon (12). When the stress is mild, autophagy can degrade and recycle damaged or unwanted cellular constituents in autophagolysosomal vesicles to provide additional energy supply during stress, which has an essential effect in quality control of organelles and cellular adaptation to stress (13). However, several reports demonstrate that various drugs in combination with radiation promote autophagic cell death rather than apoptosis significantly contributing to the antitumor effects of radiosensitizing treatment or radiotherapy in glioma (14). Hence, autophagy induced by radiation also functions as a pro-death mechanism. Besides dual activity of autophagy on tumor cell fate *in vitro*, recently *in vivo* studies demonstrate that irradiation-induced autophagy exerts a crucial activity on tumor clearance by the immune system. Autophagy induced by radiation contributes to the release of cell death-associated danger signals ATP and HMGB1 that trigger antitumor host immune responses. Furthermore, autophagy inhibition reduces radioresponses *in vivo* due to deficient immunogenic signaling (15,16).

The initiation signal of autophagy induced by radiation have not been completely elucidated, although biochemical analysis performed during the last few years has identified several proteins in DNA damage repair signaling pathway or oxidative stress signaling pathway participated in modulation of autophagy. Clarifying crosstalk between autophagy and intracellular radiation response is beneficial to provide novel targets for exploiting novel radiosensitizers to enhance the effects of anticancer therapies for cancer patient.

2. The role of autophagy in radiotherapy

Function of autophagy on tumor cell fate in vitro. A large amount of recent studies have shown that tumor resistance to radiation therapy is often associated with the upregulation of autophagy in many kinds of tumor cell lines, such as colon cancer cells, prostate cancer cells, malignant glioma cells, nasopharyngeal carcinoma cells, and breast cancer cells (17,18). The putative cytoprotective function of autophagy induced by radiation is generally considered to reflect the capacity of the cell to eliminate toxic species such as free radicals and damaged and unwanted proteins or organelles to generate energy and metabolic precursors (19). Due to the cytoprotective function of autophagy, autophagy inhibitors (such as chloroquine, bafilomycin, 3-methyl adenine, or ammonium chloride) and genetic silencing or knockdown of autophagy-associated genes (such as BECN1, ATG 5, 7 or 12) have the potential to be exploited to increase tumor cell radiosensitivity, usually via the promotion of apoptosis (15).

Moreover, some studies reported that autophagy enhanced the anticancer effects of radiotherapy on patients with oral squamous cell carcinoma and glioblastoma cells (20,21). Vitamin D and its analogs such as EB 1089 could enhance the response to radiation in breast cancer and non-small cell lung cancer through the promotion of a cytotoxic form of autophagy (22,23). Hence, autophagy is also recognized as having the potential to contribute to cell killing in response to radiation. This kind of cell death was defined as autophagic cell death or type II apoptosis (24). Autophagic cell death can serve as a part of backup cell death form when stress-induced apoptosis is blocked with broad spectrum caspase inhibitor, z-VAD-Fmk, autophagy can provide an alternative cell death mechanism (25). Moreover, autophagy is important to the

