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NEWS AND VIEWS

Animal genes identification and mTOR signaling reactivation in autophagy

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Autophagy (self-eating) is a self-degradation process essential for survival, differentiation, development, and homeostasis. Conceiving that a process of cellular self-eating could be beneficial may appear bewildering. In its simplest form, however, autophagy is probably a single cell's adjustment to starvation; the cell is forced to break down part of its own reserves to keep alive until circumstances improve (Mizushima and Klionsky, 2007). Based on its mechanism, physiological function, and cargo specificity, autophagy can be classified into at least three forms, chaperone-mediated autophagy, microautophagy, and macroautophagy (Klionsky, 2005), among which macroautophagy is the best characterized. Autophagy was discovered in mammalian cells and has been extensively investigated in yeast (Huang and Klionsky, 2002). Original studies in yeast *Saccharomyces cerevisiae* identified a group of autophagy (ATG) genes that are required for survival during starvation (Klionsky et al., 2003). Accumulating evidence indicates that many ATG genes are functionally conserved from yeast to mammals; nevertheless, autophagy is more complicated in multicellular organisms and probably requires factors that are absent in yeast. Until now, little is known about the mechanism of autophagy specific to mammals. In a groundbreaking investigation, Tian et al. (2010) discovered four novel genes particularly required for autophagy in multicellular organisms, and established *Caenorhabditis elegans* as one of the premier genetic models for uncovering new autophagy genes. The authors also isolated numerous new mutations in genes homologous to yeast autophagy genes, which confirmed the results of the study. These new mutations not only provide a valuable resource for inquiring the structure and function of autophagy proteins, but also set up *C. elegans* as a preeminent system for investigating the role and regulation of autophagy in multicellular organisms.

During autophagy, cytoplasmic proteins and organelles such as mitochondria are engulfed by a double-membrane autophagosome. The newly formed double-membrane vacuole consequently fuse with lysosomes to form the autophagolysosome, in which the engulfed cytoplasmic material is hydrolyzed by acid-dependent enzymes, ensuring amino acids and other macromolecular precursors can be recycled. In addition to recycling cytoplasmic material in response to starvation or stress, autophagy (mainly macroautophagy) also clears protein aggregates, eliminates or benefits invaded pathogens (Zhou et al., 2009), and influences or participates in cell death (Maiuri et al., 2007). Although it serves as a cell survival mechanism, once overactivated and allowed to progress, autophagy will eventually lead to cell death because of the depletion of cell organelles and critical proteins (Levine and Kroemer, 2008).

Cell death plays essential roles in organ development, tissue homeostasis, and degenerative diseases. Based on the distinct cell morphology, cell death has been categorized into apoptosis, autophagy, and necrosis. Apoptotic cell death is characterized by the activation of caspase and formation of apoptotic bodies, whereas autophagic cell death is distinguished by large-scale sequestration of a portion of the cytoplasm into autophagosomes, leading to a characteristic vacuolated manifestation in cells (Baehrecke, 2005). Accumulating evidence suggests the existence of molecular connections during apoptosis, autophagy, and necrosis. In response to specific perturbations, the same input signal can cause cells to shift from one cell death mode to another, and a mixed type of cell death was also observed in other cases. Depending on cell type, stimulus, and environmental conditions, three different types of crosstalk between autophagy and apoptosis have been suggested: autophagy can act as a partner, a suppressor, or a stimulator of apoptosis (Eisenberg-Lerner et al., 2009).

Autophagy is regulated by multiple signaling pathways, including GTPase Gi3, class III phosphatidyl inositol 3-kinase, serine/threonine kinases mammalian target of rapamycin (mTOR), protein kinase C, Erk, and MAPK p38. Among them, mTOR is best characterized for its negative regulatory role in

autophagy (Pattingre et al., 2008). While phosphatidylinositol 3-kinase (PI3K)/Akt can be an upstream activator, the phosphorylated 70-kDa ribosomal protein kinase (P70S6K) is one of the well-recognized downstream targets of mTOR. Despite the established role of mTOR/P70S6K in autophagy, recent findings revealed that the process can be induced independently of mTOR signaling. Furthermore, P70S6K can be phosphorylated without the involvement of mTOR kinase.

