Q&A

CELL BIOLOGY

Autophagy and cancer

Beth Levine

Autophagy is the degradation of redundant or faulty cell components. It occurs as part of a cell's everyday activities and as a response to stressful stimuli, such as starvation. Connections with cellular life-and-death decisions and with cancer are now emerging.

How is autophagy induced?

Autophagy occurs when cells need to 'self-cannibalize' or degrade their constituents. Underlying 'housekeeping' levels of autophagy probably occur in most normal cells to prevent the accumulation of protein aggregates and defective cellular substructures. Certain environmental cues (such as starvation, high temperature, low oxygen, hormonal stimulation) or intracellular stress (damaged organelles, accumulation of mutant proteins, microbial invasion) activate signalling pathways that increase autophagy. Classically, most research on how autophagy is induced has focused on an enzyme called TOR kinase. This enzyme is a sensor of nutrient status and a master regulator of cell growth; it negatively regulates autophagy through its effects on a set of proteins known as autophagy-execution proteins. However, it is now clear that numerous signalling pathways, such as those involved in the control of cell growth, DNA-damage repair, a form of programmed cell death called apoptosis, and immunity, can also induce autophagy. It is still a mystery how these specific signals turn on the autophagic machinery.

What happens once autophagy is induced?

Once the cell receives the appropriate signal, the autophagy-execution proteins trigger a cascade of reactions that result in membrane rearrangements to form a double-membrane-bound vesicle called an autophagosome (Fig. 1). Initially, an 'isolation membrane' forms, although its origin is still controversial. The membrane surrounds the cytoplasmic contents to be degraded, and its edges fuse to form the autophagosome. This vesicle then fuses with a lysosome (or a vacuole in yeast), with the release of lysosomal digestive enzymes into the lumen of the resulting autolysosome. The sequestered cytoplasmic contents are degraded inside the autolysosome into free nucleotides, amino acids and fatty acids, which are reused by the cell to maintain macromolecular synthesis and to fuel energy production. The nutrient recycling and housekeeping functions of autophagy promote cell survival, although in certain contexts autophagy may also promote cell death (Fig. 2, overleaf).

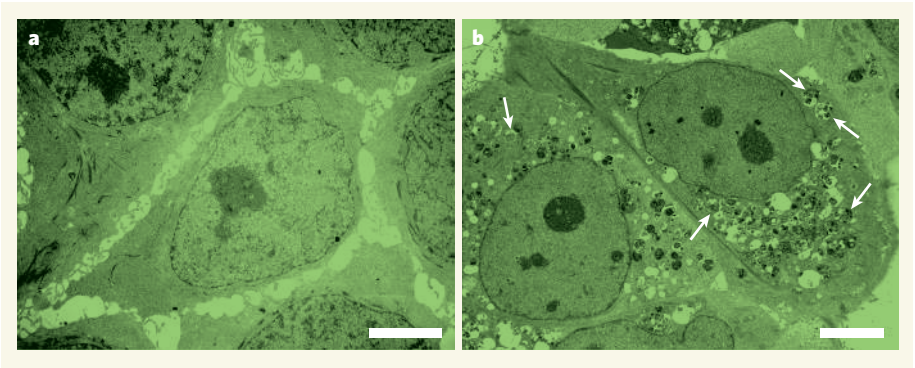

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Figure 1 | Autophagy in action. **a**, Untreated breast cancer cell (lacking autophagosomes); **b**, breast cancer cell treated with the drug tamoxifen, and containing numerous autophagosomes (arrowed). Scale bars, 5 μm.

Does autophagy also stop protein synthesis?

No. On the contrary, one of its evolutionarily conserved functions, through protein recycling, is to help maintain the synthesis of essential proteins when external nutrients are limited. Although certain stress stimuli that induce autophagy, such as starvation, turn off general protein synthesis, they also turn on the synthesis of specific stress-response proteins, including autophagy-execution proteins. So in this setting, the cell uses a coordinated strategy. To ensure that it has enough amino acids to synthesize the proteins that are essential for its survival, general protein translation is shut down and autophagy is activated.

