



AUTOPHAGY: A JANUS-FACED ROLE IN INFLAMMATION AND CANCER

Mohannad A. Elkhider and Bob Chaudhuri

De Montfort University, The Gateway, Leicester, LE1 9BH United Kingdom

ABSTRACT

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Correspondence:

Mohannad A. Elkhider, De Montfort University, the Gateway, Leicester, LE1 9BH United Kingdom

Email: hanoodelkhider@gmail.com

Plan: This review focus on the role of autophagy, in up regulation, in the innate and adaptive immune response, in controlling carcinogenesis, and in supporting neuronal cell growth, development & remodeling. Also the review covers the therapeutic interventions involved in the cancer management through autophagy modulations.

Preface: Autophagy is a cellular degradative pathway where unwanted and weary cytosolic components are recycled. Any defects interfering with the integrity of the autophagic machinery would compromises the cells defenses leaving the cell susceptible to infection by circulating pathogens. Current literature points out that the dysregulation of autophagy may be associated with the genesis of cancer. Accumulation of aberrant organelles and proteins increases the chances of triggering an inflammatory microenvironment favoring chromosomal instability and mutagenesis. The aggregation of certain proteins yields cellular toxicity which eventually leads to cell death and neurodegeneration. Therefore, the autophagic duty of continuously monitoring and clearing out aggregated proteins is indispensable in neuronal cells.

Outcome: The accumulation of autophagosomes is an established assurance in a number of neurodegenerative diseases. However, this observation has triggered controversy whereas one opinion considers the activated autophagic pathway to act as an executioner by initiating neuronal cell death while the other explains the presence of autophagosomes as a final attempt by the cell to sustain viability against the increasing amount of stress.

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1. INTRODUCTION

Over 50 years ago autophagy was discovered as a metabolic process of generating energy when the cell is under starvation conditions. To date, testing on yeast cells with the modern genetic investigational tools made available revealed the implication of autophagy in numerous cellular activities that are vital for maintaining viability.



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Principally, unwanted cellular components are delivered to the lysosome and are recycled to basic building blocks for the synthesis of essential proteins. Blockade of this pathway compromises the cells integrity leaving it prone to stressors that ultimately trigger cell death. Current knowledge focuses on the process selecting and delivering cellular components to the lysosome; however, only little is known with regard to the molecular pathway of how the delivered cargo is recycled in the lysosome. Eukaryotic cells depend on two degradative pathways.

The first is the ubiquitin-proteasomal system which degrades short-lived proteins, and the other is autophagy which is the major catabolic recycling pathway targeting long-lived proteins. The role of autophagy is not only as an emergency generator of energy, but it also participates in cellular housekeeping duties via eliminating redundant, weary and unwanted cellular components. Also autophagy contributes to cell remodeling during development stages, and takes part in mediating immunological response against invading pathogens (Levine and Klionsky 2004).

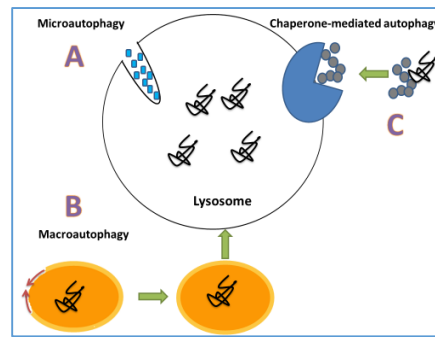
In this review we shed the light on a lysosomal degradative process termed autophagy. The etymology of this term is derived from Greek roots where “auto” means self, and “phagy” means to eat. The course of this metabolic pathway consists of delivering intracellular organelles and proteins towards the lysosome for degradation. Investigating the process of autophagy was mainly by electron microscopy, and was shown to be initiated as a general response during periods of nutritional shortage. When the cell is faced with nutrient starvation, it responds by degrading non-essential cytoplasmic organelles and proteins in bulks. The generated building blocks produced via the breakdown of these cellular components is utilized as a source of energy to support vital cellular processes during periods of limited nutritional supplies (Kopitz et al 1990). Only recently investigators have been able to precisely pinpoint the molecular elements which run the autophagic machinery, and correlate the occurrence of defects in these regulatory factors to the development of different states of disease (Mijaljica 2010).

This review outlines the network of molecular pathways involved in autophagy, how autophagy orchestrates immunity and inflammatory response, its dubious involvement in the genesis of cancer, and finally how the dysregulation of this pathway contributes to neurodegenerative diseases.

2. DIFFERENT MODES OF AUTOPHAGY

The entire autophagy pathway incorporates three different modes of substrate elimination which are: (1) Chaperone mediated autophagy (CMA), (2) macroautophagy, and (3) microautophagy (Figure1). CMA is the most unique form of autophagy out of the three modes; CMA is only observed in mammals and takes part in degrading proteins that exist solely in a soluble form (Cuervo 2011). In contrast, both micro- and macroautophagy are observed in a wider range of eukaryotic species including plants, fungi, and mammals. These two processes are involved in the degradation of proteinaceous chunks existing in the cytoplasm, as well as cellular organelles (Tania I 2011).

The degradation of mitochondria via autophagy in *Saccharomyces cerevisiae* illustrates how the media constituents influence the process under conditions of nitrogen starvation. When the cells are grown in a medium rich in lactate, microautophagy targets the mitochondria for degradation with very high selectivity. In contrast, when cells are grown in a glucose rich medium organelles are non-selectively degraded via macroautophagy along with other cytosolic components (Kissová et al 2007).



