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Review article

DNA Damage Response and Autophagy: An Exclusive Meeting in Cancer

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Abstract

Healthy cells maintain genome integrity by activating a conserved DNA damage response (DDR) pathway that halts the progression of the cell cycle and activates DNA repair. Molecular disorders preventing DDR functioning properly often predispose to cancer. Therefore DDR acts as a tumor suppressor barrier. DDR often leads to not only cell cycle arrest and DNA repair, but also induces cellular senescence and apoptosis. Ultimately, "autophagy" as a self-degradation and recycling program of cellular components can be induced by DDR. In healthy cells and the initial stage of cancer, autophagy appears to have a tumor suppressor function by eliminating damaged organelles, and protein aggregates to promote genomic instability. However, in advanced tumors, autophagy s activated, particularly as a result of hypoxia and metabolic stress, to promote tumor survival under these conditions. Autophagy can also be induced by DNA damaging chemotherapy agents in tumor cells, which mostly results in resistance to conventional cancer therapies. In addition, activation of certain oncogenes in advanced tumors may promote autophagy activation and guarantee the persistence of tumors. Thus, currently development of inhibitors targeting autophagy with potential clinical use is increasing rapidly. In this review, the DDR and autophagy signaling mechanisms, as well as the interconnecting pathways of both are highlighted. Moreover, the biological consequences of the companion of these two important cellular responses in cancer are discussed.

Keywords: DNA damage response, autophagy, cancer, apoptosis, sensecence, Wip1.

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INTRODUCTION

Eukaryotic cells are constantly exposed to various internal (replicative stress), and external environmental stress factors (ionizing radiation IR, UV-radiation) that cause lesions in DNA and cause genomic instability. In response, eukaryotic organisms activate a highly conserved DNA damage response (hereafter DDR) that halts cell cycle progression and coordinates DNA repair to maintain genome integrity (Bartkova et al.2006; Bartek and Lukas 2007; Bartek et al. al. 2007). The term "DNA damage response" (DDR) refers to an intracellular signal network that detects and decodes damaged DNA. DDR has a complex mechanism including the activation of cell cycle checkpoints, which allows the repair of damaged DNA and maintains genomic integrity. (Liang et al.2009; Broustas and Lieberman 2014). When left unrepaired, DNA lesions can be an important source of genomic instability (Halazonetis et al. 2008). However, DDR plays also a critical role in directing cells that did not have an effective DNA repair to apoptosis or senescence and thereby prevents the replication of the damaged genome and the transmission of mutations to subsequent progeny. Defects occurring in one or more of the mechanisms that enable DDR activation, such as sensing of DNA damage, activation of cell cycle control, DNA repair, apoptosis or senescence, or their functional inactivation contribute to cancer initiation by allowing the accumulation of mutations (Liang et al. 2009). Thus, DDR acts as a tumor suppressor barrier in preventing tumorigenesis (Broustas and Lieberman 2014; Hosoya and Miyagawa 2014).

Recently, “cell’s self-degradative process” autophagy has been shown to be activated by DNA damage and involved in numerous cellular response activated by DDR signaling, including mainly in DNA repair, senescence and cell death. Autophagy and the DNA damage response (DDR) are key cellular programs necessary for protection of cellular and organismal homeostasis. Here, we review the mechanisms of DDR, induction of autophagy and discuss the relationship between DDR and autophagy. A detailed understanding of the mechanisms linking DDR and autophagy may provide new insights in pathology of diseases such as aging and cancer and also may lead to development of new and more effective, targeted solution to cancer therapy.

DNA Damage Response Signaling

DDR has a signal network regulated by phosphorylation and dephosphorylation events which is driven through three main components involving the sensors, signal transmitters and effector proteins. When DNA damage occurs, post-translational histone modifications (including poly-ADP-ribosylation, phosphorylation, and acetylation) take place via polymerases (poly ADP-ribose, PARPs) in order to provide relaxation to the chromatin (Lukas & Bartek 2011). These changes in chromatin lead to termination of transcription and replication around the DNA damage site and facilitate repair in the next step. Detection of DNA damage is primarily carried out by DNA damage sensors such as the MRN complex (MRE11-RAD50-NBS1), replication protein A (RPA), 53BP1 and γ-H2AX. Following the

detection of DNA damage, ATM (ataxia telangiectasia mutated kinase) and ATR (ataxia telangiectasia and Rad3-related kinase) signal transmitters are autophosphorylated to activate the effector proteins involved in downstream pathways such as p53, BRCA1 / 2, Chk1, Chk2, p21, p-Rb, CDC2 (El-Awady et al., 2016).