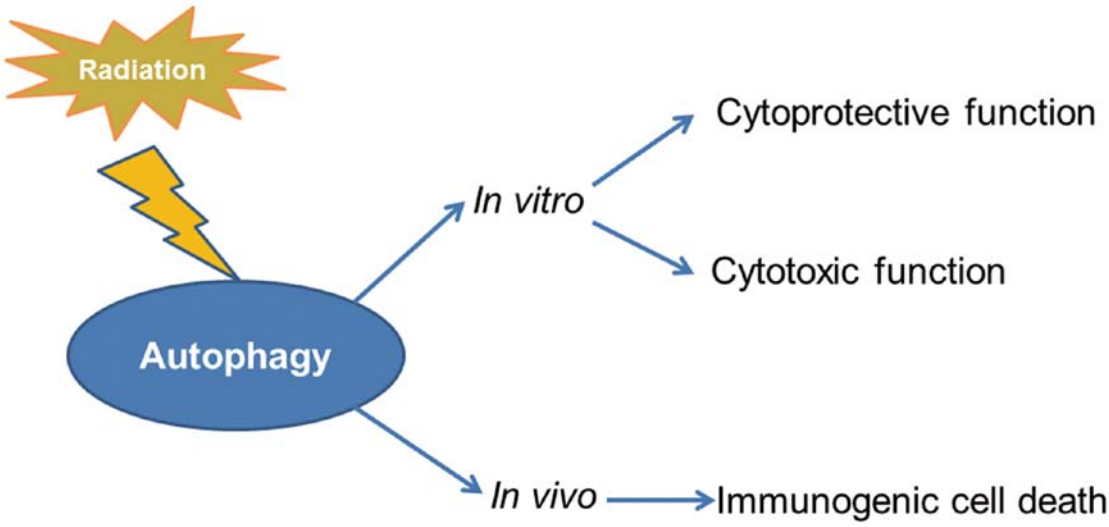

Figure 1. The role of radiation-induced autophagy *in vivo* and *in vitro*. Autophagy induced by radiation plays cytoprotective and cytotoxic function on tumor cell fate *in vitro*, yet exerts a crucial activity on immunogenic cell death *in vivo*.

radiation-induced senescence. Inhibition of autophagy results in a switch from radiation-induced senescence to apoptosis in breast cancer cells (26).

Function of autophagy on tumor cell fate in vivo. Besides dual activity of autophagy on tumor cell fate *in vitro*, recent *in vivo* studies showed autophagy play a crucial role in immunogenic cell death (ICD) induced by radiation (27). After exposure to radiation, autophagy contributes to the release of cell death-associated danger signals such as calreticulin exposure and HMGB1 and ATP release from dying cells which in turn is required for attracting immune cells including dedritic protein into the tumor that trigger antitumor host immune responses, the molecular machinery that underlies this crucial manifestation of ICD has not yet been elucidated in detail (16,28). The ATP release from dying cells depends on autophagy (29). Recent evidence shows that blocking the autophagic machinery with small interfering RNA prevents the release of the immune stimulating 'danger signal', ATP, in chemotherapy treated tumor cells undergoing ICD (30). Combining radiotherapy with specific chemotherapies or immunotherapies is able to induce a repertoire of cancer specific immunogens, which not only potentiate tumor control by enhancing cell kill, but also through the induction of a successful antitumor vaccination. However, the release of ATP may be just a part of an immune stimulating process after exposure to radiation (27). Chloroquine, an autophagy inhibitor which can reduce the release of ATP, still can improve the efficacy of breast cancer radiation therapy by blocking endosomal pathways, which promote an immunogenic form of cell death and better antigen cross-presentation to increase intrinsic radiosensitivity (31). An overview of the effect of autophagy induced by radiation *in vivo* and *in vitro* described in this section is presented in Fig. 1.

3. DNA damage repair and autophagy

ATM and autophagy. Radiation injury to DNA is caused directly or indirectly and is known to be repaired immediately (32). ATM, a primary sensor of DNA damage after exposure to radiation, plays a crucial role in initiating autophagy (33). In response to genotoxic stress, DNA damage is detected by MNR (comprising MRE11, NBS1 and Rad50 proteins) and RPA (human replication protein A) complexes which act as sensors and recruit ATM and ATR (ataxia-telangiectasia and RAD3 related) to the site of the lesion, these kinases are activated by DNA lesions and direct the DDR pathways through phosphorylation of downstream targets (34). The activation of ATM/ATR triggers phosphorylation of its downstream targets such as p53, mTOR and FOXO3a, which could trigger radiation-induced autophagy (35).

