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Autophagy in exposure to environmental chemicals

Pesonen, Maija

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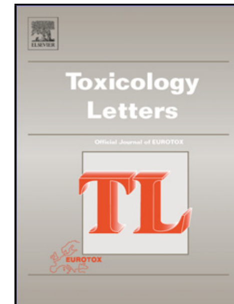

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Authors: Maija Pesonen, Kirsi Vähäkangas

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Autophagy in exposure to environmental chemicals

Maija Pesonen* and Kirsi Vähäkangas

Faculty of Health Science, School of Pharmacy/Toxicology, University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland

***Corresponding author:**
Maija Pesonen
Faculty of Health Science,
School of Pharmacy/Toxicology,
University of Eastern Finland, P.O. Box 1627,
70211 Kuopio, Finland
+358405105825
E-mail:Maija.Pesonen@uef.fi (M. Pesonen)

Highlights

- Autophagy is a catabolic process that degrades and recycles cellular organelles and proteins
- The level of autophagy is increased under stress conditions including chemical stress
- Many environmental chemicals have been found to affect autophagy
- Increase of autophagy can increase adaptation into stress and decrease genetic damage
- Inhibition or depletion of autophagy process can either increase or decrease toxicity depending on the context

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Abstract

Autophagy is a catabolic pathway, which breaks down old and damaged cytoplasmic material into basic biomolecules through lysosome-mediated digestion thereby recycling cellular material. In this way, autophagy prevents the accumulation of damaged cellular organs inside cells and reduces metabolic stress and toxicity. The basal level of autophagy is generally low but essential for maintaining the turnover of proteins and other molecules. The level is, however, increased in response to various stress conditions including chemical stress. This elevation in autophagy is intended to restore energy balance and improve cell survival in stress conditions. However, aberrant and/or deficient autophagy may also be involved in the aggravation of chemical-caused insults. Thus, the overall role of autophagy in chemical-induced toxicity is complex and only a limited number of environmental chemicals have been studied from this point of view. Autophagy is associated with many of the chemical-caused cytotoxic mechanisms, including mitochondrial dysfunction, DNA damage, oxidative stress, changes in the endoplasmic reticulum, impairment of lysosomal functions, and inflammation. This mini-review describes autophagy and its involvement in the responses to some common environmental exposures including airborne particulate matter, nanoparticles and tobacco smoke as well as to some common single environmental chemicals.

Key words: autophagosome, pesticides, particulate matter, nanoparticles, tobacco smoke

Abbreviations:

Atg, autophagy-related gene Atg; ATG, autophagy-related protein ATG; LC3, microtubule-associated protein 1 light chain-3 protein; mTOR, mammalian target of rapamycin. (mTOR is a member of the phosphatidylinositol 3-kinase-related kinase family of proteins); p53, the tumour suppressor protein p53; p62, ubiquitin-binding protein p62, encoded by SQSTM1 gene; ULK1/2, unc-51 like autophagy kinase 1/kinase 2; TFEB, transcriptionfactor-EB

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1. Introduction

Autophagy is a catabolic cellular process in which cells degrade old and damaged cytoplasmic components via lysosomes into basic biomolecules thereby recycling cellular material. Autophagy supports cellular energy balance during stress and starvation. It is predominantly involved in removing the proteins with long half-lives along with protein aggregates, and damaged organelles, while the short-lived proteins are mainly degraded by the ubiquitin-proteasome system (for a review see He and Klionsky 2009). A basic low level of autophagy is present in nearly all eukaryotic cells since this is necessary for maintaining the turnover of proteins and organelles (constitutive autophagy). This level is, however, increased (inducible autophagy) in various physiological and pathological stress conditions such as depletion of growth factors, starvation, and hypoxia, including chemically induced stress. Autophagy has been conserved throughout evolution and it plays an important role in many crucial physiological processes such as differentiation, development, and immunity (He and Klionsky 2009; Dikic and Elazar 2018).

There are at least three different types of autophagy present in eukaryotic cells: micro-autophagy, chaperone-mediated autophagy and macro-autophagy. Macro-autophagy is the major mechanism that eukaryotic cells exploit to transport and degrade damaged proteins and organelles. This form of autophagy is characterized by the sequestration of cytoplasmic material inside of a double-membrane vesicle called the autophagy vacuole (autophagosome) that delivers cargo to the lysosomes for degradation (for a review see He and Klionsky 2009). In this review, the term autophagy refers to macro-autophagy.

Autophagy functions primarily as a self-protecting, adaptive system maintaining cellular homeostasis and thus, deficiencies or impairments in autophagy increase the risk of metabolic stress, inflammation and cell/tissue injury. Defective autophagy has been associated with the pathogenesis of many human diseases such as neurodegenerative diseases, aging and the metabolic syndrome. In addition, deficiencies in core autophagy genes have been reported to increase the risk for cancer (Takamura et al. 2011; Damme et al. 2015). Under normal conditions, autophagy is intended to reduce genomic instability and cytotoxicity by eliminating old and damaged cell products (Mathew et al. 2007; Eskelinen 2011). However, in cancer it may also contribute to growth and survival of malignant cells by recycling energy and nutrient components (Degenhadt et al. 2006; Kimmelman and White 2017).