In brief, upon DNA damage, MRN complex binds to double strand breaks and brings ATM kinase to the damage site and replication protein A (RPA), predominantly reacts to single strand breaks and brings the other kinase ATR to damaged sites in DNA. Upon formation of a damaged single strand DNA (ssDNA) break, ATR is activated by PI-3K (phosphatidyl inositol-3-kinase). Following its activation by autophosphorylation at Ser482 residue, ATR activates checkpoint kinase 1 (Chkl) by phosphorylating Ser317 and Ser345 residues (Blackford & Jackson, 2017; Olcina et al. 2014). In addition to Chk1, ATR-related protein ATRIP, replication protein A RPA, RAD17, 9-1-1 complex (Rad9-Rad1-Hus1) and TopBP1 are phosphorylated by ATR during checkpoint activation (Shimada and Nakanishi, 2013). Thus, ATR plays an important role in maintaining genome integrity during DNA replication through regulation of DNA damage response (Blackford & Jackson, 2017). Formation of DNA double strand breaks (DSBs), induce ATM's autophosphorylation at Ser1981 and DNA dependent kinase (DNA-PK) becomes also activated. ATM activates the DNA damage marker H2AX from the Ser139 residue (γ-H2AX) and the checkpoint kinase 2 (Chk2) from Thr68 by phosphorylation. Consecutively, they initiate downstream signaling by performing the phosphorylation of many proteins in the DDR including MDC1, 53BP1, BRCA1 (Blackford & Jackson, 2017; Olcina et al. 2014). Thus, activation of ATM/ATR kinases upon DNA damage triggers the phosphorylation of additional proteins transmitting the signals for DNA replication, checkpoints of cell cycle, DNA repair, cell survival and apoptosis. Recent proteomic studies reveal that ATM and ATR kinases can phosphorylate up to several hundred target proteins and trigger intense changes in gene expression involved in chromatin organization. On the other hand, ATM phosphorylates checkpoint kinase Chk2 whereas ATR, Chk1, and thus both play important roles in DNA damage-induced checkpoint activation. Chk1/2 temporarily halt the progression of the cell cycle by inactivating three essential phosphatases (Cdc25A/B/C) which inhibit the activation of cyclin-dependent kinases. In addition, ATM/ATR kinases provide stabilization and transcriptional activation of tumor suppressor protein p53 by directly inhibiting Mdm2. Accordingly, p53 contributes to the arrest of the cell cycle by inducing the activation of p21 and by suppressing the expression of many proteins that stimulate the cell cycle (Bartek and Lukas 2007; Medema and Macurek 2012). Activation of DNA repair emerges as the final point of DDR. DNA repair systems are various and involved in maintaining the genomic integrity. The decision on which repair program should be activated mainly is defined by the type of damage and the status of the cell cycle (Bartek & Lukas, 2007). When DNA repair is completed, inactivation of p53 by protein phosphatases is required at the right time for cells' re-entry to the cycle (Lowe et al., 2012; Mirzayans et al., 2013). In contrast, when DNA damage is not repairable, chronic DDR signaling triggers induction of apoptosis or senescence which also acts as a tumor

suppressor barrier within the cell (Roos et al., 2016). Recent studies show that autophagy is another cellular response also triggered by DNA damage and appears to play an important role in cellular protection. Evidences indicate the presence of DDR-autophagy linkage in various types of eukaryotic cell types. The relationship between autophagy and DNA damage response may be necessary for several basic processes such as induction of DNA damage repair, senescence, apoptosis, and cytokine secretion (Eliopoulos et al.2016; Czarny et al., 2015). Taken together, DDR is a conserved cellular response mainly involved in maintaining genomic stability in eukaryotic cells via activating appropriate cellular responses upon DNA damage.