p53 and autophagy. p53, a critical component of the cellular reaction to radiation, integrates DNA damage signals and radiation-responsive autophagy (36). In unstressed cells, p53 is maintained at low levels by the action of MDM2, an oncogenic E3 ligase, which is essential for both degradation and nuclear export of p53. Following irradiation p53 tends to be rapidly stabilized by reversible post-translational modifications (37). The function of p53 in response to radiation is ATM-dependent. Activation of ATM results from the DNA DSBs, which in turn phosphorylates p53 at Ser15 and Ser20. Whereas, p53 and MDM2 are phosphorylated by ATM directly at Ser395. Both phosphorylated p53 at Ser15 and Ser20 and phosphorylated MDM2 interrupt the binding of MDM2 with p53, and protect p53 from ubiquitination and degradation. At the same time p53 levels are increased, and majority of p53 translocates to the nucleus. As an important transfection factor, p53 promotes autophagy by transactivating its target genes that involved the diversity of radiation responsive pathways in mammalian cells (38). One mechanism for p53-induced autophagy is through activation of energy sensor AMPK pathway and inhibition of the mTOR pathway. Another mechanism of p53-induced autophagy is mediated by transactivating multiple genes with proautophagic roles, including DRAM, PTEN (an inhibitor of the PI3K/AKT signaling pathway), IGF-BP3, DAPK-1 ARF, JNK, Sestrin1/2,

IGF-BP3 and PUMA (39-41). Recently, Cui *et al*, verified radiation induced autophagic cell death via the p53/DRAM signaling pathway in breast cancer cells (42).

mTOR and autophagy. mTOR, an atypical serine/threonine kinase, can modify downstream molecules and inhibits autophagy. As an evolutionarily conserved kinase, mTOR functions largely as the catalytic subunit of two distinct protein kinase complexes the mTORC1 (comprising mTOR, mLST8 and Raptor) which is a rapamycin sensitive complex (mTORC1) and the mTORC2 (comprising mTOR, mLST8 and Rictor), which is a rapamycin insensitive complex. Raptor and Rictor serve as scaffolding proteins to regulate the assembly, localization and substrate binding of mTORC1 and mTORC2, respectively (43). Previous studies have reported that altered mTORC1 signaling pathways were mostly found in many human cancers, whereas mTORC2 was less correlated (44). Once mTORC1 is activated in DNA damage repair signaling pathway, mTORC1-mediated phosphorylation prevents activation of ULK1 and its interacting partner ATG13 as well as AMBRA1, the key link between the ULK1 and BECN1 complex. In addition, the activation of mTORC1 prevents AMPK-mediated phosphorylation which is required for full activation of ULK1, which is a serine/threonine kinase that plays a key role in initiator of autophagosome formation in mammalian systems (45,46). The blockade of the mTORC1 can activate the ULK1 complex and promote the interaction with the PtdIns3K complex which is necessary for the formation of new autophagosomes (47). Several studies have reported on the positive effects of mTOR inhibitor on sensitizing cancers to radiation therapy and chemotherapy in lung cancer, pancreatic carcinoma cells and esophageal carcinoma cells (48-51).

FOXO3a and autophagy. Under normal conditions, FOXO3a is attached to DNA. The carboxyl-terminal domain (amino acids 616-623) of FOXO3a binds to the FAT domain of ATM, (amino acids 1960-2566), which has been postulated as a protein-binding domain, activation has been shown to depend on its autophosphorylation at Ser 1981 contained in the FAT domain (52). In response to DNA damage induced by radiation the transcriptional activity and protein expression level of FOXO3a increase. Furthermore, radiation can induce the nuclear translocation of FOXO3a in Saos2 cells (53). FOXO3a localization within the nuclear phospho-ATM (Ser1981) foci in irradiated cells is affected by its posttranslational modification (phospho-Thr32) (54). Nuclear transcription factor FOXO3a detaches from DNA to interact with ATM and activated ATM via phosphorylation, which can trigger autophagy as describe above. Additionally, FOXO3a regulates transcription of autophagy-related genes, including LC3 or BNIP3 (55).

PARP-1 and autophagy. PARP-1 is another protein of DDR involved in the regulation of autophagy. PARP-1 is a member of a family of 18 such proteins which are known to bind to DNA and function in DNA damage repair. Irradiation is known to induce DNA damage and activate PARP-1. The important role of PARP-1 in radiation response and the efficacy of PARP-1 inhibitors as radiosensitizers have been investigated for more than 30 years (56). Following DNA binding, The PARP-1 enzymatic activity is triggered caused by depletion of ATP and activation of AMPK. The PARP-1 activation can not only lead to the inhibition of mTOR which induces autophagy but also promotes HMGB1-mediated autophagy (57,58). Chen, *et al* reported that radiation activates PARP-1 which regulates autophagy via the activation of AMPK or inhibition mTOR in CNE-2 human nasopharyngeal carcinoma cells (59,60).