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Despite its role as a protective process, autophagy has been linked to cell death pathways. The relationship between cell death and autophagy is, however, complex. Autophagic cell death is regarded as cell death with autophagy rather than by autophagy (Kroemer and Levine 2008). Autophagy may facilitate other forms of cell death rather than acting as the form of cell death (Doherty and Baehrecke 2018). In many cases, cell death is preceded with the excessive activation of autophagy by a cytotoxic chemical or other stress stimuli. Autophagy signalling can undertake a crosstalk with the cell death pathways, apoptosis, necrosis and necroptosis and this crosstalk is critical in determining the cell's fate (Nikoletopoulou et al. 2013; Doherty and Baehrecke 2018). In particular, a crosstalk between autophagy and apoptosis has often been reported. In some conditions, autophagy signalling can suppress apoptosis and in others, it can activate apoptotic cell death. For example, if autophagy is inhibited during its early stages e.g. at the level of the formation of autophagosome, then cells generally undergo apoptosis. If autophagy process is inhibited at a later stage, cells can accumulate massive numbers of autophagic vacuoles and then undergo cell death with biochemical hall markers of apoptosis. Actually, the molecular pathways underlying the forms of cell death are multifaceted so that it is often difficult to distinguish one cell death form from another (Kroemer and Jäättelä 2005; Orrenius et al. 2013; Nikoletopoulou et al. 2013; Doherty and Baehrecke 2018).

Since autophagy is an important process for cellular repair and survival, it is not surprising that the stress and toxicity evoked by environmental chemicals can affect autophagy. There are many mechanisms, including damage to mitochondria and DNA (oxidative stress), or changes in endoplasmic reticulum (ER) and lysosomal functions that are associated with the activation and also impairment of the autophagy process. So far, only a limited number of environmental chemicals have been studied from this point of view. This mini-review describes autophagy and its involvement in the responses to some of the most common environmental exposures: airborne particulate matter, nanoparticles, tobacco smoke and some common single environmental chemicals. Pharmacological agents that are used for research or therapeutic purposes have been described in other reviews (e.g. Cheng et al. 2013; Triola 2015)

2. Cellular mechanisms and the main regulators of autophagy

The autophagy pathway is a dynamic process involving initiation, elongation, fusion, and degradation of the autolysosome. Initiation consists of the formation of a small cisterna, called an isolation membrane (phagophore) (Fig.1). This then expands, surrounds ubiquitinated cytoplasmic material and closes to form a double-membrane vesicle, the autophagy vacuole

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(autophagosome), which contains the sequestered cargo. Once formed, the autophagosome either fuses first with an endosome (this intermediate product is called the amphisome) or fuses directly with a lysosome to form an autolysosome where lysosomal enzymes degrade the cargo and the breakdown products are released into the cytoplasm (He and Klionsky 2009; Ktistakis and Tooze 2016). The dynamic rearrangement of the autophagosome requires multiple proteins (ATGs), which are encoded by over 30 autophagy genes (Atgs). During the formation of the autophagosome proteins form complexes (Table 1) and undergo various post-translational modifications (e.g. phosphorylation, acetylation, ubiquitination, and proteolytic cleavage) (He and Klionsky 2009; Mizushima et al. 2011).

The best known of the several autophagy core proteins used as markers for autophagy, are microtubule-associated protein 1 light chain-3 protein (LC3), p62 and Beclin-1 (Klionsky et al. 2016). LC3 is essential for autophagosome maturation and fusion with a lysosome. During the autophagic process, LC3 is cleaved by the protease ATG4 to produce LC3-I and this is further conjugated to phosphatidylethanolamine to form LC3-II that becomes anchored on the phagophore membrane. The membrane-bound LC3-II functions as an adaptor and interacts with the p62 protein to allow the engulfment of the cargo into the phagophore (Mizushima et al. 2011). The p62 protein, also known as sequestosome 1 (SQSTM1), is another common marker of the autophagy process. It acts as a receptor protein that binds to ubiquitinated proteins and delivers them via the membrane-bound LC3II-protein to the phagophore for degradation. p62 itself is degraded by autophagy and thus its accumulation in the cytoplasm has been used as a marker for reduced autophagy flux (Komatsu et al. 2012). Beclin-1 is the third common marker protein of autophagy. This protein is a component of the PI3K-complex. It is used as a marker of autophagy induction and it mediates a crosstalk between autophagy and apoptosis (Kang et al. 2011). The guidelines for the use of autophagy markers have previously been reported by Klionsky et al. (2016). The detailed description of the complex protein and molecular events in vacuole formation has been reported elsewhere in several outstanding reviews (He and Klionsky 2009; Mizushima et al. 2011; Dikic and Elazar 2018).

Two kinases, 5’AMP-activated protein kinase (AMPK) and serine/threonine kinase (mTOR), are the best-known cytoplasmic sensors and regulators of the autophagy pathway. AMPK senses energy depletion (decreased ATP/AMP-ratio) and induces autophagy either indirectly through phosphorylation of the autophagy initiating ULK-complex or directly by inhibiting the mTOR-complex1 (mTORC1), which is a central integrator for multiple autophagy-related

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signals. Inhibition of the mTORC1-complex leads to activation of the autophagy pathway while activation of the mTORC1-complex results in an inhibition of autophagy (He and Klionsky 2009; Rabanal-Ruiz et al. 2017). In addition to cytoplasmic AMPK and mTORC1, a network of transcription factors (e.g. TFEB, TP53/p53, FOXO1, E2F1, STAT3, NF-κB), microRNAs (miRNAs) and histone modifications have been associated with the regulation of autophagy (for reviews see Frankel and Lund 2012; Füllgrabe et al. 2014). Many miRNAs regulate autophagy targeting predominantly the early stages of the autophagy pathway. However, the physiological significance of miRNAs in autophagy is not still fully elucidated (Frankel and Lund 2012). Dysregulation of the autophagy-connected miRNAs has been associated with diseases, including cancer (Frankel and Lund 2012; Fesler et al. 2017). The transcription factor EB (TFEB) controls the genes that belong to the coordinated lysosomal expression and regulation (CLEAR) network (Settembre et al. 2011). These genes stimulate the biogenesis of lysosomes, autophagosome formation and autophagosome-lysosome fusion as well as the degradation of stored material in autolysosomes (Martin-Stoica et al. 2016). The p53 protein is activated in response to various types of stress, particularly genotoxic stress to maintain genomic stability (for a review see e.g. Vousden and Lane 2007), and among its many regulatory functions is also involved in regulation of autophagy, especially in cancer (Denisenko et al. 2018). Many of the p53-regulated gene products can stimulate autophagy through the AMPK-mTORC1 pathway (Feng et al. 2005) or encode directly core autophagy and lysosomal proteins (e.g. ULK1, ULK2, DRAM) (Crighton et.al. 2006; Kenzelmann-Broz et al. 2013). In contrast to the promotion of autophagy, p53 also inhibits autophagy in unstressed conditions. The stimulation of autophagy is mediated by the nuclear p53, whereas inhibition is mediated by p53 when it is present in the cytoplasm (Tasdemir et al. 2008).