Different modes of autophagy

- A. *Microautophagy mediates direct internalisation of organelles and cytosolic material by the lysosome.*
- B. *Macroautophagy is hired for degrading larger components since it's the most efficient amongst the three in bulk degradation. A double membrane vesicle, the autophagosome, engulfs the targeted protein/organelle aimed for degradation, once fully sequestered the autophagosome along with the substrate is delivered to the lysosome where the cargo is released.*
- C. *Chaperone-mediated autophagy is unique for being the most selective form of degradation. Targeted substrates existing solely in the cytosol bind to autophagic chaperone via a special recognition motif. The chaperone/substrate complex then travels towards the lysosome and binds with lysosomal receptor in a manner independent of any membrane sequestration steps.*

2.1. The molecular network behind macroautophagy

The first stage of the macro autophagy pathway consists of engulfing targeted cellular components by a vesicular body made up of a double membrane. These autophagic vesicles are known as autophagosomes and the targeted cellular components are either misfolded proteins, or redundant organelles (Wong et al 2011). Afterwards, the autophagosome sequestering cargo intended for degradation is delivered through the cytosol towards the lysosome. Finally, the autophagosome docks at the lysosomal membrane forming what is known as the autolysosome. The vesicular structures are released into the lysosomal lumen and are characterised by a unique rapid Brownian motion. The residing degradative hydrolases breakdown the delivered cargo generating raw building blocks that are delivered from the lysosome to the site of highest demand (Ohsumi 1999).

2.1.1. Mammalian target of rapamycin signaling: Autophagy induction

The mammalian target of rapamycin (mTOR) is a Ser / Thr kinase participating in cellular signaling which is hired during starvation; it takes part in the progression of the cell cycle, amino acid synthesis, nutrient importation, and cell proliferation. This kinase acts a regulator of all downstream genes that are vital in the initiation of the whole autophagic pathway.

These genes are grouped under the name of Autophagy-related genes (atg); so far approximately 17 of them have been discovered in yeast with orthologues in higher eukaryotic species (Levine and Klionsky 2004). mTOR constantly hyperphosphorylates downstream atg13. Presumably when the cell's energy demands are met with sufficient supplies of nutrients, the hyperphosphorylated atg13 is incapable of associating with atg1. The atg13-atg1 complex formation is a vital step in autophagy induction; without this complex the synthesis and maturation of autophagosomes, the vesicular bodies which engulf delivered cargo, is inconceivable.

Therefore, the inhibition of mTOR during nutrients' shortage will allow atg13 to remain in a hypophosphorylated state which facilitates its binding with atg1 and consequently allows autophagosome formation in macroautophagy (Lum et al 2005).

2.1.2. Examples of selective macroautophagy

2.1.3. Macropexophagy

The Peroxisomes, also known as micro-bodies, is a degradative organelle which is mainly involved in the breakdown of different types of fatty acids (Wanders and Waterham 2006). Although they are the most recent sub-cellular organelles discovered in the eukaryotic cell, yet they are the first to be identified as targets of the selective macroautophagic pathway. Macroautophagic degradation of peroxisomes has been mainly investigated in yeast species, namely *Candida boidinii*, *Hansenula polymorpha*, and *Pichia pastoris*.

Initially the cells are grown in a medium containing methanol. Peroxisomes developing in this environment adopt key enzymes responsible for the metabolic processing of methanol. The same cells are then transferred to a glucose-rich medium making the enzymatic duty of these peroxisomal enzymes redundant. In this scenario, autophagy is not activated upon exposure to nutrient starvation, but occurs in response to a shift in surrounding metabolic conditions serving as an intracellular remodeling pathway for dismissing organelles which catalyse metabolic pathways that are made redundant (Veenhuis et al 1983).

Successful degradation of peroxisomes via macropexophagy depends on two elements, the *peroxisomal membrane protein 3* (Pex3) and the *peroxisomal membrane protein 14* (Pex14). Pex14 is responsible for importing the peroxisomal proteins that makes up the organelles' matrix, while Pex3 takes part in the formation of the peroxisomal membranes. These two proteins are believed to take part in the initial steps of intracellular signaling and the recognition of the organelle by the autophagic machinery (Bellu et al 2001; Bellu et al 2002).

2.2. The molecular network behind microautophagy

Microautophagy is characterised by direct internalisation of components destined for degradation by the lysosomal membrane independent of autophagosomal sequestration and transportation of cargo.

When the target is relatively small the lysosomal membrane creates a tubular invagination surrounding the marked targets and then pinches off in the form of small vesicles within the lysosome. On the other hand, when larger particles are targeted for degradation, finger like protrusions act by sequestering targeted cargo and delivers it into the lysosomal lumen (Todde *et al* 2009).

Microautophagy participates in the turnover of a variety of cellular components; one of the targets of microautophagy is the lysosomal membrane itself which is degraded as a means of organelle size reduction. The main trigger of microautophagy is nitrogen starvation which annihilates the inhibitory effects on autophagy by mTOR in a manner similar to the one described in macroautophagy. However, the activated downstream regulatory proteins responsible for activating pathway function behave in a different manner since microautophagy does not depend on autophagosome formation (Lum *et al* 2005).

2.2.1. EGO complex: Coregulator in microautophagy

Besides the mTOR signaling pathway, recent studies identified a secondary regulatory complex involved in the regulation of the pathway known as the EGO complex which is made of three proteins, the GTPase Grr2, and two members of the RNA-dependent RNA polymerase (RdRP) family Ego1 and Ego3 (Dubouloz *et al* 2005). The EGO complex has two roles in microautophagy regulation. The first is to assist cells through exiting the stationary phase when the inhibitory effect of mTOR is terminated. The second role is to mediate the sorting of permeases which are transport proteins integrated in the membrane that allow the passage of recycled autophagy products from the lysosomal lumen to the cytosol (Uttenweiler *et al* 2007). When compared to macroautophagy, findings with regard to molecular elements taking part in this pathway still remain in its infancy. Dedicated clinical efforts are encouraged in order to precisely outline the different steps that take part in this degradative pathway.