SIRT1 and autophagy. SIRT1, a member of the mammalian sirtuin family, plays an important role in autophagy. The mechanism of SIRT1 in regulating this process is due to its ability to deacetylate histones and non-histone proteins, such as p53, FOXO3a and H4K16 (lysine 16 on histone H4) in response to stresses and DNA damage (61). SIRT1 interacts with p53 and affects the transcriptional activity of p53 which can be, directly or indirectly, involved in DDR. SIRT1 regulates autophagy by both epigenetic and posttranslational mechanisms (62). SIRT1 regulates autophagy gene H4K16 (lysine 16 on histone H4) expression through histone deacetylation. H4K16 deacetylation inhibits the transcription of genes involved in the early and late steps of autophagy in multiple cell types, resulting in decreased autophagic flux. Moreover, SIRT1 indirectly regulates autophagy by deacetylation of FOXO3a, leading to increased expression of autophagy-related genes, including BNIP3, which are critical for autophagy induction.

4. Mitochondrial damage and autophagy

ATP and autophagy. Growing evidence has suggested that mitochondria may also be an important extranuclear target in mediating the cytotoxic effects of radiation (63,64). Mitochondria are essential organelles which perform multiple functions. During the process of tricarboxylic acid cycle and oxidative phosphorylation, mitochondria utilize oxygen to generate ATP from organic fuel molecules using the electrochemical gradient generated across the inner of two membranes by the electron transport chain, but in the process also produce ROS (65). Radiation promote the accumulation of ROS, which acts straightforwardly by direct modifications of biological molecules like proteins, lipids and nucleic acids and leads to mitochondrial dysfunction post-irradiation and causes an energy imbalance.

Once the AMP/ATP ratio increases, AMPK, a genuine sensor of the energetic state of the cell, was activated. AMPK which directly responds to the so-called adenylate energy charge as the enzyme is activated by very low increases of AMP levels (and, to certain extent, of ADP), and deactivated by ATP, and could be activated by upstream kinases. Phospho-active AMPK activates autophagy to restore the correct adenylate energy charge. At the molecular level, active AMPK stimulates autophagy by means of at least four distinct mechanisms. These include: i) phosphorylation of the mTORC1 inhibitor, TSC2 at Ser1387, which induces RHEB GTPase activity; RHEB-GDP strongly inhibits the activity of mTOR and activate autophagy, and does so both *in vitro* and *in vivo* (66); ii) phosphorylation of ULK1 at Ser317 and Ser777. ULK1 is then free to interact with and to be phosphorylated by AMPK (67); iii) AMPK regulates autophagy by phosphorylating PIK3C3 and BECN1 at Thr388, S90 and S93; iv) AMPK regulates autophagy not only by promoting formation of the PIK3C3-BECN1 complex, but also by inhibiting

the interaction of BECN1 and BCL2 to release more available BECN1 to form PtdIns3K complexes (68).

Mitochondrial ROS and mitophagy. Mitochondria are one of target organelles for low-dose radiation (69). Recently, Shimura *et al* reported that low-dose, long-term fractionated radiation induces oxidative stress in irradiated normal human fibroblasts via accumulation of mitochondrial ROS (70). Mitochondria ROS act as essential signaling molecules in the regulation of the mitophagy and tumor development (71,72). Mitophagy is a process of mitochondria-selective autophagy in response to various signals, including oxidative stress, starvation, and modification of mitochondrial proteins (73). It has been shown that degradation of mitochondria and mtDNA can be executed by mitophagy. Mitophagy is mediated by two different molecular pathways: BNIP3/NIX and Parkin (PARK2)/PINK1 (PTEN induced putative kinase 1). BNIP3L/NIX, localizes to mitochondria, and regulates the aggregation of damaged mitochondria to autophagosomes by directly interacting with LC3 or GABARAP (GABA[A] receptor-associated protein). ^{125}I-irradiation elevated the expression of BNIP3 (69). BNIP3 is one of the primary death promoting proteins, which competes with BECN1 for binding to BCL2, and hence releases 'free' BECN1 to induce autophagy. Additionally, radiation increased mitochondrial mass and caused accumulation of mitochondrial ROS, which resulted in mitochondrial damage that was in turn recognized by Parkin. Parkin/PINK1 allows the selective degradation of damaged and dysfunctional mitochondria in response to mitochondrial membrane de-polarization induced by ROS. Parkin accumulated in depolarized fragmented mitochondria by the recruitment of PINK1 to control mitochondrial quality via induction of mitophagy (69,74). As an E3 ubiquitin ligase, the ligase activity of Parkin was activated by PINK1. This process involves the ubiquitination of VDAC1 (voltage-dependent anion channel 1) and its combination with p62, which subsequently interacts with LC3 and directs this complex to the autophagosome (75).