3. Xenobiotics and autophagy

A number of environmental chemicals such as pesticides and metals have been shown to interfere with autophagy. One chemical may affect differently the autophagy pathway depending e.g. on the cell type, duration of exposure and dose.

3.1 Autophagy, ROS and mitochondrial damage

Mitochondrial dysfunctions lead to elevated ROS levels and generally to activation of autophagy. Superoxide ($O_2^{\bullet-}$) has been shown to be the major reactive oxygen species in the activation of autophagy (Cheng et al. 2009). Autophagy has been shown to protect against

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clorpyrifos (CPF) caused ROS-mediated toxicity (Park et al. 2013a; Table 2). CPF is a widely used organophosphate insecticide that induces ROS-production, autophagy and mitochondria-mediated apoptosis in human neuroblastoma (SH-SY5Y) cells. Pre-treatment of the cells with the autophagy inducer, rapamycin increased significantly the viability of the CPF-treated cells inhibiting apoptosis via a decrease of cleaved caspase-3 level. In addition, rapamycin pre-treatment decreased also the expression of the pro-apoptotic protein (BAX) and increased the anti-apoptotic Bcl-2 expression in mitochondria whereas treatment with the autophagy inhibitor, 3-MA, remarkably increased CPF-caused cytotoxicity. The increased cytotoxicity showed a correlation with the increased BAX expression and the decreased Bcl-2 expression in mitochondria (Park et al. 2013a). Several of the other chemicals in Table 2 (e.g. TCDD, fibronil, 4-nonylphenol, paraquat, TCDD + endosulfan) induce autophagy but trigger apoptotic cell death when the autophagy has been inhibited by the model inhibitor 3-methyladenine (3-MA), indicating that autophagy is pro-survival and combats cell death. 3-MA is an inhibitor of autophagy that reduces the formation of the PI3K complex, which is needed in the initiation of the autophagy process (Blommaart et al. 1997). Similarly autophagy has been shown to protect SH-SY5Y-cells from fibronil-induced cytotoxicity and apoptosis (Park et al. 2013b; Table 2). Fibronil is a neurotoxic phenylpyrazole insecticide, used in agriculture. Pre-treatment of SH-SY5Y cells with the autophagy inducer, rapamycin, increased significantly the cell viability after fibronil-treatments and alleviated apoptosis (Park et al. 2013b). Fibronil-induced autophagy and apoptosis were also effectively inhibited by the ROS-scavenger; N-acetylcysteine indicating that oxidative stress is critical for both toxicity and autophagy (Park et al. 2013b). Oxidative stress has also been associated with the induction of autophagy after exposure to polybrominated diphenyl ethers (PBDEs). PBDEs are a group of flame-retardants, which have been incorporated into many products (e.g. foams, plastics, resins) and are known as lipophilic and persistent global contaminants (de Wit et al. 2010). Two members of the PBDE-family, BDE-153 and BDE-100 were shown to induce autophagy through oxidative stress and impairment of mitochondria (mitophagy) in human liver hepG2 cells (Pereira et al. 2017a; 2017b). In addition to the activation of autophagy, oxidants may also impair the maturation of autophagy and this lead to the loss of the catabolic processes. A recent study by Frudd et al. (2018) suggested that direct oxidation of catalytic thiol-groups on the core autophagy proteins, Atg3 and Atg7 could prevents the conjugation with phosphatidylethanolamine of LC3 that is required for functional autophagy.

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Mitochondrial damage and ROS-production induced by most of the chemicals in table 2 are early stimulators of autophagy and when these become sufficiently severe, dysfunctional mitochondria should to be removed by autophagy (mitophagy) in order to protect cells against the further release of ROS and the production of pro-apoptotic proteins. Damaged mitochondria are recognized by the mitochondrial serine/threonine protein kinase (PINK1) that becomes stabilized in response to the loss of mitochondrial membrane potential (Narendra et al. 2008). PINK1 subsequently activates the cytosolic E3 ubiquitin ligase (Parkin) that builds an ubiquitin chain on the mitochondrial membrane, labelling damaged mitochondria for autophagy. Additionally, several receptors (e.g. p62, OPTN, NBR1, TAX1BP1), which contain a region for recognising the autophagy adapter (LC3II), recruit the labelled mitochondria and mediate the engulfment of mitochondria by the autophagy vacuole (Lazarou et al. 2015; Bingol and Sheng 2016). Mitochondrial integrity is also controlled by the members of Bcl-2 protein family, which consists of both anti-apoptotic (Bcl-2, Bcl-xL) and pro-apoptotic (Bax/Bak) proteins. These proteins are involved in the complex crosstalk between autophagy and apoptosis (Hardwick and Soane 2013). The critical link in this crosstalk is the anti-apoptotic proteins that undergo interactions with both the autophagy protein beclin-1 and the pro-apoptotic proteins, Bax/Bak (Pattingre et al. 2005). Beclin-1 is a component of the multi-protein complex (PI3K) acting as an essential activator of autophagy (He and Klionsky 2009). Under normal conditions, beclin-1 interacts physically with a member of anti-apoptotic proteins (e.g. Bcl-2) inhibiting autophagy. However, cellular stress disrupts the interaction, which promotes autophagy. Moreover, if the stress is extensive, it also disrupts the interaction between Bcl-2 and the pro-apoptotic proteins, Bax/Bak accelerating apoptosis (Pattingre et al. 2005). Furthermore, during excessive and prolonged stress, beclin-1 is cleaved by the caspase-mediated reaction and the truncated beclin-1 becomes translocated to the mitochondria where it accelerates apoptosis (Wirawan et al. 2010). Thus, beclin-1 can function both in autophagy and apoptosis, depending on its presence either in its full-length (promotes autophagy) or shortened (promotes apoptosis) form. In addition to beclin-1, two other autophagy proteins, Atg5 and ambra-1, are cleaved by calpain or caspases in stressed cells and this shifts autophagy towards apoptosis (Yousefi et al. 2006; Gorazzari et al. 2012).

