Autophagy: Cell death or survive mechanism

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1. Mechanisms of cellular death

Cell death has been traditionally thought to occur via one of two mechanisms: “programmed” (genetically controlled) or “uncontrolled”. Apoptosis is considered to represent the major form of programmed cell death and is under the control of specialized genes and pathways. In contrast, necrosis involves uncontrolled cellular death occurring after acute physical trauma or stressors overwhelming the cellular survival mechanisms. However, recent studies have shown that necrosis may also occur in a programmed manner in certain circumstances (necroptosis). Again, cancer cells have been recently shown to exploit impaired programmed cellular death mechanisms in order to develop tolerance against stress and starvation via certain pathways such as the catabolic autophagy pathway. Subsequently, cancer cells have the need of impaired programmed cellular death mechanisms in order to survive and maintain mobility in physiological conditions, even if it is not for maintaining viability.

2. Autophagy

Autophagy, meaning self (auto) devouring (phagy) in ancient Greek, is the physiological degradation of the intracellular structures in order to provide nutrition for the cell under starvation. This leads to a recycling of intracellular molecules and maintenance of the homeostasis. Also, autophagy has been recently found to play a role in a number of processes including metabolism, morphogenesis, differentiation, ageing, cellular death, and immunity. On the other hand, abnormalities of autophagy have been associated with the development of a number of conditions such as cancer, infectious diseases, and neurodegenerative diseases.

Currently, three distinct mechanisms of autophagy have been identified (Fig. 1):

1. Macro-autophagy: It results in the disintegration of proteins and damaged cellular organelles. It occurs via the formation of autophagosome.
2. Micro-autophagy: It occurs via the invagination, protrusion, and septation of the lysosomal membrane.

However, autophagy mainly refers to macro-autophagy.

Autophagy related proteins (also known as Atg proteins) have been initially identified in studies involving yeasts, and more than 30 Atg genes have been described up to now. Some of these proteins are involved in the formation of autophagosomes (isolation membrane/autophagic vacuole). Autophagosomes develop in structures referred as pre-autophagosomal structure (PAS). Mechanisms of autophagy may be summarized in 4 steps as follows:

1. mTor complex: Atg1-Atg13-Atg17 kinase complex (Fig. 2)
2. PI3K complex: Atg6 protein (beclin-1) complex regulating the activity of Vps34 (Fig. 3)
3. Two ubiquitin like systems (Fig. 4)
4. Atg9 cycle system

As a result of these steps, autophagy proceeds as follows in the cell:

1. Nucleation
2. Elongation of the membrane
3. Fusion with the lysosome
4. Degradation

These steps may be schematized as below (Fig. 5):

The autophagic vacuole goes through elongation after its formation, followed by the degradation of its content through fusion with the late endosome or lysosome. The building blocks such as...
amino acids or fatty acids released after degradation are recycled for cellular processes. These steps may be summarized as follows using electron microscopic images (Fig. 6):

3. Regulation of autophagy

The most important stimuli in the regulation of autophagy include starvation, hypoxia, and stress.
The tor protein complex also plays a significant role in the regulation of autophagy. Tor is a kinase that controls the growth and protein synthesis in the cells, and its suppression results in the activation of autophagy. Starvation is associated with reduced Tor activity as well as de-phosphorylation of Atg13, leading to its binding with Atg1 and induction of autophagy. In mammals...
the ULK:Atg13:FIP200 protein. ULK1 and ULK2 proteins have been found to stimulate autophagy through interaction with mTor kinase and AMPK, while phosphorylation of S6K protein was found to suppress autophagy. Another important signaling pathway in cellular growth is class I PI3K, activating the Akt/PKB pathway, which in turn inhibits autophagy through activation of Tor. On the other hand, PTEN, which has an opposite action to that of PI3K/Akt pathway, induces autophagy. The mutations of PTEN are associated with a suppressive effect on autophagy. The PI3K complex consists of Atg6 (beclin-1), Atg14, Vp15 and Vps34 proteins and in conjunction with PI3K binding protein, initiates the formation of the autophagic vacoule. 3-methyl adenine (3-MA) is a PI3K inhibitor that is widely used to inhibit autophagy. Also, certain oncogen and tumor suppressor proteins have been found to play a role in the regulation of autophagy. For instance, overexpression of DAPk protein family in cancer cells resulted in increase in autophagy and cell death.