5. ER stress and autophagy

As previously indicated ER is another target of radiation, radiation initially induces ROS production, which would activate ER membrane sensors of ER stress in IEC-6 cells and breast cancer MDA-MB-231 and MCF-7 cells (76,77). ER stress induced by radiation triggers signal transduction pathways, known as unfolded protein response (UPR) also through the induction of DNA damage (78,79). ER stress and UPR related genes are required for stress induced autophagy. The last few years have evidenced that the ER stress and autophagy processes are closely related as some of the signaling routes activated during the ER stress response are involved in stimulating autophagy.

Although the main signaling pathway of ER stress that is activated following irradiation is still a debatable one; some recent evidence suggests that PERK-eIF2a and/or IRE1a may serve as the main executing pathways of ER stress in irradiation scenarios (80-82). A link between autophagy and the ER stress has been further substantiated by the PERK-eIF2α pathway which is essential for autophagy induction after ER stress. PERK phosphorylates the eukaryotic initiation factor 2α (eIF2α) on residue serine 51, which then initiates a cascade of events that decreases the overload of misfolded proteins, thereby alleviating ER stress (83). eIF2α phosphorylation stimulates the selective translation of the ATF4 transcription factor (although general translation is shut off) and CHOP (a transcription factor induced by ATF4), which were shown to transcriptionally regulate more than a dozen ATG genes. These genes are necessary for sustained autophagy (84). PERK activated by oxidative stress can also directly inhibit mTOR or indirectly inhibits mTOR via activation of PARP1, which can induce autophagy (85). In addition, IRE1 was activated in response to a variety of cellular stressors after exposure to radiation. In mammalian cells, IRE1-JNK pathway was required for autophagy activation after ER stress (86,87). Activation of IRE1 and JNK causes Bcl-2 phosphorylation, dissociation of BECN1, activation of the PI3K complex and induces autophagy. Moreover, dysregulated autophagy may also trigger the IRE1 activity with concomitant activation of UPR, thereby dampening excessive autophagy triggered via the PERK/eIF2α pathway and pointing to a plausible feedback mechanism in the control of UPR signaling (88).

6. ROS and autophagy

ROS is a family of highly reactive molecules which includes free oxygen radicals, like superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), and non-radical oxygen derivatives, like the stable hydrogen peroxide (H_2O_2). ROS are unstable molecules which have the capacity to readily convert to many different viable and active forms. The superoxide radicals react to form other ROS, namely, hydrogen peroxides and hydroxyl radicals, and interconvert with RNS, which generate effects similar to ROS (89). Radiation exposure has been intimately linked to increased reactive ROS production and persistent oxidative stress in cells. Accumulating data implicate accumulation of ROS from radiolysis of water molecules, damage mitochondria and ER regulation of autophagy in oxidative stress response (66,90). Although multiple studies reported that accumulation of ROS regulated autophagy by pathways such as: i) oxidization of ATG4 leading to accumulation of autophagosomes; ii) activation of the AMPK signaling cascade inducing the initiation of autophagy through the ULK1 complex; iii) disruption of BECN1-BCL-2 interaction leading to the initiation of autophagy; or iv) alteration of mitochondria homeostasis leading to mitophagy activation (75). Furthermore, reactive oxygen/nitrogen species (ROS/RNS) generated in the context of radiation exposure are essential activators of cytoplasmic signaling cascades such as p38 MAPK, JNK, HIF-1α, which play essential roles in the regulation of autophagy (91).