3.2 Autophagy and ER-stress

Mitochondrial dysfunction and oxidative stress are closely linked to ER-stress that also accelerates ROS generation and can impair redox homeostasis in the cell. Many of the chemicals listed in table 2 (e.g. paraquat, chloropicrin, PFOA, cadmium, tobacco smoke) have

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been shown to cause ER-stress along with the activation of autophagy. ER-stress is triggered either when protein folding is incomplete, or the capacity of ER to fold proteins is reduced. This leads to the unfolded protein response (UPR) and Ca^{2+}-signalling, which try to prevent the accumulation of unfolded or misfolded proteins by reducing global protein synthesis and inducing the production of chaperones. The level of unfolded proteins is sensed by three signalling pathways (PERK-elF2α, IRE1, ATF6) and signalling via PERK-elF2α and IRE1-JNK has been shown to activate the core autophagy genes (e.g. Atgs, Beclin-1, p62) (Høyer-Hansen and Jäättelä 2007). Paraquat (PQ) is a widely used non-selective herbicide, which has been shown to induce early ER-stress and subsequent activation of autophagy in SH-SY5Y cells (Niso-Santano et al. 2011). Autophagy was suggested to be protective against PQ-induced toxicity since an inhibition of autophagy caused an exacerbation of apoptosis (Niso-Santano et al. 2011). In contrast, another study in which the paraquat-induced cardiac contractile and mitochondrial injury was studied using transgenic mice and cardiomyocytes, suggested an opposite effect on autophagy activity. Inhibition of PQ-activated autophagy and AMP-activated kinase AMPK (by 3-MA or compound C) was protective against PQ-induced cardiomyocyte-dysfunction (Wang et al. 2014). In low level of ER-stress, autophagy is generally protective in preventing the accumulation of misfolded proteins, while extensive or sustained stress often leads to the removal of the affected cell by apoptosis (Høyer-Hansen and Jäättelä 2007; Smith and Wilkinson 2017).

3.3 Autophagy and lysosomal damage

An excessive number of autophagosomes can result from a decrease or deficiency in lysosomal capacity and not necessarily from an increase in the biogenesis of autophagosomes. This is generally indicated by the cytoplasmic increase in the levels of the p62 protein (Komatzu et al. 2012). Lysosomes contain over 50 acid hydrolases that degrade and recycle the macromolecules processed by autophagy and membrane trafficking. The lysosomal membrane and its ATP-dependent proton pump maintain the lumen in an acidic condition (pH 4.5-5.0), optimal for the activities of these enzymes (Aki et al. 2012). Defective lysosome capacity often results from a change in the lumen pH and/or alteration in the permeability of the lysosomal membrane. This can also disturb the fusion between the autophagosomes and lysosomes. Extensive changes in membrane permeability can cause cytosolic acidification and cellular necrosis (Kroemer and Jäätelä 2005; Martini-Stoica et al. 2016).

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Some pesticides, perfluorooctanoic acid, arsenic and cadmium are examples of types of chemicals, which impair lysosomal functions (Table 2). Rotenone is a widely used pesticide that inhibits the mitochondrial electron chain, increases ROS-production and induces early (at 6 h) autophagy in the neuroblastoma cells (SH-SY5Y) (Xiong et al. 2011). However, longer exposure to this pesticide leads to accumulation of autophagosomes and a disturbance of the lysosomal pH (Mader et al. 2012). Similarly, the organophosphate pesticide, malathion, evokes an accumulation of autophagosomes in SH-SY5Y-cells (Venkatesan et al. 2017). Malathion is neurotoxic through irreversible inhibition of acetylcholinesterase activity. The accumulation of autophagosomes has been shown to result from malathion-induced destabilization of lysosomal membranes and the subsequent impairment of autophagosome-lysosome fusion (Venkatesan et al. 2017). Lindane is an organochlorine insecticide that disturbs the maturation of the autophagosome into a functional autolysosome. The molecular mechanisms leading to disruption have been associated with the activation of the MAPK/ERK-pathway and inhibition of beclin-1 expression (Corcelle et al. 2006). Lindane also disrupts apoptosis by enhancing the expression of the anti-apoptotic proteins (Bcl-xL) as well as down-regulating the levels of pro-apoptotic Bax, preventing cytochrome c release, and inhibiting the activities of caspase-9 and caspase-3 (Corcelle et al. 2006; Zucchini-Bascal et al. 2008).

Perfluorooctanoic acid (PFOA) is a member of the family of perfluorinated alkyl compounds. These chemicals are ubiquitous in the environment, and PFOA has been detected in the serum of inhabitants of many industrialized countries. PFOA has a long half-life; in humans it has been estimated to be around 3-4 years and toxic responses can detected, particularly in the liver, pancreas and testicles (Lindstrom et al. 2011). PFOA has been demonstrated to disturb lipid metabolism, increase ROS-levels and cause ER-stress (Lau et al. 2007; Yan et al. 2015). It also causes the accumulation of autophagic vacuoles as well as disturbing autophagosome-lysosome fusion in both mouse liver *in vivo* and in a human hepatocyte culture model *in vitro* (Table 2). A proteomic analysis further revealed that PFOA-exposure alters the expression of 54 proteins related to autophagy, vesicular trafficking and membrane fusions in mouse liver (Yan et al. 2017).