2.2.2. Piecemeal nuclear autophagy

The nucleus, being an essential cellular component, was previously thought to be exempted from autophagic targeting. Recently, investigations by Roberts *et al* (2003) revealed that the nucleus is also listed with other organelles subject to degradation via autophagy. Only minor segments of the nucleus are targeted for degradation, hence the name piecemeal nuclear autophagy (PMN) is given.

The portions of the nucleus marked for degradation are engulfed by the lysosome in a pattern similar to that of microautophagy; however, the general microautophagic machinery is not required in this pathway since mutations of microautophagic elements does not interfere with this pathway (Roberts *et al* 2003).

2.3. The molecular network behind chaperone mediated autophagy

Chaperone mediated autophagy (CMA) is a selective degradative process responsible for eliminating 30% of total cytosolic proteins in a molecule-by-molecule type of delivery during extended periods of starvation.

The delivery of CMA substrates to the lysosome is not driven by vesicular sequestration of cargo, but through protein translocation from the cytosol into the lysosomal lumen via a proteinaceous pore (Bejarano and Cuervo 2010). Substrates targeted by CMA contain a unique motif sequence consisting of the amino acids KFERQ. These motifs are possibly generated via posttranslational modification, such as deamidation of the targeted cytosolic proteins (Gracy et al 1998). The protein-chaperone complex travels towards the lysosome where it interacts with the cytosolic domain of the lysosomal-associated membrane protein 2 (LAMP-2A). The structure of LAMP-2A is comprised of a transmembrane domain integrated within the lysosomal membrane, a small cytosolic tail, and a sizable highly glycosylated region which lies within the lumen of the lysosomes.

3. ROLE OF AUTOPHAGY IN FINE-TUNING IMMUNITY AND INFLAMMATION

The role of autophagy is not limited to energy homeostasis through catabolic breakdown of organelles and proteins in order to replenish energy. It is also involved in regulating molecular functions that are essential for mediating both innate and adaptive immunological responses.

While macroautophagy is known to take part in both the innate and adaptive immune responses, CMA is only associated with the latter form of immunity (Tanida 2011; Zhou et al 2005). The molecular machinery utilized for targeting pathogens is much similar to one seen in the previously discussed macroautophagic pathway.

3.1. Autophagy in innate and adaptive immunity

3.1.2 Targeting *Listeria monocytogenes*

One good example for the utilization of macroautophagy is cellular targeting of the virulent pathogenic bacteria *Listeria monocytogenes* which is the causative of listeriosis, a rare form of infection sourcing from different contaminated food products. Immune-compromised individuals are most susceptible to listeriosis. Intracellular pathogenesis of listeriosis. The bacteria hijack's the cells' endocytic pathway in a Trojan horse manner as means to gain entrance to the cytosol. Once internalised, the bacteria exploit the cells' phagosomes as incubators until complete maturation is reached. The mature pathogen then releases listeriolysin, a non-enzymatic cytolytic pore-forming protein, and phospholipase B which aids in puncturing the phagosomes. The formed pores facilitate the pathogen's escape into the cytosol just before the sequestering phagosome fuses to the lysosome. Hence, it escapes from lysosomal degradation and spreads to neighboring cells (Geoffroy et al 1987).

As *L. monocytogenes* fully mature, it attempts to escape from the phagosome where it is sequestered and finds its way to the cytosol. During the process of escaping the maturation of phagosome, it releases a cholesterol-dependent pore-forming cytolysin known as *listeriolysin* which acidifies the harboring phagosome and destroys it (Meyer-Morse et al 2010).

Once the pathogen escapes, it is recognised by the *peptidoglycan recognition proteins* (PRPs) which further transduce signals triggering autophagosome formation sequestering the pathogen (Yano and Kurata 2008). Macroautophagic autophagosomes are then hired to form a double membrane surrounding the pathogen. The pathogen is then delivered to the lysosome and degraded through the classical autophagic pathway as shown in Figure 2.

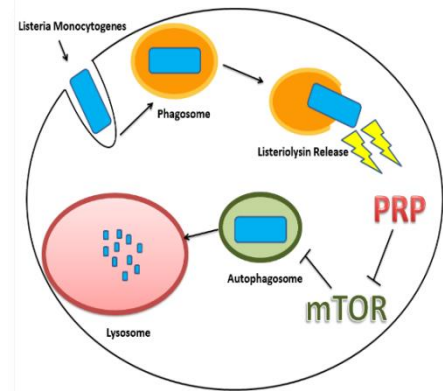


Fig.2

A secondary involvement of basal autophagy is observed in cells that depend on autophagy as means of clearing out *Mycobacterium*, *Streptococcus*, *Shigella*, *Legionella*, and *Salmonella* infections. Ubiquitin-conjugated proteins (E2 enzymes) accumulate on a regular basis within the lysosome as an end product from the degradation of ubiquitinated proteins. Basal levels of autophagy results in accumulation of ubiquitin conjugates in autophagy efficient cells. These ubiquitin bodies have proven to possess a noteworthy bactericidal activity working synergistically with the lipases and proteases that already reside within the soluble fraction of the lysosome enhancing its degradative efficacy (Alonso et al 2007).

Autophagy also contributes to the innate immune response in eradicating viral invading pathogens. The autophagic machinery is triggered when viral pathogens are sensed in the cytosol. Endosomes containing TLR7 (Toll-like receptor 7) fuse with autophagosomes sequestering viral pathogens. This fusion step produces a vesicular body known as the amphisome where pathogens come in contact with TLR7 and activate them. The activated TLR7 signals the synthesis of the inflammatory cytokine Type I IFN (type 1 Interferons) which is accountable for translating certain genes responsible for arresting the replication of viral pathogens by initiating multiple defensive measures grouped under the term “antiviral state”

The current understanding of the role of autophagy in the area of adaptive immunity is still a new-born and demands a great deal of investigation in order to fully comprehend its importance. Current reports indicate that autophagy takes part in presenting antigens to T cells that continuously monitor the presence of foreign invaders while patrolling different organ systems. The *major histocompatibility complex* (MHC) class II antigen presentation displays exogenous proteins that are taken up by antigen presenting dendritic, macrophage, and B cells (Bryant and Ploegh 2004). The internalised antigens are processed and are loaded up on the extracellular portion of the MHC peptide forming a complex which then migrates towards the membrane. These antigens are presented to T helper cells responsible for orchestrating immunological responses by hiring various specialised pathogen combating cells. T helper cells do not directly engage in eradicating pathogens; however, they mediate the maturation *cytotoxic T cells* (CTL) which are capable of terminating infected cells (Chicz et al 1993).