p38 MAPK and JNK are two of the major MAPK subfamilies, which are serine/threonine kinases that mediate responses to various extracellular stimuli. Once activated in response to various stresses, p38 MAPK and JNK lead to phosphorylation-dependent activation of other kinases and transcription factors and play a crucial role in the modulation of autophagy (92). It has been previously reported that disruption of ROS clearance activates the p38 MAPK pathway which acts as a key regulator in ROS-mediated autophagy (93). The activation of p38 MAPK was in part responsible for the downregulation of phosphory-

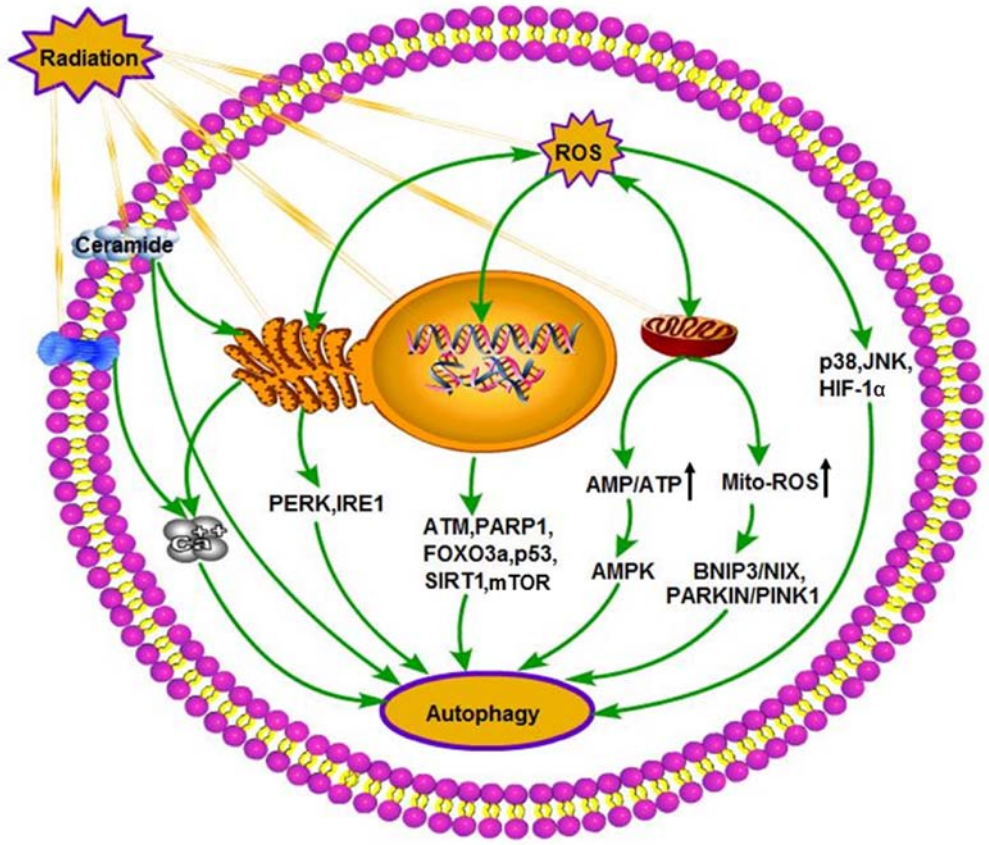

Figure 2. Overview of the major intracellular autophagic triggers induced by radiation. Radiation can directly or indirectly damage DNA which can activate DNA damage repair signaling pathway, a large number of proteins participating in DNA damage repair signaling pathways such as p53, ATM, PARP, FOXO3a and mTOR are involved in autophagy regulation. Radiation can also cause injury to extanuclear targets such as plasma membrane, mitochondria and endoplasmic reticulum which induce accumulation of ceramide, Ca^{2+} concentration and ROS, activates multiple stress signaling pathway to modulate autophagy. Ceramide can induce ER stress and mitochondrial dysfunction, which are two important autophagic triggers. ROS is an essential activator of cytoplasmic signaling cascades such as p38, JNK, HIF-1α which activate autophagy related signaling pathways. ROS can also cause injury to mitochondria and ER which elevate the levels of ROS and Ca^{2+} concentration and decrease ATP. The change to these molecules is important to induce autophagy. Moreover, ROS production activates ER membrane sensors of ER stress and triggers autophagy.