As mentioned earlier, autophagy was first described in yeasts, and subsequent work identified similar mechanisms in mammals, despite certain differences. In this regard, the basic differences are
outlined below (Fig. 7):

4. The relationship between autophagy and apoptosis
   (Table 1)

1 Autophagy may lead to cell death in parallel with apoptosis, e.g. in Kaposi sarcoma treated with imatinib and prostate cancer cells treated with MG132.17–19

2 Autophagy may allow cell survival through suppression of apoptosis, e.g. DNA damage, gene amplification, and chromosomal abnormalities occur at an increased rate in tumor cells in the absence of autophagy. Inhibition of autophagy results in increased responsivity to radiotherapy in breast, prostate, and colon cancer.17,20,21

3 Autophagy may also represent a pre-requisite for apoptosis, e.g. autophagy has been shown to be required for caspase-dependent cell death in HIV infected CD4+ T cells.17,22

4.1. Molecules with an impact on autophagy

4.1.1. Akt/PKB and Tor pathway
   It is the most important pathway for the regulation autophagy. While a positive effect on mTor activity is seen with Rheb (GTP binding small protein), a negative effect is observed with TSC1/ TSC2 (Tuberous sclerosis complex 1 and 2). The activation of the Tor pathway is associated with cell growth and survival, as opposed to subsequent autophagy when inactivated.

   mTor pathway is controlled by Akt/PKB pathway, which phosphorylates TSC1/TSC2 and inactivates mTOR inhibitors. In other words, activation of Akt/PKB leads to the inhibition of autophagy.23,24 At the same time, Akt/PKB activation blocks apoptosis via its effects on NF-kB pathway.25,26

4.1.2. Bcl-2 and Bcl-XL
   These anti-apoptotic proteins inhibit both apoptosis and autophagy. They prevent the formation of autophagic vacuole through binding the BH3 site on Beclin-1.27,28 BNIP-3 is a protein belonging to the family of Bcl-2 proteins that activates autophagy.29 (Fig. 8)

4.1.3. Atg4D, Atg5 and Beclin
   Proteolysis of these proteins induces apoptosis. It has been found that certain autophagy molecules may also be used by apoptotic pathways through this process.30–33

4.1.4. P53
   p53 stimulates apoptosis, increases the transcription of pro-apoptotic genes such as Bax, PUMA, and NOXA, while down-regulates anti-apoptotic proteins including Bcl-2.34 Activation of p53 leads to the inhibition of mTor pathway, ultimately resulting in the activation of autophagy.35 Despite its positive effects on autophagy, complete dysfunction of p53 causes full activation of autophagy. Nuclear p53 has been shown to promote autophagy, while cytoplasmic p53 was found to have the opposite effect.36

4.1.5. Reactive oxygen species (ROS) and Atg4
   Increase in intracellular ROS stimulates apoptosis, while decreased intracellular ROS promotes cell growth and survival. ROS affects autophagy through oxidation of Atg4. Oxidized Atg4 is inactivated, suppressing the formation of autophagosome.37

4.1.6. ARF
   It is a tumor suppressor gene, with an effect on both autophagy and apoptosis.38

4.1.7. FADD, caspase-8; FLIP
   Over-activation of autophagy leading to cell death has been observed in T-cells devoid of FADD and caspase-8 activity.39 On the other hand, FLIP is an inhibitory protein that inhibits both apoptosis and autophagy.40

4.1.8. DAPk
   DAPk is a serine/threonine kinase regulated by calcium/calmodulin binding that activates apoptosis and autophagy.41

4.1.9. E2F1
   It activates apoptosis and autophagy in the presence of DNA-damaging agents.42
4.2. The role of autophagy in tumorigenesis

Autophagy should be defective in tumorigenesis. In human breast and prostate cancer cells, mono-allelic deletion of becn1 required for the regulation of autophagy has been shown in addition to a decrease in beclin 1 in certain breast cancer lines.\textsuperscript{43,44} Beclin1 is the counterpart of Atg6/vps30 in mammalian tissues and is required for the formation of autophagosome.\textsuperscript{45} Beclin1 /−/ mice die early during embryogenesis, while becn1 ± mice experience an increased occurrence of lymphoma, lung cancer, and liver cancer. Additionally, hyperproliferative pre-neoplastic alterations have been identified in the breast tissue samples of becn1 ± mice, which have also been found to express wild-type beclin 1. This suggests that becn1 may represent a haplo-deficient tumor suppressor gene.\textsuperscript{46,47} Recent studies have also shown that autophagy is related with increased in vivo and in vitro survival, particularly when apoptosis is inactivated.\textsuperscript{26,44} Under circumstances with insufficient angiogenesis, autophagy is inactivated in tumor areas of hypoxia leading to maintenance of the survival of tumor cells. In starvation, this function of autophagy buys the cell some extra time until optimum nutrition, oxygenation, and growth factor conditions are restored. Thus, autophagy should be representing a mechanism necessary for tumor survival.\textsuperscript{48}

It is still unclear how the inactivation of a survival pathway plays a role in tumorigenesis. The answer to this question is most likely to be related with the fact that although apoptosis and necrosis are irreversible processes once initiated, autophagy involves mechanisms that can be deferred and re-initiated if required.