Autophagy plays a governing role in harmonizing the inflammatory signaling pathways. Disruption of this balance can cause uncertain effects either through limited or over activation of the immune response.

Usually inadequate immune response resulting from poor autophagy would interfere with the cells' ability to overcome invading viral, bacterial, and protozoan pathogens. Similarly, exaggerated immunological response due to poorly regulated autophagy would serve as grounds for unwanted chronic inflammation. Besides, autophagy takes part in the adaptive immune response through modulating the inflammatory roles of IFN- γ (Chemali et al 2011).

Decline in basal autophagy activity is coupled with increased levels of freely available cytokine, IL-1 β (Interleukin Beta). This cytokine is responsible for cyclooxygenase-2 (COX-2) synthesis, as well as modulating cellular growth functions comprising proliferation and differentiation.

The overall outcome yields acceleration in differentiation, proliferation, and intensified sensitivity to pain during inflammatory periods. Tests on peripheral blood mononuclear cells (PBMCs) treated with 3-methyl adenine (3MA), a pharmacological inhibitor of autophagy, showed a relative increase in the levels of circulating IL-1 β (Crişan et al 2011). Also functional autophagy efficiently and selectively degrades p62 (nucleoporin p62) in healthy cells. Studies identified p62 accumulation, a supporting adaptor protein, to serve as the basis for pathogenesis and disease progression. (Nezis et al 2008, Komatsu et al 2007).

3.3. Nucleoporin p 62 in NF- κ B transcription pathway

Nucleoporin p 62 or p 62 has got an additional importance in initiating the transcription through nuclear factor kappa- B cells (NF- κ B) pathway. As tumor necrosis factor (TNF) binds to its receptor (TNFR), a complex downstream of the TNFR and upstream of NF- κ B is formed. This complex is comprised of TNF receptor-associated factor 2 (TRAF2), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), RIP, and a typical *protein kinase C* (aPKC) (Chen and Goeddel 2002).

NF- κ B is liberated during transcription from its inhibitor known as nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor (I κ B α) (Verma et al 1995) and the free NF- κ B translocates into the nucleus and transcribes pro inflammatory cytokines and chemokines responsible for creating an inflammatory microenvironment. The nucleoporin p62 is responsible for gluing TRAF2, TRADD, and aPKC, forming a conglomerate specifically by acting as a linker between RIP and aPKC (Sanz et al 1999). As a result, deregulation of autophagy inhibits the degradative pathway of p62 causing it to accumulate. The high levels of accumulated p62 would then increase the chances of triggering an inflammatory response against exposure to minimal levels of TNF.

4. AUTOPHAGY AND APOPTOSIS

Malfunction in autophagy could indirectly act as a causative factor to chronic inflammation and disease. Autophagy may act as an adjusting rheostat which constantly monitors and takes part in cellular activities. This can be seen when cells undergo the programmed cell death (PCD) known as apoptosis. During apoptosis cells express certain markers known as the 'eat me' and 'come and get me' on their surface (Li 2011).

These signals facilitate the chemotaxis and engulfment marked cells by phagocytes (Lauber et al 2004). In order to synthesise and export these markers, energy required is generated from recycling redundant organelles and proteins via autophagy. Thus, defective autophagy would interfere with the expression of these chemotaxis markers. As a result, the ability of phagocytic cells to recognise cellular corpse is drastically diminished causing their accumulation (Qu et al 2007). Cells lining the intestinal mucosa are the ones most likely to accumulate as these cells constantly grow, proliferate and regenerate in a higher rate when compared to other types of specialised cells. The accumulation of cellular corpse indicates that apoptosis is no longer efficient in cell clearance; this would cause a shift from apoptosis to another type of cell death known as the necrotic cell death. The main concern with regard to necrotic cell death is the secondary necrosis witnessed at the end stage of the process. This creates an inflammatory microenvironment suitable for the cancerogenesis via the *damage associated molecular pattern molecule* (DAMP) released by dying cells (Levine et al 2011).

An important housekeeping duty of autophagy in preventing inflammation is the excretion of α -1-antitrypsin (AT) from hepatic cells. AT is a serine protease inhibitor which protects pulmonary connective tissue matrix from degradation by neutrophil protease. Defective autophagy stands against the secretion of AT from hepatic cells to the bloodstream. Consequently, individuals with mutations in autophagy are prone to chronic inflammation and emphysema caused by damage to lungs by circulating neutrophils (Perlmutter 2009). Likewise, failure to secrete AT yields toxic effects on the hepatic cells themselves. Malfunctioning mitochondrial autophagy leads to AT retention within the endoplasmic reticulum and mitochondrial injury.

4.1. Role of healthy autophagy in the prevention of cancer

Current advances in cancer research concerned with an imbalance of the metabolic involvement in tumourigenesis and progression. Metabolic imbalance caused by environmental factors, exposure to certain agents, diet, and industrialised life style could participate in unravelling of disease in individuals who are already at high risk of cancer development (Burton et al 2010, Payne et al 2009). Many molecular factors responsible for orchestrating the body's metabolic processes are susceptible to alteration and disease progression, more interest is drawn to autophagy and its role in cancer.