lated mTOR and the subsequent activation of autophagy (94). p38 MAPK depletion is also found to regulate the trafficking of ATG9, a multi-spanning membrane protein that is essential for autophagy (95). Fractionated radiation activated p38 MAPK which is involved in radiation-induced stabilization of HIF-1α which play an important role in switching autophagy. Consistently, inhibition of p38 MAPK also effectively attenuated radiation-induced stabilization of HIF-1α (96).

After exposure to radiation, the expression of HIF-1α increased, then HIF-1α translocated into the nucleus. In the nucleus, HIF-1α bound to the hypoxia response element in its target promoter and promoted the transcription of its target (97). HIF-1α is a subunit of HIF1 which consists of a regulatory HIF-1α subunit and the constitutively expressed HIF-1β subunit, both are members of the basic helix-loop-helix and PER-ARNT-SIM families of transcription factors (98). Pervious opinion considered that while HIF-1β is constitutively expressed, HIF-1α is an oxygen sensitive subunit and its expression is induced under hypoxic conditions (99). Accumulating evidence has recently revealed that HIF-1α is activated in cancer cells not only under hypoxic conditions, but also in the presence of oxygen when the following conditions are satisfied. In addition to post-translational mechanisms, regulation at the level of transcription and translation initiation is also important for the activation of HIF-1α under normoxic conditions (100). Koshikawa *et al* reported that ROS generated in mitochondria upregulated the transcription of the HIF-1α gene via the PI3K-Akt/PKC/ HDAC pathway, leading to the accumulation and activation of HIF-1α in tumor cells (101). ROS upregulates HIF-1α transcription by activating non-hypoxic factors in a redox-sensitive manner (102). HIF-1α induces autophagy by a mechanism involving the upregulated expression of its target genes BNIP3 and its homologue NIX and the dissociation of the BECN1-BCL2 complex, leading to release of BECN1, which is capable of triggering autophagy (103,104).

7. Ceramide and autophagy

Radiation activates the cell surface glycohydrolases which play a crucial role in the production of ceramide at the plasma membrane. Ceramide is localized within lipid rafts in the plasma membrane, which are specialized membrane microdomains that regulate various signaling pathways (105). Ceramide is also a central molecule of sphingolipid metabolism and involved in the regulation of autophagy at various levels (106,107). Ceramide downregulates the expression of

amino acid and nutrient transporters in the context of starvation, a state that induces survival autophagy by reducing mTOR signaling pathway or activating AMPK (108,109). Further evidence indicated that ceramide increases BECN1 expression by activating JNK kinase, which in turn activates c-Jun, a known transcription factor for BECN1 expression (110). Ceramide accumulation also can induce ER stress, mTOR inhibition via TRB3 (tribbles homologue 3), which triggered lethal autophagy (111-113). Moreover, ceramide can be generated by neutral sphingomyelinase in mitochondria, which is a late event upon the cell irradiation following the early phase of ceramide accumulation at the cell plasma membrane (106). Ceramide accumulation in the mitochondria can lead to ceramide stress-induced mitochondrial fragmentation, and decrease in ATP production (114). These important mitochondrial parameter changes can induce mitophagy (115).

