

Autophagy in Cancer: Good, Bad, or Both?

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Abstract

Autophagy has been recognized as an important cellular process for at least 50 years; however, it is only with the recent identification of key regulators of autophagy (*Atg* genes) that we have begun a mechanistic exploration of its importance in cancer. Recent studies suggest that autophagy may be important in the regulation of cancer development and progression and in determining the response of tumor cells to anticancer therapy. However, the role of autophagy in these processes is complicated and may, depending on the circumstances, have diametrically opposite consequences for the tumor. In this article, we discuss recent discoveries regarding autophagy in cancer. (Cancer Res 2006; 66(19): 9349-51)

Introduction

Autophagy or "self-eating" is becoming an important area in cancer research, and we are seeing exponential growth in the number of publications on this topic. There have been several recent reviews about autophagy as it relates to cancer and other diseases (1, 2), and the 2006 AACR annual meeting held its first symposium devoted to autophagy and cancer. Autophagy is a genetically programmed, evolutionarily conserved process that degrades long-lived cellular proteins and organelles. Autophagy is important in normal development and response to changing environmental stimuli and, in addition to its role in cancer, is important in numerous diseases, including bacterial and viral infections, neurodegenerative disorders, and cardiovascular disease (2). Autophagy involves the formation of a double-membraned vesicle, which encapsulates cytoplasm and organelles and then fuses with lysosomes, thus degrading the contents of the vesicle. The formation of the double-membraned vesicle is a complex process involving 16 autophagy-related proteins (Atg proteins; ref. 3). Two ubiquitin-like conjugation systems are involved in autophagy. These systems produce modified complexes of autophagy regulators (Atg8-PE and Atg5-Atg12-Atg16) that may determine the formation and size of the autophagosome. Nucleation, expansion, uncoating, and completion of the autophagosome then occurs, priming it to fuse with lysosomes (3). The initiating signal for autophagosome formation is poorly understood, but the mammalian target of rapamycin (mTOR) is a negative regulator, and the extent of autophagy is regulated by proteins upstream of mTOR signaling, including PTEN, PDK1, Akt, and TSC1/2 (4). For example, PTEN and TSC1/2 positively regulate autophagy, whereas Akt inhibits it. Downstream targets of mTOR,

including elongation factor-2 kinase (5) and S6 kinase (4), have been shown to regulate autophagy.

The role (or more likely roles because as we will discuss, distinct functions for autophagy occur at different times) of autophagy in cancer is a topic of intense debate. As mentioned above, autophagy allows a cell to respond to changing environmental conditions, such as nutrient deprivation. On starvation, autophagy is greatly increased, allowing the cell to degrade proteins and organelles and thus obtain a source of macromolecular precursors, such as amino acids, fatty acids, and nucleotides, which would not be available otherwise. Thus, autophagy serves a protective role, allowing cells to survive during nutrient deprivation. A dramatic demonstration of this was provided by Mizushima and colleagues who showed that perhaps the most abrupt nutritional stress that a mammal experiences, being cut off from the mother's blood supply at birth, will lead to death if autophagy cannot proceed (6). Further evidence for the importance of autophagy in protecting against nutritional stress comes from studies where tumor cells are deprived of growth/survival factors, leading to an increase in autophagy that prevents the cells from dying. Moreover, when autophagy is prevented under these conditions, the cells undergo apoptosis (7, 8). Thus, when tumor cells are starved, autophagy stops them from dying by inhibiting apoptosis. In a tumor, this may mean that autophagy keeps tumor cells alive when limited angiogenesis leads to nutrient deprivation and hypoxia; therefore, we would expect that increased autophagy would promote the growth of solid tumors, whereas reduced autophagy might provide a useful way to limit tumor growth.

In stark contrast to this potential cancer-promoting effect of autophagy, numerous lines of evidence indicate an anticancer role for autophagy. The autophagy gene *Beclin 1* (the mammalian counterpart of the yeast *Atg 6* gene), which is part of a type III phosphatidylinositol 3-kinase complex required for autophagic vesicle formation, is a haploinsufficient tumor suppressor in mice (9, 10) and is monoallelically lost in human breast, ovarian, and other tumors (1). Moreover, *p53* and *PTEN*, two of the most commonly mutated tumor suppressor genes, both induce autophagy (11, 12). Conversely, the oncogenic protein Bcl-2 directly interacts with Beclin 1 to inhibit autophagy (13). Because oncogenes can inhibit autophagy and tumor suppressors induce autophagy whereas a bona fide autophagy regulator is itself a tumor suppressor, these data suggest that autophagy serves an anticancer role. The mechanism through which autophagy inhibits tumor development is unclear. Possibilities include limiting tumor cell growth or reducing mutagenesis or other damage caused by reactive oxygen species by removal of damaged mitochondria and other organelles. Alternatively, autophagy may kill developing tumor cells. In support of this idea, a cell death pathway that involves both autophagy and apoptosis is selectively inactivated when primary epithelial cells become immortal (14), and in model systems of mammary acini formation, both apoptosis and autophagy are involved in the removal of epithelial cells to form luminal structures (15), suggesting that autophagy prevents early