Importance of Beclin -1 in auto phagosome initiation

The foremost relationship established between autophagy and cancer was via Beclin-1 (Atg 6 protein). This protein takes part in autophagosome initiation which is the first step in the autophagic degradative pathway. However, prior recognition of its importance in the initiation of autophagosomes, Beclin-1 was identified as a tumor suppressor protein which interacts with the oncoprotein, known as the *B-cell lymphoma 2* (Bcl-2) (Liang et al 1998).

The binding of Beclin-1 inhibits Bcl-2's role of mitigating the pores formed by the pro-apoptotic protein *Bcl-2-associated X protein (Bax)* on the mitochondrial membrane. As a result, **Bax** is left to freely bore holes throughout the mitochondrial membrane allowing mitochondrial components to ooze out which is one pathway that triggers apoptosis (Oltvai et al 1993)

4.2. Reactive oxygen species (ROS) and the induction of cancer

Autophagy manages metabolic stress by acting as a buffer reducing the intensity of damages during stressful periods. Certain pathways are triggered as means of counteracting causative factors behind the state of metabolic stress that the cell goes through. A principal mechanism involves selective autophagy of the energy generating semi-independent organelle known as the mitochondria (Vanhorebeek et al 2011).The most important and efficient mode of the mitochondrial ATP generation pathways is the oxidative phosphorylation cycle.

At the end of the cycle, ROS (*Reactive oxygen species*)are generated as by-products of this pathway. In healthy mitochondria this would not cause any complication; however, if the mitochondrial membrane is defective, the generated ROS by-products tend to leak out reaching the cellular cytosol (Wallace 2005).Chronic leakage of ROS would cause its accumulation and interference with other molecular components of the cell, most importantly is its injurious interaction with nuclear DNA favouring mutagenesis and thereby cellular malignancy.(Harman 1956)In normal healthy cells, ROS damage arising from mitochondrial damage, is arrested by autophagy that specifically targets the mitochondria; this form of autophagy is known as mitophagy. Successful elimination of leaky mitochondria via mitophagy would stand against ROS escape into the cytosol inhibiting its harmful interaction with nuclear DNA.

4.3. Unfolded proteins and autophagy

When the Endoplasmic Reticulum (ER) is unable to cope up with the amount of accumulation misfolded proteins, a signalling pathway known as the *unfolded protein response (UPR)* is triggered by the stressed ER(Høyer-Hansen and Jäättelä 2007). This response calls on chaperones and activates autophagy towards misfolded proteins as additional aid in order to annihilate their existence by transporting them towards the lysosome for degradation. Out of all proteins that are prone to misfolding during metabolic stress, p62 is categorised as the most problematic.

As mentioned in the section of autophagy and inflammation earlier, accumulation of p62 would amplify NF-κB (Nuclear Factor kappa B) transcription. Apart from NF-κB activation, p62 also plays a role in elevating ROS production as cells undergo periods of oxidative stress hence compromising genomic stability (Mathew et al 2009).

5. THE LINEAR RELATIONSHIP BETWEEN INFLAMMATION, AUTOPHAGY, AND CANCER

Substantial research in cancer etiology has established links between chronic inflammation and cancer susceptibility. Chances of patients experiencing chronic inflammation, either from persistent infection or due to endogenous imbalance of cytokine activity, considerably escalate the likelihood of cancerogenesis (Coussens and Werb 2002). During inflammation, cells undergo lipid peroxidation, oxidative, and nitrosative stress; these factors positively contribute to the generation of ROS (Bartsch and Nair 2006). ROS are responsible for DNA damage through base alterations, strand breaking, and positively modulating oncogenes while negatively modulating tumor suppressants (Wiseman and Halliwell 1996).

5.1. The role of pathogens in inflammation

If unsuccessfully eradicated, pathogens could trigger tumorigenesis for e.g., Epstein-Barr virus, which infects more than 90% of the world's population, was the first pathogen to be suspected to take part in the pathogenesis of Burkitt's lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma, and nasopharyngeal carcinoma (Thompson and Kurzrock 2004). Another pathogen which might be linked to cancer progression is the gram negative bacteria *Streptococcus anginosus*. DNA fragments from this specific strain were present in samples taken from cervix, lung, and kidney cancers (Sasaki et al 1998).

Similarly, *Streptococcus* eradication is enhanced by ubiquitin accumulating in the lysosome as basal autophagy delivers ubiquitinated proteins for degradation. A defect in autophagy would weaken the cell's defense against these pathogens and others including *Mycobacterium*, *Shigella*, *Legionella*, and *Salmonella*. This would ultimately increase susceptibility to cancerogenesis through prolonging the period of infection accompanied by persistent inflammation.

5.1.1. Inflammation and routes to cancer

Inflammation, as one of the identified promoters in cancer progression, intrinsic defects initiating constant inflammation, accounts for much more cases when compared to inflammation triggered by invading pathogens.

During chronic inflammatory states, uncontrolled and constant cytokine expression is accountable for ungoverned cellular growth and proliferation featuring tumorigenesis (Hussain and Harris 2007). An investigational study by Crişan et al (2011) exploring the complementary inhibition and induction of basal autophagy, revealed its significance in the regulation of the two inflammatory cytokines, IL-18 and IL-1 β . The former is responsible for mediating severe inflammatory reactions upon activation attracting further chemokines to the tumor site. On the other hand IL-1 β takes part in differentiation, proliferation and the synthesis of the anti-apoptotic and pro angiogenic COX-2 (Crişan et al 2011). IL-1 β is also associated with over 50% of all gastric cancer cases and reported in a large number of patients with liver metastasis (Tomimastu et al 2001).