What are autophagy-specific genes?

These are genes that are required for the execution of the autophagy pathway. Several of them encode proteins that are components of kinase complexes, which regulate the activity of proteins and lipids through the addition of a phosphate group. Alternatively, they encode components of protein-conjugation systems, which attach to each other or to membrane lipids to form the membrane of the autophagosome. Deletion of an autophagy-specific gene blocks autophagy in a cell or organism. These genes were first identified through genetic screens in yeast that included a search for genes that are essential for survival during starvation. Many of these yeast genes are also present in higher organisms, as are the underlying molecular mechanisms of autophagy. Although there seems to be a universal requirement in autophagy for 'autophagy-specific' genes, this does not mean that these genes are not involved in other cellular processes.

What is the difference between autophagy and apoptosis?

These two processes have long been classified as different forms of programmed cell death. But whereas apoptosis invariably leads to cell death, autophagy (despite its frequent occurrence in dying cells) commonly contributes to cell survival. Deletion of autophagy-specific genes in cells of diverse organisms increases cell death during development, as well as susceptibility to starvation and other apoptotic stimuli. This is not to say that autophagy cannot also be a death programme. However, it seems to take on this function primarily when the cellular apoptotic machinery is crippled, or when autophagy is induced to such high levels that cells literally eat themselves to death.