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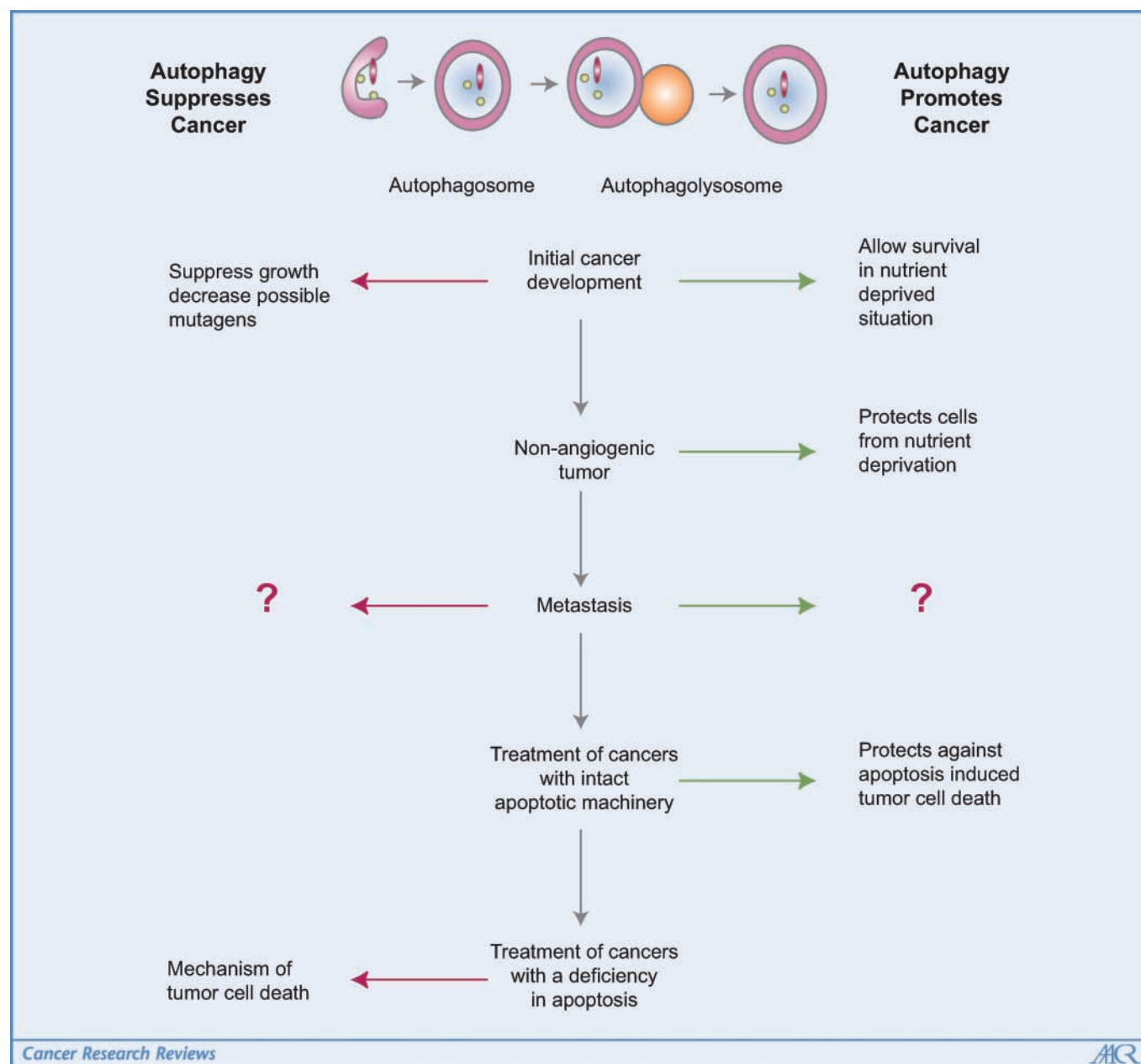


Figure 1. Contrasting roles for autophagy during cancer development, progression, and treatment. Stimulation of autophagy suppresses cancer development but may promote tumor growth and survival under conditions of nutrient deprivation in poorly angiogenic tumors. Autophagy may protect tumor cells from undergoing apoptosis in response to treatment with anticancer agents but may be a mechanism of tumor cell death in cells with defective apoptotic machinery.

steps in epithelial tumor development. Taken together, these data suggest that autophagy can both stimulate and prevent cancer depending on the context. To further test this idea, it will be interesting to examine mice with defects in other *Atg* genes to see if they too have a cancer predisposition phenotype similar to the *Beclin 1*^{+/-} mice.