5.2. Autophagy, NF- κ B, inflammation, cancer and possible treatment

The transcription factor NF- κ B is a central regulator of the immune system. It regulates the expression of more than 150 genes in response to stress caused by infection. Its core duty is to strengthen the cellular response against viruses and bacteria by expressing cytokines, chemokines, and immunoreceptors. It is also accountable for intensifying the cells' integrity by promoting cellular differentiation, proliferation and inhibiting apoptosis (Pahl 1999). More recently, NF- κ B dysregulation has been associated with the pre-neoplastic state and the alteration of healthy cells causing malignancy.

Cancerous cells possess aberrant kinases that endlessly phosphorylate the NF- κ B inhibitor I κ B α . The phosphorylated inhibitor is then delivered to proteasomal degradation allowing NF- κ B to induce proliferation, differentiation, and anti-apoptotic genes supporting angiogenesis and the progression of malignant cells (Sun and Xia 2003).

Transcription by NF- κ B, like many other pathways, requires mediators that contribute to successful transduction of signals. NF- κ B activation is only feasible when I κ B α is phosphorylated and degraded; the kinases responsible for this phosphorylation step are I κ B kinase (IKK), and NF-kappaB-inducing kinase (NIK). However, a prerequisite for I κ B α phosphorylation by these kinases is their proper folding to obtain the active form.

The chaperone responsible for the proper folding of IKK and NIK is known as Heat shock protein 90 (Hsp90). Only when both IKK and NIK are structurally functional in order to phosphorylate I κ B α liberating NF- κ B, initiate transcription.

The anticancer drug geldanamycin's (GA's) mode of action is based on interfering with the chaperone duties of Hsp90. By restraining Hsp90, the proper folding of IKK and NIK is inhibited producing a non-functional protein residing in the cytosol. Consequently, the autophagic lysosomal pathway is triggered as means of degrading both kinases since they are marked as aberrant proteins and need to be discarded for the cell to remain burden. In the absence of NIK and IKK, NF- κ B then remains bound to its inhibitor I κ B α rendered unable to transcribe cytokines and anti-apoptotic genes. Cells treated with a pharmacologic inhibitor of autophagy or had their autophagy genes silenced showed an increase in the stabilisation of the two kinases IKK and NIK folding by Hsp90 confirming the detrimental role of autophagy in the NF- κ B transcription pathway (Qing et al 2007).

A relevant example demonstrating the magnitude of poor autophagy is seen in Crohn's disease and Ulcerative Colitis. Patients experiencing prolonged periods of intermittent inflammation, and patients with poor compliance to medication are placed in the category of being at an increased risk of developing cancer (Ullman and Itzkowitz 2011, Desreumaux and Colombel 2003).

5.4. Two Distinct forms of programmed cell death: Apoptosis and Autophagy

Apoptosis in healthy cells acts as a security checkpoint monitoring the cells' division cycles. As long as the cells exist in a healthy environment, apoptosis remains dormant and cells continue to divide in their own habitual manner. However, jeopardised cellular genomic stability either by extrinsic factors (chemical agents, radiation or physical stress) or an intrinsic malfunction in cellular processes, would serve as a stimuli that triggers apoptosis in response to sensed damage. (Lips and Kaina 2001; Gómez-Lechón 2002; Kowaltowski, and Vercesi 1999). The initiation of apoptosis follows two distinctive pathways. The first is characterised by an increase in mitochondrial permeability releasing the pro-apoptotic factor cytochrome-c (Korsmeyer 1999). The second apoptotic pathway is modulated by death receptors on the cells' surface. Upon their activation death-inducing signalling pathways (DISC) are initiated and are responsible for triggering apoptosis (Sheikh and Fornace 2000). The final stage of apoptosis is marked by morphological alterations such as chromatin condensation, nuclear DNA fragmentation, membrane blebbing (bulging) and shrinkage. The cellular remains, which are known as apoptotic corpse, are phagocytosed by circulating phagocytes that act as patrolling scavengers (Loo et al 1993)..

A number of anti-cancer agents that promote apoptosis in malignant cells has been introduced as cytotoxic agents, some of these include: glucocorticoids, anti-hormones, alkylating agents, anti-metabolites, and topoisomerase inhibitors (Loo et al 1993).

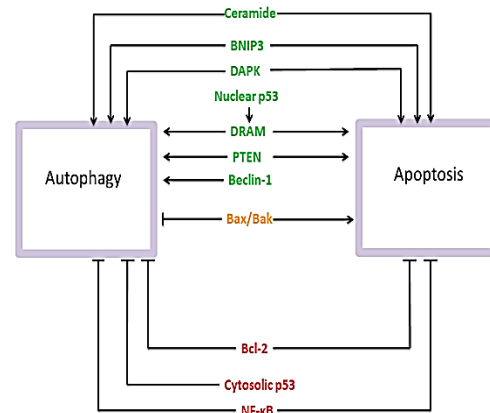
Compared to the well-established apoptotic pathway (type I PCD), a relatively new form of secondary death known as type II PCD is emerging. This form of cell death is dependent on the autophagic machinery as an alternative route to cell death when apoptosis is inefficient.

An investigational study by Fazi et al (2008) on the effects of the synthetic derivative of retinoic acid (fenretinide) demonstrated the liability of autophagy in the absence of apoptosis. When fenretinide was administered to apoptosis defective MCF-7 breast cancer cells, the cells went through what seemed as an autophagic form of cell death.

The molecular pathway of type II PCD is identical to autophagic activation during the period of starvation when nutrients are inadequate. However, the main difference between type II PCD and autophagy as means of survival is the extent of the pathway's activation. Autophagy is maintained within basal levels when the purpose behind its activation is the maintenance of homeostasis and energy regeneration through catabolic breakdown of unessential cellular components during periods of starvation. Apoptosis and autophagy signalling pathways are managed by common regulators. Some tumor suppressor proteins, which are well established as triggers of apoptosis, were also identified as positive regulators of autophagy.

Furthermore, oncogenes that impede apoptosis and promote cancerogenesis were recognised as genuine inhibitors of autophagy. The common signalling pathways shared by the two forms of programmed cell death are summarized in Figure 3.