Autophagy

Macroautophagy (autophagy) is a highly conserved catabolic process that prevents cell damage and enables survival in an energy or nutrient shortage and also stimulated against various cytotoxic events and under different cellular stress conditions. Autophagy is primarily involved in protection of the cell and therefore needs to be an extensive regulation and adapted to the ever-changing environment of cells to respond accurately to the different stimuli they encounter. Primarily, long lived proteins and damaged organelles are directed to lysosomal degradation to maintain cell metabolism, genomic integrity, and cell survival (Dikic et al., 2018). Functions in the repertoire of routine cleansing functions performed by autophagy are of great importance for autophagy-mediated cellular protection against aging, neurodegenerative diseases, infection and cancer. Thus, deregulated autophagy may be involved in various human pathologies, including cancer and neurodegeneration and its modulation may be a promising therapeutic approach.

The molecular mechanism of autophagy is best characterized in response to nutrient deprivation. In nutrient deprived conditions, autophagy is induced by functional complexes containing autophagy-associated (Atg) proteins leading to the formation of autophagosomes. In the induction of autophagy, the Ulk1 complex, which is composed of a serine / threonine kinase, Unc-51-like kinase 1 (Ulk1) and Ulk2, Flk200, Atg13 and Atg101, plays an important role in attracting the Atg proteins from downstream to the upstream stage of autophagy. Under healthy conditions, Ulk1 is phosphorylated and thereby inhibited by mTOR (mammalian target of rapamycin) complex 1 (mTORc) and AMP-activated protein kinase (AMPK) from various serine/threonine residues. However, under nutrient deprived conditions, ULK1 is released from the mTORC1 complex, thus it is dephosphorylated and activated. It has been reported that PP2A-B55α phosphatase complex, which is activated during fasting, plays an important role in the dephosphorylation of Ulk1 (Wong et al. 2015). With the complex formed by the release of Ulk1, precursor-autophagosomal membranes that will turn into autophagosome are formed. Class III PI3 kinase (PI3KIII) Beclin-1 and Vps15 / p150 and Vps34 complexes are important in phagophore formation. The maturation process of autophagosome membranes and autophagosome formation is provided by ubiquitylation-like processes. The initial step of these processes is started with the Atg5 -

Atg12 - Atg16L complex. Atg5 is an E3 ubiquitin ligase conjugated to the Atg12 protein and in this complex E3 exhibits ligase-like activity. Accordingly, LC3-I (Microtubule-associated protein 1A/1B-light chain 3) / Atg8 is also drawn into the Atg-5-Atg12-Atg16L complex. The second process is achieved by lipidating LC3-I by covalent bonding with phosphatidyl-ethanolamine (PE) to form LC3-II. This process is also supported by the Atg5 - Atg12 - Atg16L complex. The resulting PE-conjugated ("lipidized") LC3, known as LC3-II, contributes to the elongation of membranes by settling on mature autophagosomes targeting lysosomes. The LC3 protein is widely recognized as a marker of autophagy. Once the autophagosome is established, it fuses with the lysosome and thereby proteins and organelles will be degraded into building blocks such as amino acids, fatty acids and etc. The process of autophagy starting from nucleation to degradation step is called autophagic flux. Auphagic flux represents the ability of autophagosomes for degradation of intracellular components. Autophagic flux can also be followed by monitoring the degradation of specific autophagic substrates that interact with LC3-II, such as the autophagy cargo receptor p62 (SQSTM1), and bind to Lys63-ubiquitylated proteins that target autophagic degradation. After combining autophagosomes with lysosomes for the destruction of targeted proteins, p62 itself is also accepted as another marker of autophagy (Feng et al.2014; Czarny et al.2015, Dikic et al., 2018).