4. Autophagy and exposures to arsenic or cadmium

Two metals, arsenic and cadmium are good representatives of toxic metals inducing and impairing autophagy (Table 2), which in the case of these environmental metals has been associated with the mechanisms of cell transformation (Qi et al. 2014; Son et al. 2014). They

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are known to be contaminants in soil, air and water in many parts of the world, posing a health risk for humans and wildlife (Prüss-Ustün et al. 2011). Arsenic (As^{3+} and As^{5+}) damages many proteins, particularly those involved in cellular energy pathways and it triggers the generation of ROS. This increase in ROS production and inhibition of the mTOR-system activate the autophagy pathway. The induction of autophagy by arsenic has been shown to be protective against apoptosis if the exposure is short-term. However, prolonged exposures to the environmentally relevant doses have been shown to lead to the impairment of autophagy process (Lau et al. 2013; Qi et al 2014). Cadmium exerts a variety of toxic molecular effects in living systems e.g. it increases DNA strand-breaks, elevates ER-stress, increases ROS-production, and disturbs calcium homeostasis. Various signalling pathways e.g. calcium-ERK and PERK-elF2α, have been reported to be involved in cadmium-activated autophagy (Messner et al. 2016; Lee et al. 2017). Exposure to cadmium has also been shown to reduce the expression of two autophagy-related proteins (ATG5, LC3) and to disturb autophagy-lysosome fusion after prolonged exposure (Messner et al. 2012; Messner et al. 2016).

Prolonged exposures to low levels of arsenic or cadmium lead to cell transformation in their target tissues. Although the mechanism of cell transformation is not fully understood, defective autophagy leading to the accumulation of genetic mutations and epigenetic modifications (Mathew et al. 2007; Jensen et al. 2008) may contribute to cell transformation. A long exposure to these metals leads to the accumulation of the p62 protein due to its decreased degradation via autophagy. Various stress conditions can induce phosphorylation of p62 (at serine 349) and this has been shown to activate the Keap1-Nrf2 pathway within the antioxidant system (Ichimura et al. 2013). In addition, p62/SQSTM1 is a target for Nrf2 (Jain et al. 2010) and thus, these alterations can produce an amplifying loop, which sustains activation of Nrf2 and p62. This further promotes the formation of the nuclear factor κβ and mTORC1 complex in nutrient-rich conditions and facilitates cell transformation (Komatsu et al. 2012; Lau et al. 2013; Son et al. 2014). The p62 protein has also been shown to accumulate in the premalignant liver diseases and in most hepatocellular carcinomas (Umemura et al. 2016). Furthermore, both a decline and an overexpression of the essential autophagic genes such as Atg7, Atg5 or beclin-1 have been reported in the development and occurrence of hepatocellular carcinoma (Liu et al. 2017a). Accumulation of p62 by exposure to arsenic also results in the expression of several adhesion proteins (Snail, E-cadherin, vimentin), which are essential for arsenic-induced cell transformation (Liu et al. 2017). The function of autophagy in cell transformation is, however, multifaceted since transformation also depends on other biological factors e.g. cell and tissue

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type, the activation of oncogenes and the inhibition of tumour suppressor genes (Amaravadi et al. 2016; Kimmelman and White 2017).

5. Autophagy in the exposure to airborne particulate matter

Airborne particulate matter (PM) is a complex mixture of small particles (diameter < 10 µm, PM_{10}, $PM_{2.5}$, $PM_{0.1}$) and liquid droplets that are suspended in the air; these particles can be inhaled and gain access to even deep parts of the lungs. PM is heterogenic in chemical composition, containing, for instance, black carbon, metals, organic aerosols, nitrate, sulphate, PAHs, and automobile exhaust (Kelly and Fussell 2015). PM has been associated with many health issues in humans, particularly with respiratory disorders (Brunekreef and Holgate 2002; Kelly and Fussell 2011).

A number of studies have reported induction of autophagy as well as impairment of airway functions after exposure to PM (Wessels et al. 2010; Deng et al. 2014; Chen et al. 2016; Xu et al. 2017). For example, Deng et al. (2014) showed that airborne fine particulate matter ($PM_{2.5}$) increased autophagy, elevated oxidative stress, and activated the tumour necrosis factor alpha (TNF-α)-pathway, as well as evoked cytotoxicity in human lung epithelial cells. On the other hand, treatment with an autophagy inhibitor, 3-methyladenine (3-MA) enhanced the $PM_{2.5}$-induced cytotoxicity and apoptosis suggesting a protective effect of autophagy. In contrast, treatment with the ROS-scavenger, N-acetylcysteine, nearly completely abolished the activity through TNF-α-pathway and reduced the number of autophagic and apoptotic cells. Interestingly, neutralization of the TNF-α-pathway with a specific anti-TNF-α-antibody not only abolished the activity of an apoptotic marker (caspase-8-enzyme) but also reduced the expression of an autophagy marker (Deng et al. 2014).