Ceramide, BNIP3, DAPK, Nuclear p53 (via DRAM activation), and PTEN are all activators of both autophagy and apoptosis. Beclin-1 activates autophagy while Bax/Bak activates apoptosis only. The inhibitors of both pathways are NF- κ B and Bcl-2. Cytosolic p53 only acts by inhibiting autophagy. Although Bax/Bak are activators of the apoptotic pathway, they act opposingly as negative modulators in the autophagic pathway.



Apoptosis and autophagy share common signalling pathways. Certain regulatory elements that trigger apoptosis are also responsible for triggering autophagy. Then again, negative regulators of apoptosis can also be responsible for inhibiting autophagy. Furthermore, the two pathways implement a rheostat like function to one another where the activity of one could negatively regulate the other and vice versa. The positive regulators of both pathways identified so far are Ceramide, BNIP3, death-associated protein kinase (DAPK), the Phosphatase and tensin homologue (PTEN) and nuclear protein 53 (p53).

Recent studies have provided evidence for death associated with protein kinase (DAPK), which is a well-established tumor suppressor protein, to play a part in triggering autophagy. The importance of DAPK was first realised in both apoptotic and necrotic forms of cell death; however, a review by Bialik and Kimchi (2010) brought to light the significance of DAPK in the initiation step of the autophagosome and its trafficking. In the initiation step Beclin-1 is phosphorylated by DAPK, this would allow its dissociation from Bcl-2 and promoting autophagy. Moreover, DAPK phosphorylates microtubule-associated protein1B (MAP1B) which specifically associates with localised LC3-II, an important element in the transport of the autophagosome to the lysosome (Bialik and Kimchi 2010).

One more tumour suppressor protein that plays a dual role in the management of autophagy and apoptosis depending on its site of localisation is p53. The transcription duties of p53, localised in the nucleus, triggers autophagy in two distinct pathways. The first pathway is through the activation of the homeostatic enzyme adenosine monophosphate-activated protein kinase (AMPK). Both metabolic stress and genetic damage activate p53 which in turn phosphorylates AMPK, the phosphorylated AMPK interacts with autophagy's key inhibitor mTOR. During physiological periods, mTOR receives constant activating signals and continuously inhibits autophagy allowing cellular anabolic processes to take place.

Nevertheless, DNA damage sensed by p53 initiates the activation of AMPK which alleviates the inhibition of autophagy imposed by mTOR (Feng et al 2005).

6. THE ALTER EGO OF AUTOPHAGY IN CANCER

Recent findings suggest that autophagy is not to be viewed only as a beneficial process preventing pathogenesis via its cytoprotective duties. As mentioned above, substantial research confirms the significance of autophagy in counteracting tumourigenesis as an alternative death form when the primary apoptotic pathway is inhibited. The use of the soil amoeba *Dictyostelium* in investigational studies should be accepted as the golden rule in testing autophagic cell death. Some of the advantages of *Dictyostelium* are: its robust physiology facilitating genetic manipulation, the type of media required for its growth is relatively cheap, and its phylogenetic conservation of differentiation and other cellular functions. Most importantly, *Dictyostelium* species lacks the apoptotic death machinery and solely depends on non-apoptotic forms of cell death (Giusti et al 2009).

Differentiation between autophagic and apoptotic cell death is quite intricate because of the common molecular elements that both pathways share. For instance, the recognised apoptotic pathway inhibitors the Bcl-2 family of proteins are also recognised as inhibitors of Beclin-1 which is responsible for the initiation of autophagy. Another example of a shared molecular element between the two pathways which is missing in *Dictyostelium* is pro-apoptotic caspase-8. The dual role of caspase-8 consists of triggering apoptosis on one hand and inhibiting autophagy on the other (Giusti et al 2009).

A study by Sy et al (2011) investigated the activity of Timosaponin A-III as a promising cytotoxic drug by testing on HeLa cells. The results of this study provided a better understanding of the relationship between autophagy and apoptosis whether they occur mutually independent of each other, sequentially, or concurrently. The treated cells were observed for morphological changes in their ultrastructure to determine whether the cells are going through autophagy, which is characterised by vacuolization, or through apoptosis, where chromatin condensation occurs.

A steroidal saponin from the rhizomes of *Anemarrhena asphodeloides* called Timosaponin A-III, acts by traversing the mitochondrial envelope causing the release of ROS due to the transition in mitochondrial permeability leading to the leakiness of the membrane. Initially, cells displayed signs of autophagy identified by the visual increase in the rate of vacuolization; the triggered autophagy was in response to the released ROS, more specifically H₂O₂. The observed manner of autophagy had the standard molecular basis of the starvation-induced autophagic pathway. Minimal amounts of ROS are responsible for activating mitophagy which regulates mitochondrial turnover, this process precludes the activation of apoptosis via the increasing level of intracellular ROS. However, as the treatment period is prolonged the autophagic pathway is incapable of keeping up with the increasing rate of mitochondrial damage which eventually triggers apoptotic cell death.

The notion of cancer adopting autophagy to serve its metabolic needs is quite remarkable. In healthy cells apoptosis is fully functional and serves as the primary checkpoint that assures the retirement of defective cells from the cell division cycle. However, tumour cells evolve means of circumventing apoptosis and triggering autophagy to support growth in an environment with limited glucose, oxygen, amino acids and growth factors for long periods of time that could reach up to several weeks (White and Dipaola 2009).

Initially, the catabolic self-eating autophagic process consumes up to two-thirds of the tumour mass leaving only robust cells that are known as minimal cells capable of recovery. At this point, cells undergo a forced state of dormancy depending on minimal amounts of nutrition. Only the basic cellular activities are maintained to support mere survival. Other cellular processes that are considered as additional sources of energy expenditure such as motility and amino acid synthesis are paused as a form of energy conservation until sufficient energy supplies are restored (Mathew et al 2007).