The DDR-autophagy linkage

Autophagy can be stimulated not only by nutrient deprivation but also by several other stress factors, including genotoxic stress. Recent studies have shown that DNA damaging agents (etoposide, camptothecin, temozolomide or p-anilioaniline, ionizing radiation), including ionizing radiation (IR), can induce autophagy as well as arrest the cell cycle (Katayama et al. 2007; Abedin et al. 2007; ; Rieber and Rieber 2008). The data obtained from previous studies conducted so far, suggesting ATM, which is the main sensor of DNA double strand breaks, also plays a key role in DNA damage induced autophagy, similar to apoptosis and senescence. Activation of ATM after exposure to genotoxic or oxidative stress leads to suppression of mTORC1 and induction of autophagy. AMPK mediates the transduction of the signal from ATM to mTORC1 in the suppression of mTORC1 through tuberous sclerosis complex 1 and 2 (TSC1/2). In healthy conditions, mTORC1 suppresses this complex through phosphorylation of ULK1/2 and Atg13, but upon inhibition of mTORC1, the phosphorylation level Ulk1 / 2 and Atg13 decrease and the kinase activity of Ulk1 increases. Following the formation of the Ulk1/2-Atg13-FIP200 complex occurs. Ulk1/2-Atg13-FIP200 complex translocates to the precursor-autophagosomes and starts the induction of autophagy.

Post-translational modifications including phosphorylation, ubiquitination, acetylation, and lipidation mediate the prompt induction of autophagy. However autophagy regulation depends also on the execution of specific transcriptional programs. In this context, P53 protein, known as an important component of DDR and tumor suppression, also plays a central role in induction of autophagy (Czarny

et al. 2015, Eliopoulos et al., 2016). Previous studies have reported that p53 inhibits the mTOR signal by transcriptionally raising the levels of the negative regulators of mTOR such as AMPK-1/β2, TSC2, PTEN and Sesn1/2 and 9, thus leading to autophagy induction (Stambolic et al. 2001; Feng et al.2007; Budanov and Karin 2008). On the other hand, p53 can trigger induction of autophagy by activating the transcription, of the lysosomal protein DRAM (Crighton et al. 2006). Furthermore, DAPK, the transcriptional target of p53, phosphorylates Beclin-1 to release it from BCL-2 and BCL-XL, and simultaneously phosphorylate protein kinase D (PKD), which activates Vps34 and thereby trigger induction of autophagy (Eisenberg-Lerner and Kimchi 2012). It has been reported that p53 transcriptionally activates ULK1 and ULK2, their upregulation leads to sustained autophagy in response to DNA damage and subsequently contributes to cell death (Pietrocola et al., 2013). Furthermore, PARP-1 is another important component of DDR which has been also involved in the regulation of autophagy (Rodriguez-Vargas et al. 2012).

In a recent study it was shown that the Ser/Thr phosphatase PPM1D/Wip1 also plays important role in genotoxic stress induced autophagy (Figure 1). It has been reported that dephosphorization of ULK1 via Wip1 phosphatase is required for the formation of the Ulk1 complex. Wip1 was induced by genotoxic stress in a p53 dependent manner and subsequently interacts with Ulk1 and dephosphorylates it from Ser637, and thus triggering puncta formation and induces autophagy. Interestingly, overexpression of Wip1 in wt MEF without any genotoxic stress was shown to be sufficient to induce autophagy. On the other hand, genetic ablation of Wip1 phosphatase in primary thymocytes, prevented dephosphorylation of Ulk1 from Ser637 thus autophagy was inhibited and accordingly radiation induced apoptosis is accelerated. Importantly, acceleration of apoptosis is driven by the proapoptotic molecule Noxa as inhibition of the autophagic process leads to prevention of its degradation. The study by Torii et al. conducted in primary fibroblasts and thymocytes so far has been the first showing that the Wip1-Ulk1 axis plays a role in genotoxic stress-induced autophagy (Torii et al. 2016) (Figure 1). Additional evidence indicating the relationship between Wip1 phosphatase and autophagy has been obtained from a study investigating the relationship between Wip1 and ATM, particularly in metabolic disorders, obesity and atherosclerosis (Le Guezennec et al. 2012). However, in this study, in contrast to the autophagy induced by genotoxic stress, Wip1 was shown to inhibit selective autophagic flux in macrophages in mice via suppression of Atm-mTOR-dependent signaling pathway. Unlike autophagy induced by starvation, there are still many questions about genotoxic stress-induced autophagy (Lin et al.2015; Kang et al.2015; Sing et al.2012; Eliopoulos et al.2016). Clarification of the mechanisms and outcomes of autophagy induction in DDR can make important contributions to the characterization of human pathologies, especially cancer and aging, which are related to the accumulation of DNA damage.