Recently, autophagy was shown to be essential for PM-activation of the NF-κβ pathway as well as being involved in airway inflammation and mucus hyper-production in mice and in human bronchial epithelial (HBE) cells (Chen et al. 2016). Blockade of autophagy reduced markedly the expression of inflammatory cytokines IL8 and IL6 as well as the mucus coding gene, MUC5AC, in HBE-cells *in vitro*. In addition, mice with knockdown of autophagy-related genes, Becn1 or Lc3b, showed reduced airway inflammation and mucus hyper-production in response to PM-exposure *in vivo* (Chen et al. 2016). Furthermore, the study by Chen and co-workers showed that the inhibition of lysosomal functions stimulated the NF-κβ-signaling but attenuated the AP-1 pathway. The study of Xu et al. (2017) also reported

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activation of autophagy and inflammation in human bronchial epithelial cells *in vitro* and in mice *in vivo* after exposure to PM. Exposure to autophagy inhibitors (Spautin-1 or 3-MA) reduced the PM-induced expression of inflammatory cytokines *in vitro* and decreased the neutrophil influx as well as reduced the *in vivo* expression of pro-inflammatory cytokines in the PM-treated mouse. In addition, the autophagy inhibitors reduced the PM-induced inflammation via suppression of the NF-κβ-pathway.

6. Manmade nanoparticles

Nanoparticles (NPs) are very small in size or at least one dimension is between 1-100 nm. Several studies report toxicity and activation of autophagy in response to exposure to many kinds of engineered nanoparticles in various cell types, much as encountered with the PM-exposures (Liu et al. 2011; Li et al. 2015; Mittal et al. 2017; Wang et al. 2017). Autophagy was associated with an acute lung injury in mice after administration of the cationic Starburst polyamidoamine (PAMAM) dendrimers (Li et al. 2009). PAMAMs were found to induce autophagy and cell death via the Akt-TSC2-mTOR pathway. The autophagy inhibitor, 3-MA reduced nanomaterial-evoked cell death and ameliorated acute lung injury in mice. Knocking down of TSC2 by using siRNA also significantly improved cell viability, suggesting that PAMAMs dysregulate the Akt-TSC2-mTOR signalling pathway (Li et al. 2009). PAMAMs were also toxic to human liver cells causing mitochondrial damage and apoptosis. An inhibition of autophagy rescued the viability of hepatocytes (Li et al. 2015). Similarly, single-walled carbon nanotubes, which have several biomedical applications, induced autophagy and this was associated with the damage to airway cells in the exposed mice (Liu et al. 2011). The autophagy inhibitor, 3-MA significantly inhibited the cell death and ameliorated the acute lung injury in these mice (Liu et al. 2011). In addition, nanoparticles often accumulate in lysosomes. For instance, exposure to graphite carbon nanofibers (GCNF) has been shown to trigger oxidative stress, autophagosome-lysosomal damage and disruption of the cytoskeleton in human lung cells (Mittal et al. 2017). The lysosomal dysfunction was the major cause for the accumulation of autophagosomes and the subsequent apoptosis. Inhibition of autophagy by 3-MA or treatment with the antioxidant, N-acetylcysteine reduced autophagy and cell death (Mittal et al. 2017). Silver nanoparticles (AgNPs) were shown to disturb autophagy-lysosome pathway, which was dependent on the size of the stimulating particles. The disturbance caused in the autophagy-lysosome pathway resulted in inflammation, oxidative stress and apoptosis in human hepatocytes (HepG2) (Mishra et al. 2016). The responses were particle size-dependent so that the smallest particles (10 nm) were associated with the most intense toxicity and the

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strongest activation of inflammation and death pathway (Mishra et al. 2016). Gold nanoparticles (AuNPs) were also shown to accumulate in lysosomes and impair the lysosomal degradation capacity and autophagy flux in normal rat kidney cells (Ma et al. 2011). Furthermore, the study by Ma and coworkers (2011) showed that the cellular uptake of AuNPs was size-dependent with larger particles (50 nm > 25 and 10 nm) being more readily internalized into cells (Ma et al. 2011). Autophagosome-lysosome dysfunction was also observed after exposure of HepaG2 cells to silica nanoparticles (SiNPs) (Wang et al. 2017). Silica NPs blocked autophagic flux by impairing lysosomal membrane permeability and inhibiting the expression of the lysosomal protease, cathepsin B (Wang et al. 2017).

According to the examples given above, autophagy is involved in the response to PM-or NP-exposures. However, the role of autophagy in the toxicity or cell damage linked with those exposures is complex; autophagy seems to be associated with both protective and deleterious cellular pathways. PM may represent mixtures of different chemicals with variable concentrations and some components of PM as well as many types of NPs may be non-digestible by lysosomal enzymes and thus they can accumulate within lysosomes. These kinds of persistent components can overload the autophagosome/lysosome system resulting in a cellular injury, even cell death. Other properties, such as the size and probably also the charge of the activating particle (see Mishra et al. 2016) may affect the efficiency of autophagy activation and cytotoxicity.

7. Cigarette smoke and autophagy

Cigarette smoke (CS) is a chemical mixture that contains a multitude of reactive toxic components such as nitrogen and oxygen radicals, aldehydes, nicotine, cadmium and other pro-oxidants. CS is known to induce autophagy as well as triggering a wide range of immune- and oxidation-related responses (e.g. ROS, DNA-damage, lipid peroxidation, overproduction of cytokines, inflammation, proteostasis-imbalance) that damage the epithelium lining the airways and alveoli (Nyunoga et al. 2013; Clooman et al. 2014; Tran et al. 2015). These detrimental responses have also been detected in murine airway cells after exposure to nicotine or to the vapour from electronic cigarettes (Bodas et al. 2016; Shivalingappa et al. 2016).

Activation of autophagy by CS is often linked with the immune response. For example, CS-induced autophagy flux was shown to lead to a loss of ciliated cells, excess mucus production and accelerated apoptosis in *in vivo* and *in vitro* models (Chen et al. 2010; Lam et al. 2013;

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Cloonan et al. 2014; Zhou et al. 2016). The autophagy deficient mice (Becn1+/-, Map1lc3b-/-) were also found to be resistant to CS-induced cilial damage. The impaired mucociliary clearance of microbes and irritants by CS-induced autophagy may consequently increase the susceptibility of airways to infections and inflammation (Cloonan et al. 2014).