More contradictory messages come when we consider how autophagy affects the ways that tumor cells die when we treat them with anticancer agents. Programmed cell death can be divided into apoptotic (type I) and autophagic (type II) cell death. In addition, there may be forms of programmed necrosis and other even less well defined death pathways. Although the molecular pathways involved in the execution and regulation of apoptosis have been

well defined, the mechanisms of autophagic cell death have not. In fact, aside from autophagic cell death being a caspase-independent process, attempts to define autophagic cell death have been largely limited to morphologic characteristics, such as extensive autophagosomal/autolysosomal formation and Atg-8/LC3 translocation to autophagic vesicles. Recently, however, Lenardo's group has defined one mechanism of autophagic cell death. These investigators showed that autophagic cell death caused by caspase inhibition is achieved through the selective autophagic degradation of catalase, which in turn leads to the generation of reactive oxygen species that kill the cell (16).

Many anticancer agents have been reported to induce autophagy, leading to the suggestion that autophagic cell death may be an

important mechanism of tumor cell killing by these agents (1). Anticancer agents that can induce autophagy include tamoxifen, rapamycin, arsenic trioxide, temozolomide, histone deacetylase inhibitors, ionizing radiation (1), vitamin D analogues (17), and etoposide (18). However, despite these examples, it is highly controversial whether autophagy really is an important cell death mechanism. Because autophagy occurs in tumor cells before their demise, it does not necessarily follow that autophagy killed the cells; instead, autophagy may be a mechanism by which the cell is trying to survive. To address this issue, one must show that inhibition of autophagy (the most rigorous way to do this currently is by small interfering RNA knockdown of *Atg* proteins) prevents cell death (19). Moreover, because any apparent protection caused by autophagy inhibition in short-term assays might represent only a delay in cell death rather than a true protective effect, it is essential to show that autophagy inhibition causes increased tumor cell clonogenic growth after treatment with the drug. In most of the examples cited above, this has not been shown; we only know that the drug induced autophagy and then the cells died. This is an important point because a recent article shows that rapamycin-induced autophagy can protect various tumor cell lines against apoptosis induced by general apoptotic stimuli (20) and might have a similar effect on the action of anticancer agents. Moreover, when it has been shown that knockdown of *Atg* genes does confer a clonogenic survival advantage to cells after treatment with anticancer agents as was shown for etoposide (18), the cells used have had profound defects in their apoptosis machinery. These data raise the question of whether autophagy is really an important mechanism of tumor cell killing by anticancer agents in cells that have the ability to undergo apoptosis. Rigorous examination of whether bona fide cancer drugs are actually capable of killing tumor cells via autophagy is needed. The answer to this question is important because it may determine how best to develop effective combination therapies by regulating autophagy along with anticancer agents.

In conclusion, we now have good reasons to think that manipulation of autophagy may provide a useful way to prevent cancer development, limit tumor progression, and increase the efficacy of cancer treatments. This goal seems reasonable because we have drugs that induce autophagy, such as rapamycin, and are rapidly gaining a better understanding of how this process works by studying the effects of targeted inactivation of autophagy

regulators in mouse models and human tumor cells. However, the question of whether we should try to switch autophagy on or off is not straightforward; sometimes, we will want to increase autophagy, whereas at other times we may want to reduce it. Therefore, unlike some other aspects of tumor cell biology, such as cell growth, apoptosis, or angiogenesis, where we always know how we would like to manipulate the process in the tumor (less, more, and less, respectively), our goals for manipulation of autophagy will likely be context dependent (Fig. 1). Perhaps we should try to increase autophagy to prevent tumor formation in individuals at risk for cancer but reduce autophagy if a tumor is already established and subject to the environmental stresses associated with limited angiogenesis, nutrient deprivation, etc. When considering treatment, we may want to reduce autophagy to increase the efficacy of anticancer agents that induce apoptosis but increase autophagy to allow anticancer drugs to work in tumor cells with fundamental defects in the apoptotic machinery. Because it has not really been addressed, we do not know how, if at all, autophagy might alter tumor metastasis and whether we want more or less autophagy to prevent metastatic progression.

This complex picture may be simply a consequence of dealing with a process that arose through natural selection and which we are thinking about in the context of a process (tumor growth and progression) that is itself driven by Darwinian evolution. Natural selection does not have to produce something that makes intuitive sense and a neat story, it just has to produce biological mechanisms that work well enough to be retained as genetically encoded traits. Therefore, finding that, during the evolution of a tumor, the same biological process might have tumor promoting and inhibiting properties at different times should not surprise us. This fact should, however, encourage us to better understand the nuances of how autophagy affects tumor development, progression, and treatment so that we can use this information to prevent and more effectively treat cancer.

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