Generally, autophagy is inhibited by TOR-dependent signaling. Troglitazone, a synthetic ligand of peroxisome proliferators activated receptor-γ (PPARγ), was found to stimulate autophagy independent of mTOR phosphorylation (unpublished data). Sarkar et al. (2007) reported that trehalose promotes autophagy in an mTOR-independent manner. In another study, Corcelle et al. (2006) revealed that inhibition of mTOR signaling does not account for lindane (γ-hexachlorocyclohexane)-induced autophagy. Thus, under certain circumstance, these two pathways may be truly independent and may also be implemented using different components of autophagy machinery. mTOR tightly regulates all these effectors in most cases, yet exceptions exist. Tuberous sclerosis complex tumor suppressor genes inhibit P70SK phosphorylation through an mTOR-independent pathway, whereas troglitazone mediates the mTOR-independent inhibition of P70S6K phosphorylation in a cell type-dependent manner.

Recently, Yu et al. (2010) revealed a negative feedback mechanism that reverses autophagy and restores lysosome homeostasis in a variety of species. They termed it “autophagic lysosome formation” (ALR), wherein mTOR signaling can be reactivated during autophagic process. In rat kidney cells, mTOR signaling was inhibited at the initiation of autophagy, but was reactivated by prolonged starvation. They also demonstrated that the reactivation of mTOR was autophagy-dependent and required the degradation of auto-lysosomal products. The kinase reactivation is critical for proto-lysosomal tubule and vesicle generation, functional lysosome maturation, and complete lysosomal restoration.

The mTOR pathway is emerging as a key player in the etiology of certain cancers and metabolic diseases, including diabetes and obesity. Recent impressive advances in the understanding of upstream and downstream targets of mTOR offer rational explanations for the origin and progression of these diseases. For instance, insulin is a major upstream effecter of mTOR which enhances protein synthesis as part of the modulating anabolic process in response to glucose. Thus, deficiencies in mTOR signaling may lead to the development of glucose- and insulin-resistant type II diabetes. A link between mTOR pathway and cancer is also evident, as a number of the upstream and downstream components of mTOR are directly involved in cancer initiation and progression. Further knowledge of the mTOR signaling pathway could potentially lead to the design of drugs for treating diabetes and cancer.

Cancer is a major disease genetically associated to autophagy malfunction. In 40%–70% of cases of human breast, ovarian, and prostate cancer, the ATG gene beclin 1 is monoallelically deleted. Mice with a heterozygous disruption of beclin 1 have reduced autophagy and are more susceptible to develop spontaneous lymphomas, lung carcinomas, hepatocellular carcinomas, and mammary precancerous lesions (Takahashi et al., 2007). Accumulating evidence reveals that autophagy signaling regulation is tightly linked to oncogenic signaling pathways and is important for regulating cancer development and progression, as well as for determining sensitivity of tumor cells to anticancer therapy. Nevertheless, the role of autophagy in these processes is complicated and may have contrary consequences for tumor growth depending on the circumstances. Even though induction of autophagy is generally observed as a response to various experimental anticarcinogenic treatments, its effect on mediating apoptosis sensitivity or apoptosis resistance seems to depend on experimental models. Research data also support that autophagy plays a dual role in cancer (Baehrecke, 2005). Under androgen deprivation conditions, LNCap cells (prostate cancer) can resort to autophagy for survival (Li et al., 2008). In HeLa cells (human epithelial cervical cancer), down-regulation of ATG6 expression sensitizes cell to apoptotic cell death (Cho et al., 2009). Currently, we found that silencing essential autophagic genes can provide some protection for HCT116 cells (colon carcinoma cell) upon the apoptotic inducer challenge (unpublished data). Nowadays, people believe that an enhanced basal level of autophagy in highly resistant cancer cells may promote growth and therapeutic resistance; however, interfering with this fine-tuned balance between high levels of degradation and recycling in either inhibition or induction of autophagy would shift the cellular response to apoptosis or type III cell death process (Edinger and Thompson, 2004). However, inhibition of autophagy in sensitive cancer cells with a low basal autophagy level may enhance the apoptotic effect of anticancer treatment.

Analysis of the regulatory mechanism of autophagy in cell survival and cell death is clearly vital for understanding the physiological significance of autophagy in humans. Future studies toward this direction could yield valuable information on the development of effective cancer therapeutic strategies through the modulation of autophagy (Edinger and Thompson, 2004).

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