On the other hand, the disruption of autophagy by CS or the insufficient clearance of CS-damaged proteins has been reported to enhance ROS production and the accumulation of insoluble, ubiquitinated proteins (aggresomes) as well as elevaiting the p62 protein in the airway cells leading to cell damage and inflammation (Fujii et al. 2012; Tran et al. 2015; Vij et al. 2016). The multifunctional p62 protein controls the formation of the aggregates that are normally removed by autophagy (Zatloukal et al. 2002; Komatsu et al. 2012). The CS-induced impairment of autophagy and the subsequent aggresome formation have been suggested to initiate inflammatory signalling and age-related lung functions, leaving the lungs susceptible to infecting microbes and cell death (Vij et al. 2016). Recently, CS was also reported to cause an accumulation of another autophagy-related protein, the transcription factor EB (TFEB) in mouse lung *in vivo* and human bronchial epithelial cells *in vitro*. Moreover, the pharmacological TFEB-inducer, gemfibrozil was able to restore CS-impaired autophagy as well as to reducing inflammatory-oxidative stress and decreasing the formation of aggresomes. The accumulation of this transcription factor among aggresomes has also been detected in the lungs of tobacco smokers suffering from COPD (Bodas et al. 2017). TFEB regulates the genes, which control lysosome biogenesis, autophagosome-formation and autophagosome lysosome fusion (Martini-Stoica et al. 2016).

Recently, autophagy has been associated, not only with apoptosis but also with necroptosis or regulated necrosis in CS-evoked cell death in airway epithelial cells. Mizumura et al. (2014) reported that CS increased the extent of mitophagy, which caused to a stabilization of the mitophagy regulator PINK1 and induction of necroptosis in pulmonary epithelial cells. The CS-induced cell death was reduced by treatment with necroptosis/necrosis inhibitors (necrox-5, necrostatin-1). A genetic deficiency of PINK1 or alternatively treatment with the mitophagy inhibitor, Mdivi-1 protected cells against CS-induced cell death. These results suggest that mitophagy (autophagy) signalling regulates CS-induced necroptosis in pulmonary epithelial cells. Furthermore, the recent study of Wang et al. (2018) showed that the upstream regulator of autophagy, mTOR, is involved in CS-induced inflammation, autophagy, apoptosis and necroptosis in airway epithelial cells. CS-exposure caused significant decrease in the

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expression of mTOR through tuberous sclerosis 2 (TSC2). This led to induction of autophagy, apoptosis and necroptosis, which all cooperatively regulated the expression of CS-induced inflammatory cytokines (IL-6, IL-8) through NF-κβ-pathway in airway epithelial cells. In addition, an endoplasmic reticulum chaperone, the glucose-regulated protein, GRP7, was recently reported to mediate CS-induced necroptosis and cellular damage in bronchial epithelium. That study also demonstrated that GRP7 together with necroptosis is critical for up-regulating the CS-caused inflammation and mucus production via the activation of NF-κβ (Wang et al. 2018). The above studies emphasize that the function of autophagy in the CS-evoked airway epithelial damage is complex and autophagy signalling is closely connected with immune response and programmed cell death pathways.

8. Conclusion

There are several lines of evidence indicating that autophagy plays an important role in the cellular responses to environmental chemicals. In many cases, the induction of autophagy by environmental toxicants is protective and increases cell survival. However, aberrant and excessive activation of autophagy or defective autophagy functions may also be involved in cellular/tissue injury and lead to pathological changes. At present, we have insufficient knowledge about the role of autophagy in exposures to environmental toxicants. Only a small proportion of chemicals have been studied in this respect. This is particularly important for lipophilic and slowly metabolised environmental chemicals, which can remain stored in the body for a prolonged period of time. There are still many links between autophagy and immune responses and other cellular functions evoked by chemical stress, which remain to be elucidated. More basic research is needed to identify the molecular mechanisms involved in the autophagy pathway after exposures to environmental chemicals. Such research will improve understanding of the role of autophagy in chemically caused toxicity and tissue pathogenesis.

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Conflict of interest

The authors declare that there are no conflicts of interest

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Legend for the figure

Fig. 1. Schematic and simplified presentation of the macroautophagy process. Damage to cellular components e.g. in the form of protein aggregates, mitochondrial damage or chemical stress recruits initiating complexes (ULK and PI3K) to form a small cisterna, called the phagophore. During elongation, two ubiquitin-like conjugating enzymes form complexes, and the phagophore becomes expanded surrounding the damaged organelles and aggregated cell material. At the end of the elongation, the membrane is sealed to form a double-membrane vesicle called the autophagosome that contains degraded cellular material. The autophagosome moves along microtubules and then fuses with a lysosome forming an autolysosome in which the lysosomal enzymes degrade the cargo and release the degradation products into the cytoplasm. LC3II is the adapter protein that is located in the membrane of phagophore and facilitates the uptake of the ubiquitinated cargo into the phacophore. The p62 is the receptor protein that binds to the ubiquitinated cargo and mediates its degradation.