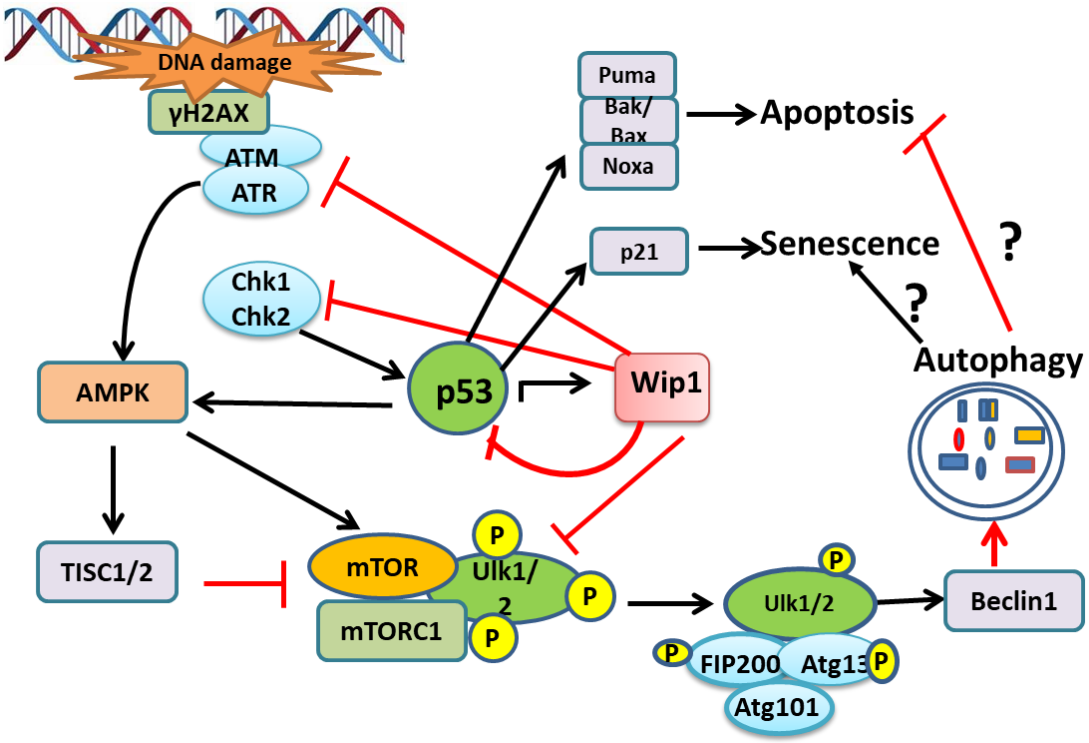

Figure 1. Key molecules involved in DNA damage induced autophagy, senescence and apoptosis. Black arrows indicate activation, red arrows inhibition.

Crossroads of DDR, autophagy and cancer

Autophagy plays a dynamic role in cancer which is mainly determined by tumor type and stage. Autophagy appears to have a suppressive role in tumor onset in healthy tissue, but in some advanced tumors, maintenance of tumor cells depend on autophagy. In this context, data from transgenic mouse models suggest that autophagy mediated suppression of tumor initiation may be regulated by DNA damage and oxidative stress. However, in advanced tumors, it is suggested that autophagy is a necessary process for tumor survival as it helps tumor cells to recover from environmental stress and provide metabolites for cell metabolism (Dikic vd. 2018; Naiara Santana-Codina vd. 2017). This suggests that in some tumors, autophagy may function as an adaptive mechanism that enables tumor progression even in the absence of important survival factors. Autophagy can also have a role in promoting cell survival when induced in response to chemotherapeutics, which can lead to resistance to anticancer drugs. Thus, targeting autophagy appears to be an attractive therapeutic option and currently being validated in clinical trials for cancer (Dikic et al. 2018; Naiara Santana-Codina et al. 2017).