Autophagy not only represents a source of energy during periods of starvation, it also allows the control of damaged intracellular organelles and proteins, preventing the accumulation of toxic proteins and organelles that may harm the cell. This may have critical implications especially for tumor cells, which rely on ineffective aerobic glycolysis and which may have inadequate blood supply due to rapid tumor growth and metastasis. Impaired autophagy in tissues and tumors may have negative consequences for the cellular integrity, paving the way for DNA damage, mutation, and genomic instability, ultimately contributing to tumor formation and progression.\textsuperscript{21,49,50} These observations suggest that stimulation of autophagy may have preventive role in cancer development, while the inhibition of autophagy-mediated survival may represent a novel therapeutic approach for the treatment of aggressive cancers.

When the role of autophagy in maintaining tumor cell survival under stressful conditions, prevention of autophagy may offer a beneficial therapeutic modality triggering cell death. Furthermore, both targeted agents and cytotoxic agents used for the treatment of cancer have been shown to induce autophagy. This possibility also has led to initiation of phase I and II studies testing the combination of standard chemotherapeutic agents with the anti-malarial agent

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<td>Apoptosis (Type I Programmed Cellular Death)</td>
<td>Chromatin condensation DNA ladder</td>
<td>Apoptotic bodies</td>
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<td>Autophagy (Type II Programmed Cellular Death)</td>
<td>Partially chromatin condensation No DNA ladder</td>
<td>Increase of autophagic vacuoles</td>
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Table 1

**Autophagy and apoptosis: Differences.**

**Fig. 8. Effective proteins via Beclin-1.**
hydroxychloroquine, which is known to inhibit autophagy through prevention of lysosome degradation.

5. Discussion

Although the association between the tumor suppressing effects of autophagy and its effects on cell death and survival has not been fully understood, it should also be noted that similar effects may also play a significant role in the efficacy of cancer treatments. In vitro, many chemotherapeutic agents have been shown to promote the formation of autophagosomes. For many years, it has been thought that these treatments caused cell death through induction of autophagy. However, inhibition of autophagy resulted in an increase in cell death rather than a decrease. Therefore, it has been postulated that inhibition, rather than induction of autophagy could be beneficial in cancer treatment. In a 2007 study by Amaravadi and co-workers, administration of chloroquine, an agent causing autophagic degradation, to mice with c-Myc associated lymphoma resulted in increased tumor cell death and tumor regression through augmentation of the effect of DNA-alkylating agents. However, it is difficult to conclude that the effects of chloroquine stem from the inhibition of autophagy, since chloroquine not only affects the multi-drug pump of tumor cells, but is also associated with the stimulation of anti-tumor response in the immune cells of the host. Further studies involving the use of autophagy-specific inhibitors may shed light on this issue. Thus, autophagy is conceived as a factor that has a role both in the promotion and prevention of cancer, and its role in cancer may differ depending on the tumor progression. Inhibition of autophagy may lead to continuous growth of pre-cancerous cells, where autophagy may act like a tumor suppressor. Later, once the tumor is large enough, cancer cells may require autophagy in order to be able to survive in a nutrient- and oxygen-poor environment, and this may be even more prominent in the inner tumor sites with less vascularization. Additionally, autophagy may also confer protection against ionizing radiation in some cancer cells; this is achieved by the removal of damaged organelles and macro-molecules such as the mitochondria as well as the prevention of apoptosis.

Certain agents used for the treatment of cancer seem to be effective through mechanisms involving autophagy. For instance, tamoxifen used for the treatment of breast cancer acts through up-regulation of beclin-1. mTOR inhibitors are among inducers of autophagy, although the mechanism of action of these agents is thought to involve effects on cell cycle regulation rather than autophagy.

6. Conclusion

In conclusion, autophagy is thought to be an efficient factor in both the promotion and prevention of cancer. The role of autophagy in cancer probably varies by tumor progression. Inhibition of autophagy may lead to continuous growth in precancerous cells and autophagy acts as a tumor suppressor in this case. When the tumor is grown sufficiently later, cancer cells need autophagia to survive under conditions poor in nutrition and oxygen. This may especially be prominent in the less vascularized internal parts of the tumor.

It is clear that autophagy has a very important role in the development, progression and treatment of cancer, like apoptosis. It is very important to continue to investigate this important pathway in further studies.

Conflict of interest

There is no conflict of interest.

References