Outstandingly, the importance of autophagy in tumourigenesis is not only limited to periods of starvation, it also provides cells with an irrepressible capacity to regenerate when unfavorable environmental conditions ameliorate. As nutritional sources are restored, cells regain their lost mass and re-establish their physiological size and functions to the ones prior to the period of starvation (Lum et al 2005).

Anoikis is a subset of apoptotic cell death which is accountable for maintaining cells with high turnover rate within normal cell count. The mechanism of this process is based on a communication process of adjacent cells either from one cell to another or by interactions with the extra-cellular matrix via specific signals.

The moment the cells receive inadequate signals or recognise inappropriate contact with other cells, apoptotic cell death is triggered in a responsive manner. Dysfunction of this important regulatory step is a root cause of neoplasia and metastatic spread of cancer cells that survive in a new location in which it is not supposed to be (Wei et al 2001).

6.1. Mediating autophagy as a chemo preventive approach

Accomplishment of essential cytoprotective duties through autophagy is regarded as an important factor in maintaining metabolic balance and disease prevention. Until now the best method of cancer management is to apply preventive measures rather than waiting till the onset of disease. According to this aspect, the maintenance of the housekeeping duties of autophagy could come in handy as a pivotal element of a healthy cellular environment.

Furthermore, cells that do not possess functional autophagic machinery are unable to recycle aberrant proteins and weary organelles to regenerate energy sufficient for DNA replication, transcription, and translation which are vital processes in cellular homeostasis.

The homeostatic role of autophagy allows the cells to exist in a non-inflammatory state when faced with metabolic stress. This could be of significant importance in cancerous cells where apoptosis is disabled and cells seek an alternative form of death known as the necrotic form of cell death. Cells that undergo the necrotic form of death yield inflammation at the end of the process via the infiltration of inflammatory cytokines, creating an ideal environment promoting tumourigenesis in a similar manner to that seen in normal cells healing from injury (Degenhardt et al 2006).

Therefore, considering the induction of autophagy as a chemo preventive measure is emerging as a possible approach in cancer management as it might yield substantial clinical outcomes. Research in this area is still lacking. However, dietary agents identified so far that up-regulate autophagy are lycopene, resveratrol, lutein, curcumin, epigallocatechin-3 and carotenoids. These naturally occurring compounds exhibit an antioxidant activity by inhibiting the accumulation of nitrogen species and ROS by the up-regulation of autophagy (Pan et al 2008).

As to pharmacological up-regulators of autophagy, FDA has approved small molecule drugs that were recently recognised as autophagy inducers. They include trifluoperazine, pimozone, fluspirilene, amiodarone, nicardipine, and nifedipine (Zhang et al 2007). Further investigations in determining whether these agents are capable of counteracting malignant transformation by means of genome stabilisation would answer the question of whether they are to be regarded as novel candidates in cancer prevention.

Therapeutic targeting of cancer cells with cytotoxic drugs coupled with angiogenesis inhibitors yields metabolic cells that eventually triggers apoptosis in cells that are still apoptosis competent (Folkman 2003). Autophagy in cancer cells antagonises the hostile microenvironment created by cytotoxic drugs leading to a form of therapeutic resistance. Compromised cells resort to autophagy in order to mitigate cellular damage leading to sub-optimal therapeutic outcomes.

Under these circumstances, the addition of autophagy inhibitors to the treatment regimen could serve as a promising approach to re-sensitize cancer cells to conventional cancer interventions by dismantling the survival advantage of over activating autophagy (Gewirtz 2009).

Bellodi et al (2009) investigated the factors that cause therapeutic resistance in cells treated with the small molecule tyrosine kinase inhibitor Imatinib (Gleevec). The results showed autophagy triggered by ER stress is responsible for resistance to treatment. Ca^{2+} depletion produced pharmacological inhibition of autophagy while RNA interference served as genetic inhibitor of autophagy. Either forms of inhibition enhanced cell death in chronic myeloid leukemia (CML) primary cells.

The final approach in implementing autophagy in cancer treatment is through inducers that activate autophagy to produce the alternative form of cell death (type 2 PCD) in cells with abolished apoptotic machinery. Yet, current literature still remains controversial in terms of whether autophagic autophagosome formation that occurs at the end stage of dying cells is strictly a sign of an alternative form of cell death or just a final attempt activated by dying cells to overcome the accumulating stress.

Hence, the future approach should aim towards differentiating between these two opposing forms of autophagy that serve completely two opposing purposes. The optimal method of assessing this would be by testing the effects of autophagy gene silencing in the target cells. Cells that show declined levels of survival are the ones that depend on autophagy as a mode of survival. Alternatively, cells that die in a slower rate in comparison to their counterpart are the ones suspected of resorting to autophagy as an alternative death pathway.

7. CONCLUSION

Autophagy is the degradation of organelles with suboptimal activity, the compilation of aberrant organelles burdens the cell which also evokes cell death. A well characterised form of organelle targeting is that of the mitochondria (mitophagy), eliminating malfunctioned mitochondria evades triggering cell death caused by proapoptotic factors escaping the damaged membrane into the cytosol. On the other hand, the degradation of other organelles still demands more research and molecular characterisation. For instance, selective ribosomal clearance (ribophagy) is emerging as a target for autophagy. In general out of the three subdivisions of autophagy, macroautophagy retains the major share with regard to clinical characterisation.

Essentially, unwanted cellular components are delivered to the lysosome and are recycled to basic building blocks for the synthesis of essential proteins. Blockage of this pathway compromises the cells integrity leaving it prone to stressors that ultimately trigger cell death. Current knowledge focuses on the process selecting and delivering cellular components to the lysosome; however, only little is known with regard to the molecular pathway of how the delivered cargo is recycled in the lysosome.

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