The relationship between DDR and autophagy becomes even more important as autophagy is closely related to other important cellular processes such as DNA repair, senescence, and apoptosis. Autophagy can usually protect the cells against apoptotic cell death, but when autophagy activation increases excessively, it can lead to self-destruction of a cell. In addition, in cancer cells, inhibition of

autophagy can lead to the activation of apoptosis, resulting in cell death. However, when the cancer cell is resistant to apoptosis, accumulation of toxic wastes that is not cleaned by autophagy may increase genomic instability and lead to a more aggressive cancer phenotype (Morselli et al. 2009). Furthermore, it has been reported that when autophagy is induced in response to genotoxic stress, it delays apoptosis due to mitophagy (destruction of damaged mitochondria by autophagy), (Abedin et al. 2007). However, in contrast, Torii et al.. reported that suppression of genotoxic stress-induced autophagy accelerates apoptosis (Torii et al.2016). The nature of the relationship between autophagy and apoptosis may depend on context and cell type, but it seems that more extensive studies both in healthy cells and cancer cells are needed to fully understand the relationship between these two.

Besides apoptosis, cells induce senescence as another major fail-safe response to DNA damaging agents, chemotherapy or radiotherapy (Kilic and Schmitt 2008; Kilic Eren and Tabor 2014). However, it is now widely known that senescent cells may become notably deleterious due to the senescence associated secreted proteins including inflammatory cytokines, chemokines, growth factors and MMPs into the adjacent tissues (Coppe et al.2014). Although autophagy is known to mediate the removal of residual products resulting from senescence, there are conflicting data indicating senescence may be promoted as a result of inhibition of autophagy or that autophagy may be necessary for induction of senescence (Mosieniak et al. 2015; Mosieniak et al. 2016). Thus, in DDR, autophagy stands in close association with both apoptosis and senescence, and in many contexts that they are induced arm-in-arm, suggesting a central role for autophagy in the regulation of DDR (Eliopoulos et al.2016). Currently the details of the relationship between autophagy, apoptosis and senescence are mostly unknown and a comprehensive study is required on the subject.

Genotoxic stress induced autophagy may play either cytoprotective or cytotoxic role in determining cellular fate which mainly depends on the aspect of DNA damage. The role played by autophagy in this process may be countered by the sensitivity of cells to genotoxic agents, which is very important for anticancer therapy (Amaravadi et al. 2016; Levy et al. 2017; Marinkovic et al. 2018). Many evidence points to the applicability of autophagy as a target in anticancer therapy and that autophagy inhibition may enhance tumor cells sensitivity to the cytotoxic effects of both chemotherapy and radiation (Chude and Amaravadi 2017; Levy et al".2017, White 2015). Autophagy emerges as a promising but complex therapeutic strategy for the development of anticancer therapies. In particular considering that autophagy is a double edged sword playing a tumor-suppressing role in the early stage of carcinogenesis, but supporting tumor survival in advanced cancers, the modulation of the autophagy process requires cautious handling. Better understanding of autophagy in tumor models may help in determining new and effective therapeutic strategies for cancer treatment.

Previous studies conducted to investigate on the relationship between autophagy and cancer have reported that advanced tumors such as pancreatic and melanoma or expressing mutant Ras exerts high