8. Ca^{2+} and autophagy

Cellular response to radiation-induced stress is generally mediated through the production of the second messenger, in-flux of Ca^{2+} (90,116,117). These radiation-induced changes in Ca^{2+} concentration can be elevations, oscillations or single transient changes within minutes to days after exposure to radiation. Radiation regulates intracellular Ca^{2+} concentration through several different pathways. Firstly, radiation can induce the accumulation of ceramide, which can regulate influx of extracellular Ca^{2+} at plasma membrane channel level (106). Secondly, radiation can activate PLC, the main enzyme localized at the plasma membrane and producing IP3 and consequently inducing Ca^{2+} release from ER (90,118). Several studies in mammalian cells and yeast models have demonstrated a potential role for mitochondrial parameters regulated by Ca^{2+} signals in modulating and/or triggering mitophagy (115,119). Ca^{2+} release events from the ER, the major intracellular Ca^{2+}-storage organelle that have an immediate effect on the physiological function of mitochondria and lysosomes (120). Ca^{2+} released from the ER activates members of the PKC family. In mammals, PKCθ, a member of the PKC family, is a novel factor that mediates Ca^{2+}-dependent induction of autophagy in response to ER stress. Increased Ca^{2+} concentrations induce PKCθ phosporylation and its localization to cytosolic LC3 puncta (121). In addition, the CAMKK2-PRKAA-mTOR pathway is an important signaling pathway for Ca^{2+}-induced autophagy, which is also a cascade with established roles in multiple cellular processes including ribosome biogenesis and transcription in addition to cell motility and metabolism (119). In addition elevation of intracellular Ca^{2+} can activate AMPK, which plays a role in Ca^{2+}-mediated signal transduction pathways (122,123). An overview of the major intracellular autophagic triggers induced by radiation is shown in Fig. 2.

9. Targeting autophagy for radiotherapy

Various preclinical models revealed that autophagy is activated in irradiated tumor cells and that ATG expression patterns are upregulated following irradiation. Inhibition of autophagy genes such as ATG5 alone or a mixture of BECN1, ATG3, ATG4b, and ATG5 together leads to the strongest, and significant sensitizing effect found in radiation (124). Recent *in vitro* and *in vivo* studies in preclinical models suggested that modulation of autophagy can be used as a therapeutic modality to enhance the efficacy of radiation therapy. Currently, multiple clinical trials have been initiated combined with autophagy inhibitors, such as hydroxychloroquine and chloroquine, which associated with increased sensitivity to radiation (125,126). However, the role of autophagy in the resistance of cancer cells to radiation therapy remains controversial. One logical outcome of this argument is that chloroquine and hydroxychloroquine are unlikely to be appropriate drugs for this purpose, because of the fact that patients with malaria are able to endure treatment with these drugs for years, which suggests that the doses of chloroquine and hydroxychloroquine that are used effectively for malaria treatment may not actually be acting to inhibit autophagy. Another logical outcome is that various studies indicated that the sensitivity of tumor cells to radiation could be enhanced by co-treatment with rapamycin, an autophagy promoter, in various kinds of tumor cells (44,50,127).

10. Conclusion

Radiotherapy is one of the best therapeutic choices for cancer treatment (26). Radiation-induced autophagy in cancer cell lines is related to cell death mechanism and autophagy-inducing agents may act especially as radio-sensitizers or radio-resistance (128). Recent studies show that radiation can damage DNA, plasma membrane and cellular organelle such as mitochondria and ER and activate several radiation responsive pathways. During periods of cellular stress, the convergence of these pathways promotes autophagy (129). To clarify cross talk between autophagy and intracellular radiation response, we focused on the proteins and molecules that link the intracellular radiation response with autophagy. A part of these proteins of radiation responsive pathway were verified to participate in the modulation of radiation-induced autophagy. However, others have not been confirmed in the regulation of autophagy. Deep experimental research is needed to further investigate the understanding of the pathways regulating autophagy induced by radiation, which will likely offer new targets for radiotherapy treatment of certain aggressive forms of tumors. Additionally, it would indicate how to regulate the autophagic process or its related pathways that protect cancer cells from undergoing autophagy and may be beneficial to exploit radio-sensitizers. There may also be a therapeutic role for autophagy in protecting normal tissues from lethal radiation exposures. In addition, a recent study showed that autophagy may yield the opposite returns from tumor immunity perspective, irradiation-induced autophagy exerts crucial activity on tumor clearance by the immune system needing further investigation. Clearly, more work is needed to clarify crosstalk between autophagy and intracellular radiation response.

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