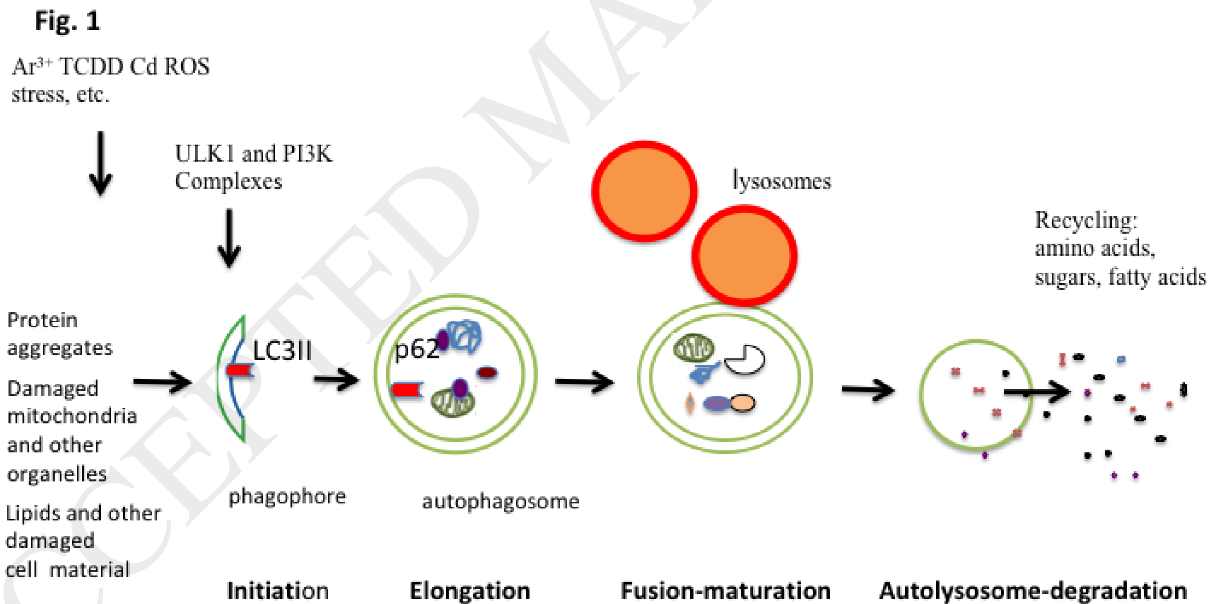

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Table 1. **Main proteins involved in the distinct stages of autophagy pathway**

(Mizushima et al. 2011; Nakamura and Yoshimori 2017

Initiation	ULK-complex: PI3K-complex: Others:	ULK1/2, FIP200, Atg13, Atg 101 VSP15, VSP34, Beclin-1, Atg14, AMBRA1 Atg2A/B, Atg9L1/2WIPI1/2/3/4, WIMP1, DFCO1
Elongation	Atg12-conjugation: LC3-conjugation:	Atg5, Atg7, Atg10, Atg12, Atg16L1 LC3A/B/C, Atg3, Atg4, Atg7, GABARAP, GABARAPL1/2/3
Fusion/Maturation		SNAREs, Syntaxin17, Atg14, Lamp-1, Dram1, VPS33A, VPS16, Atg4, Rab7, LC3, GABARAPs

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Table 2. Examples of toxic chemicals that affect autophagy

Chemical	Cell organelles/ function affected	Model	Change in autophagy	References
Arsenic	mitochondria/lysosomes, ROS	BEAS-2B L-02	increase and impairment	Lau et al. 2013; Liu et al. 2017
2,2',4,4',5,5'hexab romodiphenyl ether (BDE153)	mitochondria	HepaG2	increase	Pereira et al. 2017
Benzo(a)pyrene	mitochondria, ROS	HL-7702	increase	Yuan et al. 2017
Bisphenol A	mitochondria,ROS	rat testicle cells, human fetal lung fibroblasts	increase	Quan et al. 2016; Mahemuti et al. 2017
Cadmium	mitochondria/lysosomes/ ER-stress, ROS	renal tubular cells, endothelial cells	increase and impairment	Messner et al. 2016; Wang et al. 2016; Lee et al. 2017
Chloropicrin	ER-stress, ROS	ARPE	increase	Pesonen et al. 2012
Chlorpyrifos	mitochondria	SH-SY5Y	increase	Park et al 2013a
Dibenzofuran	mitochondria	A549	increase	Duarte et al. 2012
Endosulfan + TCDD	mitochondria, ER-stress, ROS	Caco-2	increase	Rainey et al. 2017
Fipronil	mitochondria, ROS	SH-SY5Y	increase	Park et al. 2013b
Glyphosate	mitochondria	PC12	increase	Gui et al. 2012
Lindane	mitochondria, disrupt autolysosomes, ROS	mouse sertoli cells, rat primary hepatocytes	increase and impairment	Corcelle et al. 2006; Zucchini-Pascal et al. 2009
Malathion	mitochondria/lysosomes, ROS	Na2	increase and impairment	Venkatesan et al. 2017
Nickel	mitochondria, ROS	BEAS-2B	increase	Huang et al. 2016
4-Nonylphenol	mitochondria, ROS	Sertoli cells	increase	Duan et al. 2016
Paraquat	mitochondria, ER-stress, ROS	SH-SY5Y	increase	Gonzalez-Polo et al. 2007;Niso-Santano et al. 2011
Perfluorooctanoid acid	lysosome dysfunction, ER-stress, ROS	mouse liver *in vivo*, HepG2-cells	increase and impairment	Yan et al. 2015, Yan et al. 2017
Rotenone	mitochondria/lysosome destabilization, ROS	SH-SY5Y	increase and impairment	Xiong et al. 2011; Mader et al. 2012
TCDD	mitochondria, ROS	bovine kidney cells, SH-SY5Y-cells	increase	Fiorito et al. 2011; Zhao et al. 2015
Tributyltin	mitochondria, ATP-syntetase	rat primary neuronal cells	increase	Nakatsu et al. 2010
Tobacco smoke	mitochondria, ER-stress, proteolysis, ROS	mice lung *in vivo* and human bronchial epithelial cells	increase and impairment	Bodas et al. 2016; Bodas et al. 2017

BEAS-2B, Human bronchial epithelial cells; L-02, human liver epithelial cells; HepaG2,human liver cells; HL-7702, human liver cells, ARPE, human retinal pigment epithelial cells, SH-SY5Y, human neuroblastoma cells, A549, human lung cancer cells, CaCo-2, human colon cancer cells, PC12, rat adrenal gland cells; Na2, mouse neuroblastoma cells