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Autophagy: Cancer's Friend or Foe?

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Abstract

The functional relevance of autophagy in tumor formation and progression remains controversial. Autophagy can promote tumor suppression during cancer initiation and protect tumors during progression. Autophagy-associated cell death may act as a tumor suppressor, with several autophagy-related genes deleted in cancers. Loss of autophagy induces genomic instability and necrosis with inflammation in mouse tumor models. Conversely, autophagy enhances survival of tumor cells subjected to metabolic stress and may promote metastasis by enhancing tumor cell survival under environmental stress. Unraveling the complex molecular regulation and multiple diverse roles of autophagy is pivotal in guiding development of rational and novel cancer therapies.

1. INTRODUCTION

Stress stimuli, including metabolic stress, activate cellular mechanisms for adaptation that are crucial for cells to either tolerate adverse conditions or to trigger cell suicide mechanisms to eliminate damaged and potentially dangerous cells (Hanahan & Weinberg,

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2011). Stress stimulates autophagy, in which double membrane vesicles form and engulf proteins, cytoplasm, protein aggregates, and organelles that are then transported to lysosomes where they are degraded, thereby providing energy (Klionsky & Emr, 2000; Mizushima, Ohsumi, & Yoshimori, 2002). Constitutive, basal autophagy also plays a significant homeostatic function, maintaining protein and organelle quality control and acting simultaneously with the ubiquitin proteasome degradation pathway to prevent the accumulation of polyubiquitinated and aggregated proteins (Klionsky & Emr, 2000). Autophagy-defective mice display signs of energy depletion and reduced amino acid concentrations in plasma and tissues and fail to survive in the neonatal starvation period, providing a clear example of autophagy-mediated maintenance of energy homeostasis (Kuma et al., 2004). Autophagy is also a pathway that is used for the elimination of pathogens (Colombo, 2007) and for the engulfment of apoptotic cells (Qu et al., 2007). Peptides generated from proteins degraded by autophagy can also be used for antigen presentation to T-cells for regulation of immunity and host defense (Crotzer & Blum, 2009; Levine, Mizushima, & Virgin, 2011). The importance of autophagy as a homeostatic and regulatory mechanism is underscored by the association of autophagy defects in the etiology of many diseases, including cancer (Levine & Kroemer, 2008).

Cancer is a multifaceted complex disease characterized by several defining properties, including avoidance of cell death (Hanahan & Weinberg, 2011). The ability of cancer cells to resist apoptotic cell death is a well-known mechanism that is the key to their survival and aggressiveness. Similarly, the phenomenon of autophagy in cancer has been studied extensively, and it is now firmly established that autophagy can provide both tumor-suppressive and tumor-promoting functions (Høyer-Hansen & Jäättelä, 2008; Maiuri et al., 2009). This review focuses on the tumor-suppressive and tumor-promoting properties of autophagy during different stages of cancer development. It provides insights into how autophagy's tumor-suppressive properties, which are frequently observed at the initial stage of cancer development, are later transformed into tumor-promoting potential during cancer progression.

2. AUTOPHAGY AND AUTOPHAGIC DEATH

Autophagy (from the Greek word "auto," meaning oneself and "phagy," meaning to eat) refers to a process by which cytoplasmic constituents are delivered to the lysosome for bulk degradation (Mizushima & Klionsky, 2007; Mizushima et al., 2002). The term autophagy originated when the Nobel laureate Christian de Duve used it while attending the *Ciba Foundation Symposium on Lysosomes*, which took place in London on February 12–14, 1963. Autophagy is classified into three main types depending on the different pathways in which cargo is delivered to the lysosome or vacuole: chaperone-mediated autophagy, microautophagy, and macroautophagy (Yorimitsu & Klionsky, 2005). In this review, we focus on the most widely investigated autophagic process: macroautophagy (herein, referred to as autophagy) with formation of autophagosomes and autolysosomes. Autophagosomes are double-membrane cytoplasmic vesicles, which engulf various cellular constituents, including cytoplasmic organelles. Autophagosomes fuse to lysosomes to become autolysosomes, where sequestered cellular materials are digested (Mizushima et al., 2002). The molecular basis of autophagy has been extensively studied, mainly in yeasts, through