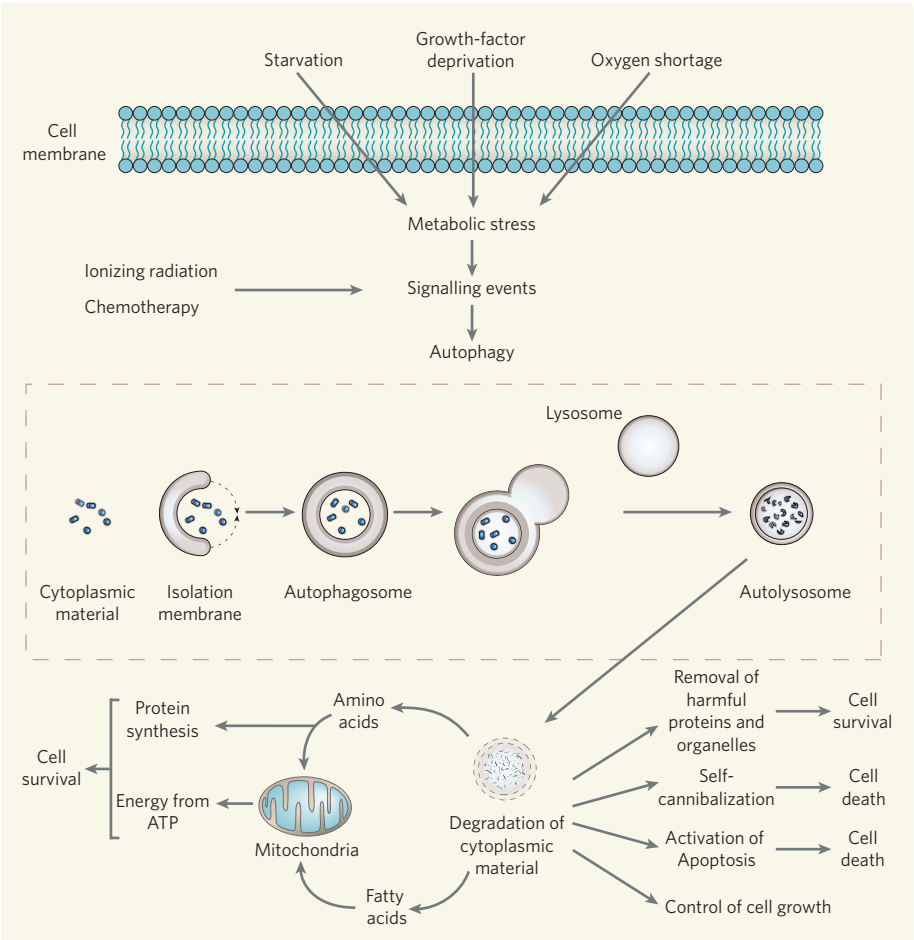

Figure 2 | The autophagy pathway and its diverse cellular functions. Both metabolic stress and cancer therapies activate signalling pathways that stimulate autophagy. The process involves the sequestration of cytoplasmic material by a membrane-bound vesicle called the autophagosome, which then fuses with a lysosome to form an autolysosome. The degradation of cytoplasmic material within the autolysosome can promote cell survival either by generating free fatty acids and amino acids, which can be reused by the cell to maintain energy production and protein synthesis, or by removing harmful proteins and organelles. It can also promote cell death independently (presumably through self-cannibalization) or together with apoptosis. Furthermore, the turnover of proteins and organelles by autophagy may contribute to the control of cell growth.

These scenarios may not be relevant to normal development or to physiological adaptations of cells and tissues to stress. However, they may be relevant to the development of cancer and to cancer therapy, because cancer cells often have mutations that confer resistance to apoptosis, and many chemotherapeutic agents that are toxic to the cell induce high levels of autophagy.

Are autophagy and apoptosis interlinked?

There is a complex, and not fully understood, relationship between them that may vary depending on the biological context. The two pathways are regulated by common factors; they share common components; they can exert overlapping functions; and one pathway may regulate and modify the activity of the other. Many signals that have long been known to activate apoptosis (sphingolipids, death-receptor signalling molecules, serine/threonine death kinases and mitochondrial-associated cell-death proteins) are now known to activate autophagy. Conversely, signalling pathways that inhibit apoptosis (the class I PI3K/Akt signalling pathway and the stress-activated NF-κB signalling pathway) also inhibit autophagy. Intriguingly, regulators of apoptosis, for example members of the anti-apoptotic Bcl-2 family, can also directly 'disarm' autophagy-execution proteins such as Beclin 1. And there is evidence that at least one 'autophagy-specific' protein (Atg5), when cleaved, can activate an apoptotic programme. Beclin 1 also has a structural domain that is considered a hallmark of pro-apoptotic proteins.

Are autophagy and apoptosis mutually exclusive?

No — they commonly occur in the same cell, both when autophagy is trying to keep cells alive and when it contributes to cell death. This co-occurrence can have different consequences. In some circumstances, including starvation and treatment with certain DNA-damaging agents, autophagy delays the onset of apoptosis. In others, such as HIV infection, autophagy is required for the onset of apoptosis in uninfected lymphocytes. During embryonic development in mammals, autophagy neither delays nor promotes apoptosis, but it is required for dying cells to generate signals that ensure the efficient removal of apoptotic corpses. The complex interplay between autophagy and apoptosis is only now beginning to be unravelled.

Is autophagy linked to disease?

Yes, it is linked to both health and disease. Normally, it contributes to adaptation to cellular stress, to development and differentiation, to immunity and to longevity. Too much or too little autophagy can contribute to certain cardiac- and skeletal-muscle diseases, liver diseases, infectious diseases, neurodegenerative disorders and cancer.

What is the cancer connection?