levels of basal autophagy activity and eventually become autophagy addicted (Yang et al.2011; Guo et al.2011). In fact, it has been also shown in early studies that these tumors involve autophagy to better proliferate both in vitro and in vivo (Amaravadi et al.2016). Apart from this, it was shown that high levels of basal autophagosomes in melanomas cause high autophagic activity which was correlated with decreased survival in melanoma patients. In addition, it has been reported that basal autophagic activity measurements can predict invasive potential, resistance to chemotherapy and survival in melanoma tumors (Ma et al. 2011). These studies indicate the importance and necessity of the clarification of mechanisms leading to increased basal autophagic activity in tumors and its potential use in terms of cancer therapy. Considering the importance of increased basal autophagic activity in tumors in terms of both anticancer therapy and predicting metastatic potential and survival, it is of great importance to reveal whether overexpressed oncogenes play role in the increase of basal autophagic activity in cancers. In this context the Ser/Thr phosphatase PPM1D/Wip1, a, appears to be an important candidate. Wip1 is actually one of the key factors that play a central role in the response to genotoxic stress-induced DNA damage in normal cells. However, Wip1 is also recognized as an oncogene as it has been reported to be upregulated, amplified and overexpressed in a wide variety of human cancers including breast, ovary, pacreatic, neuroendocrine, medulloblastoma, neuroblastoma, colon carcinoma. Oncogenic Wip1 negatively regulates global cellular stress responses such as DNA damage response, DNA repair, cell cycle checkpoints, apoptosis, and cellular senescence (Saito-Ohara et al.2003; Fuku et al.2007; Yu et al.2007; Castellino et al. 2008; Tan et al.2009; Hu et al.2010; Song et al.2013, Bulavin et al.2002; Rauta et al.2006; Kleiblova et al.2013). As mentioned above, Wip1 is also involved in genotoxic stress induced autophagy by dephosphorylating Ulk1 and thereby allowing its release and triggering puncta formation (Torii et al.2016). However, in cancers where Wip1 is overexpressed, the relationship between Wip1 and autophagy is not yet clear. In particular, it would be interesting to identify whether or not oncogenic Wip1 contributes to the increased basal autophagic activity and promotes tumor survival in established tumors (Figure 2). Recent studies indicate that modulation of the autophagy process and targeting of autophagy are promising for the development of anti-cancer therapies. In this context, investigation of Wip1-autophagy relationship in cancers deserves more attention.

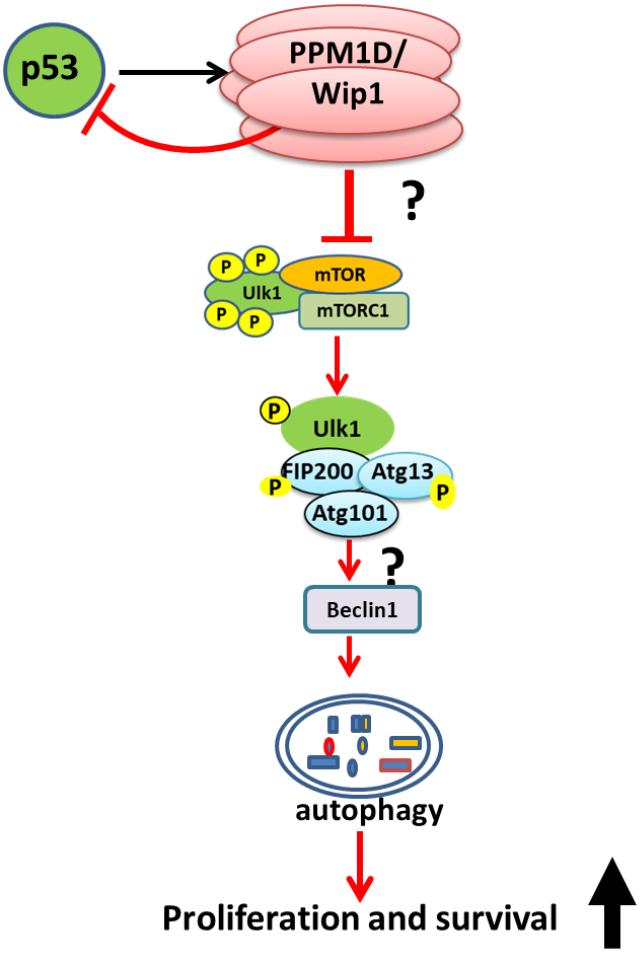

Figure 2. Possible involvement of oncogenic Wip1 in acceleration of basal autophagy in human cancers

Conclusion

Autophagy emerges as an essential component of the DNA damage response. Agents that cause DNA damage, including ionizing radiation (IR), can induce autophagy as well as arrest the cell cycle. Autophagy induced by DNA damage is also closely associated with other important cellular responses, such as apoptosis and senescence. Autophagy may play a tumor-suppressing role in the early stage of carcinogenesis, but supports tumor survival in advanced cancers. Certain oncogenes involved in modulation of DDR may play key role in promotion of autophagy in advanced tumors. Evidently, modulation of autophagy emerges as a promising but complex therapeutic strategy for anticancer therapies and requires cautious handling. Understanding of autophagy in tumor models may help in determining new and effective therapeutic strategies for cancer treatment.

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