investigation of autophagy-defective mutants to identify the responsible genes (designated as AuTophaGy; *atg*), and presently 35 *atg* genes have been discovered in yeast (Nakatogawa, Suzuki, Kamada, & Ohsumi, 2009). The basic mechanism of autophagy is well conserved during evolution as varied organisms, including plants, flies, yeast, and mammals, all of which contain a related group of *atg* genes, in spite of the fact that there are some differences between yeast and man (Klionsky, 2007).

The fundamental components of the autophagic process (Fig. 2.1) include phagophore formation, elongation and multimerization of phagosomes, cargo selection and lysosomal fusion. These components of autophagy will be discussed below.

2.1. Phagophore formation and regulation

The initial step of phagophore membrane formation in mammals remains elusive and has not been adequately defined, whereas in the yeast system this pathway is well defined. Unlike yeast, in the mammalian system there are no reports of preautophagosomal structures (Klionsky, 2007; Yorimitsu & Klionsky, 2005). Target of rapamycin (TOR) kinase acts as a molecular sensor to various stress responses, including hypoxia, insulin signaling, and energy and nutrient depletion, playing a pivotal role in cellular growth and autophagy control (Kamada et al., 2010). Initial nutrient starvation inactivates TOR kinase, resulting in a hypophosphorylated Atg13 that shows an increased affinity for Atg1 kinase (mammalian homolog of Ulk1) and forms a complex with a scaffold-like protein Atg17 (Fig. 2.1; Mizushima, 2010). Starvation treatment enhances the crosstalk between Atg13, Atg1, and Atg17. Atg13 and Atg17 are both required for appropriate monitoring of the kinase activity of Atg1. In turn, Atg1 regulates the transmembrane protein Atg9. The kinase activity of Atg1 is dispensable; however, it controls the dynamics of Atg9 recruitment to the phagophore in an Atg17-dependent pathway (Sekito, Kawamata, Ichikawa, Suzuki, & Ohsumi, 2009; Simonsen & Tooze, 2009). Atg9 is involved in lipid import from different sources like the endoplasmic reticulum (ER), endosomes, mitochondria, golgi bodies, and nuclear envelope and also helps in the assembly of the intact phagophore membrane (Axe et al., 2008; Simonsen & Tooze, 2009; Yorimitsu & Klionsky, 2005).

2.2. Elongation and multimerization of phagophores

The phagophore is elongated when Class III PI3 kinases, for example, Vps34 (vesicular protein sorting), bind to Beclin1 (mammalian homolog of yeast Atg6) increasing its catalytic activity to produce PI3P (phosphatidyl inositol-3-phosphate) (Simonsen & Tooze, 2009). PI3P acts as an important localization signal and may facilitate fusion at the final step of autophagosome formation (Axe et al., 2008). Vps34–Beclin1 interaction is upregulated by proteins like Ambra (activating molecule in Beclin1-regulated autophagy protein 1), UVRAG (ultraviolet radiation resistance-associated gene), and Bif 1 (Bax-interacting factor 1). In contrast, this interaction is downregulated by Bcl-2, Bcl-X_L, and Rubicon (RUN domain and cysteine-rich domain-containing Beclin1-interacting protein) (Funderbur, Wang, & Yue, 2010). Two ubiquitin-like conjugation systems are part of the vesicle elongation process. In one experimental system, Atg12 binds Atg7 (E1 ubiquitin-like activating enzyme) in an ATP-dependent manner. Next, Atg12 non-covalently binds Atg10 (E2-like ubiquitin carrier) linking Atg12–Atg5; Atg16 dimers conjugate with this complex via C-