There are really two connections, one at the level of cancer development and the other at the level of cancer treatment. Inactivation of autophagy-specific genes, such as *beclin 1*, results in increased tumorigenesis in mice, and enforced expression of such genes (*beclin 1*, *atg5*) inhibits the formation of human breast tumours in mouse models. Furthermore, net deletions of several autophagy-specific genes are commonly found in human malignancies. Thus, autophagy may be a tumour-suppressor pathway, and its decreased activity may contribute to the development of human cancer. Consistent with this theory, tumour-suppressor genes that are frequently mutated in human cancer (*p53*, *PTEN*) turn autophagy on, and genes that are frequently activated in cancer, such as those encoding class I PI3K and AKT, turn it off. The conflicting pro-survival and pro-death functions of autophagy make the connection to cancer treatment more complex. The pro-survival function may help cancer cells to survive in nutrient-limited environments, and to resist ionizing radiation and chemotherapies. But the pro-death function may help to kill cancer cells, either spontaneously or when they are exposed to radiation or chemotherapy. These seemingly paradoxical functions have fuelled intense debate as to whether autophagy is cancer's friend or foe.

How can autophagy prevent cancer?

There are several possible ways (Fig. 3). The most obvious one is by suppressing tumorigenesis through its death-promoting effects. However, it is unclear whether, like apoptosis, autophagy is an important cell-death pathway in tissue maintenance, because autophagy-deficient organisms often show increased, rather than decreased, cell death. Another hypothesis is that autophagy prevents DNA damage, presumably through its cellular housekeeping role in removing sources of oxidative stress such as defective cellular organelles (mitochondria or endoplasmic reticulum). This theory could mechanistically

link the tumour-suppressor and anti-ageing functions of autophagy and help to explain the increased incidence of cancer as people age and their underlying housekeeping levels of autophagy decline. A third possibility is that it negatively regulates cell growth. Thus, not only might alterations in protein degradation, which is mediated by a complex known as the proteasome, contribute to the development of cancer, but imbalances between autophagy-dependent protein degradation and synthesis might also be a factor. Interestingly, mice with a mutation in one copy of an essential autophagy gene show no defects in apoptosis, but do show increased cellular proliferation in tissues that are prone to tumour formation. This suggests that the effects of autophagy on cell growth rather than its potential pro-death effects may play a pivotal role in tumour suppression.

How can autophagy promote cancer?

Again, there are several possible ways (Fig. 3). In all eukaryotic organisms, autophagy-specific genes promote the survival of normal cells during nutrient starvation. Similarly, autophagy might enhance the survival of rapidly growing cancer cells that have outgrown their vascular supply and face oxygen shortage or metabolic stress. In other words, it may be the crutch that tumour cells use to survive the shortage of energy and nutrients. It might also promote the survival of cancer cells by targeting damaged mitochondria and other organelles for lysosomal degradation, thereby buffering oxidative stress that can be triggered by activated cancer-causing genes or by cancer treatments.

Would you turn autophagy on or off to kill a tumour cell?

Paradoxically, either action may be correct (Fig. 4). The absence of autophagy increases susceptibility to death when cells confront stressful conditions (metabolic stress, cytotoxic chemotherapy). By contrast, high levels of autophagy, or its occurrence in cells with crippled apoptotic machinery, can also lead to cell death. Yet it may be premature to conclude

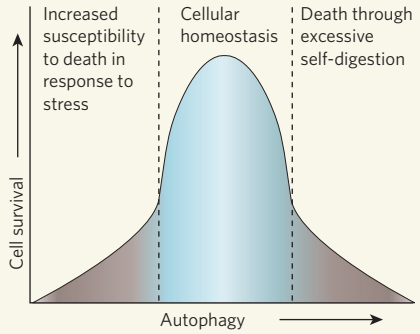

Figure 4 | Relationship between the levels of autophagy and cell death. Physiological levels of autophagy are essential for normal cellular homeostasis. The absence of autophagy increases cell death during metabolic stress and on treatment with cytotoxic chemotherapeutic agents. By contrast, excessive levels of autophagy promote cell death, presumably via self-cannibalization. Antitumour effects may be observed at all levels of autophagy, in relation either to cell death (when autophagy is very low or very high) or to cell-death-independent tumour-suppressor effects (when autophagy levels are intermediate).

that we should try to kill tumour cells by turning autophagy either on or off. Regardless of the approach taken, it is potentially risky if cell death does not invariably occur. Actions that reduce only some of autophagy's effects could result in loss of its tumour-suppressive function, whereas interventions that enhance only some of its effects could result in the increased survival of tumour cells.