terminal coiled-coil domain to facilitate the creation of the expanding phagophore. The Atg5–Atg12–Atg16 complex induces curvature formation in the growing phagophore. However, this trimeric structure dissociates when the phagophore progresses into a double-membrane ring called an "autophagosome" (Geng & Klionsky, 2008). The second ubiquitin-like system, which plays a pivotal role in autophagosome formation, helps in the processing of microtubule-associated light chain 3 (LC3) (mammalian homolog of Atg8). Cysteine proteinase Atg4 (also known as autophagin) cleaves LC3 to produce LC3BI that binds E1-like Atg7 in an energy-expending pathway resulting in activation of LC3BI. Atg3, an E2-like carrier, interacts with activated LC3BI and promotes lipidation, giving rise to LC3BI–phosphatidylethanolamine (PE) conjugate or LC3BII (Kabeya et al., 2000).

The sequential order of mammalian autophagosome biogenesis begins with activation of an Ulk1/2 (UNC-51-like kinase 1/2) complex, which associates with the initiating phagophore membrane. The Vps34 complex is recruited to the phagophore and phosphorylates phosphoinositides (PIs), leading to the production of PI3P. WIPI1/WIPI2 (WD repeat protein-interacting with PIs) and DFCP1 (double FYVE domain-containing protein), two PI3P effectors, contribute to this nascent elongating membrane. The Atg12–Atg5 complex serves an E3-type enzyme function and acts as a supporting framework to which Atg8s arrive at the phagophore (Hanada et al., 2007; Tanida, Ueno, & Kominami, 2004). Atg16L joins the Atg12–Atg5 complex and helps to govern at which site of the membrane the downstream conjugation of LC3 occurs (Fujita et al., 2008). Atg8 and GATE-16 (Golgi-associated ATPase enhancer) are recruited and conjugated to PE on the phagophore membrane, which starts elongating mediated through the action of LC3–PE. GATE-16 acts downstream of LC3 in a step coupled to the disassociation of the Atg12–Atg5 with Atg16L. Subsequently, a mature autophagosome bearing a double-membrane structure is formed (Weidberg, Shvets, & Elazar, 2011).

A recent report highlights the role of Atg14L, a subunit of PI3kinase involved in localization to the ER via four N-terminal cysteines, which accumulate in omegasomes (PI3P-rich Ω-shaped structure formed at the periphery of ER) upon autophagy induction in the process of autophagosome biogenesis (Matsunaga et al., 2010). Atg14L is able to target the PI3-kinase complex to the ER, enabling PI3P generation for omegasome and autophagosome formation. Although the role of specific kinases in autophagosome is well documented and emphasized, the role of antagonistic partners in this process, that is, phosphatases, is equally important (Vergne & Deretic, 2010). The phosphatidylinositol 3-phosphate (PI3P) phosphatase *Jumpy* (MTMR14) associates with isolated membranes during early autophagosome biogenesis that is guided by Atg16, which helps in the subsequent development and localization of autophagic organelles (Noda, Matsunaga, Taguchi-Atarashi, & Yoshimori, 2010; Vergne et al., 2009). *Jumpy* coordinates the recruitment of Atg factors in an orderly manner by interacting with PI3P through WIPI-1 (Atg18) thereby affecting the distribution of Atg9 and LC3, the factors responsible for controlling growth of the autophagic membrane.

2.3. Cargo selection

In general, autophagy has been considered a random process as it appears to engulf cytoplasm indiscriminately. Electron micrographs often show autophagosomes with wide-

ranging contents comprising mitochondria, ER, and golgi membranes. However, there is accumulating evidence that the growing phagophore membrane can interact selectively with protein aggregates and organelles. LC3B-II provides the role of “receptor” at the phagophore and interacts with “adaptor” molecules on the target including protein aggregates, damaged mitochondria, thereby helping in promotion of their selective uptake and degradation (Weidberg et al., 2011; Yorimitsu & Klionsky, 2005). The best-characterized molecule in this process is the multiadaptor molecule p62/SQSTM1, which binds Atg8/LC3 and promotes degradation of polyubiquitinated protein aggregates (Ichimura & Komatsu, 2010). Similarly, Atg32 has been identified in yeast as a protein that promotes selective uptake of mitochondria, a process known as mitophagy (Okamoto, Kondo-Okamoto, & Ohsumi, 2009).