Can targeting autophagy be exploited in cancer treatment?

Yes, but only if we can figure out whether autophagy should be turned on or off. Given the numerous paradoxes related to its role in cell death and cancer, this may prove quite difficult. Animal studies can help decipher whether the pro- or anticancer effects of autophagy prevail in any given setting. For example, in one animal model in which tumour cells have a defect in the apoptotic pathway, autophagy promotes tumour-cell survival during metabolic stress, but also prevents tumorigenesis, necrosis and inflammation. Thus, at least in this case, the tumour-suppressor effects of autophagy overshadow the autophagy-dependent survival pathway. By contrast, in another animal model in which tumours are caused by an activated cancer-causing gene, autophagy inhibition with the drug chloroquine enhances therapy-induced apoptosis and tumour regression. These examples illustrate the complexity of predicting the net effect of autophagy in cancer. They also hint at some of the variables that might be involved, including the stage of tumorigenesis, the sensitivity of the tumour cells to apoptosis and the specific molecular alterations in the cells.

What are the next steps in research?

They will be to determine more precisely *if*, *when* and *how* autophagy prevents or promotes cancer.

Will clinical trials help resolve the autophagy and cancer controversy?

Ultimately, the question of whether to turn autophagy on or off may be resolved by information from clinical trials in cancer patients. Many anticancer agents are potent inducers of autophagy. The optimist may view this as evidence that inducing autophagy is a desirable target for cancer therapy. By contrast, the pessimist can rightly argue that autophagy may function as a stress response to counter the toxic effects of antitumour drugs, and that such drugs might work even better if coupled with autophagy inhibitors. There is experimental support for each of these views. But because all current antitumour agents target pathways other than autophagy, it is difficult to dissect the role of autophagy stimulation in their therapeutic action. New agents that specifically target autophagy will have to be developed and tested to resolve this controversy. Only then will we begin to understand how to translate advances in our knowledge of the autophagy pathway into approaches to combat cancer. ■

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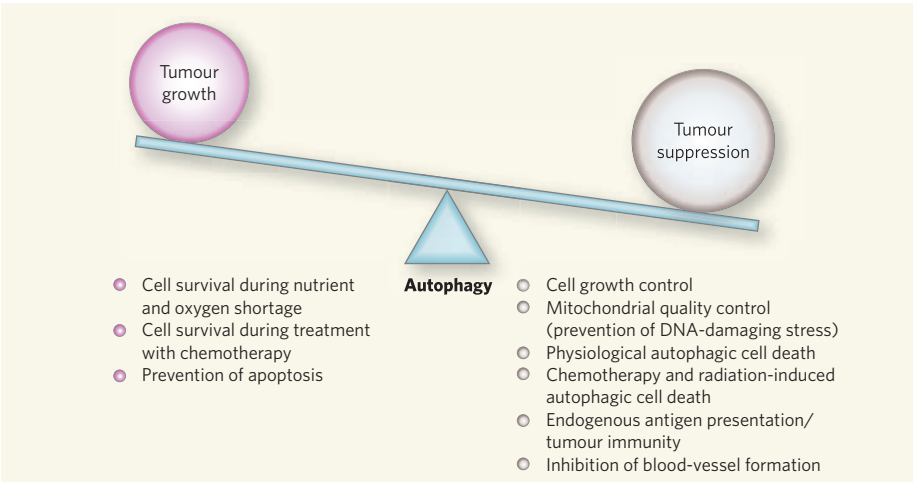

Figure 3 | Conflicting effects of autophagy on cancer. Experimental evidence supports a role for autophagy in both cancer development and suppression. What tips the balance is not clear.

FURTHER READING
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