2.4. Lysosomal fusion

Autophagosomes dock with lysosomes and fuse to give rise to structures known as “autolysosomes,” where the acidic lysosomal components digest all cargos. Migrating bidirectionally along microtubules, the autophagosomes have a natural propensity toward the lysosome-enriched microtubule organizing center, supervised by the function of dynein motor proteins (Kimura, Noda, & Yoshimori, 2008; Ravikumar et al., 2005; Williams et al., 2008). Small GTPases, like Rabs (Rab7), ESCRT (endosomal sorting complex required for transport), SNARE (soluble *N*-ethylmaleimide-sensitive factor activating protein receptor), and class C Vps proteins play key roles in the coordinated vesicular docking and fusion with target components (Atlashkin et al., 2003; Gutierrez, Munafo, Beron, & Colombo, 2004; Jager et al., 2004; Lee, Beigneux, Ahmad, Young, & Gao, 2007; Zerial & McBride, 2001). Accumulation of Rab proteins along specific intracellular niches triggers the last fusion step mediated by SNARE complexes (Martens & McMahon, 2008). It is worthwhile highlighting important proteins like ESCRT whose mutation or loss of function culminates in inhibition of autophagosome maturation. Although the role of UVRAG is most recognized as a Beclin1-interacting protein, it also has an independent function in the final maturation step by engaging the fusion machinery on autophagosomes. UVRAG is also known to engage the class C Vps proteins thereby activating Rab7, which helps to promote fusion with late endosomes and lysosomes. Another Beclin1-interacting protein Rubicon also modulates autophagosomal maturity. Rubicon remains part of a complex containing varied proteins, like UVRAG, hVps34, and hVps15, and is known to suppress autophagosomal maturation (Matsunaga et al., 2009; Zhong et al., 2009). Further research will help to clarify all of the key-interacting players involved in the crucial autophagy pathway.

Shifting the focus from the autophagosome to the lysosome, inhibiting the lysosomal H^+ ATPase by chemicals like bafilomycin A1, nocodazole, or vinblastine, will prevent the fusion of autophagosomes with endosomes/lysosomes (Fass, Shvets, Degani, Hirschberg, & Elazar, 2006; Köchl, Hu, Chan, & Tooze, 2006). A recent scientific report suggests an alternative autophagic pathway in mouse cells lacking Atg5 or Atg7 when treated with stress inducers, like etoposide. The key autophagic proteins operational in this Atg5/Atg7-independent route include Ulk1 and Beclin1 and proceed in a Rab9-dependent manner (Nishida et al., 2009).

Lysosomal permeases and transporters export essential products like fatty acids and amino acids back into the cytosolic pool. This replenishing phenomenon plays a survival role for starving cells, contributing to what is called “protective autophagy” (Mizushima et al., 2002; Yorimitsu & Klionsky, 2005).

2.5. Autophagic cell death: An elusive process

The current concept of programmed cell death involves three areas including apoptosis, autophagic cell death (ACD) and necroptosis. The autophagic pathway initially functions as an adaptive response to stress; however, when the cell continues to face extreme stress over a protracted period of time it reaches a point of no return and becomes committed to undergo cell death. This type of cell death that is associated with autophagosomes and is dependent on autophagic proteins is called “Autophagic cell death.” As indicated, extended autophagy beyond the optimal survival limit culminates in ACD (Fig. 2.1). ACD has gained immense attention among scientists since the 1990s, which has progressed rapidly due to the discovery of the *atg* genes, establishing a caspase-independent “type II programmed cell death” (Kroemer & Levine, 2008). The Nomenclature Committee on Cell Death (NCCD, 2005) classified ACD as cell death through autophagy, referring to this process as cell death with autophagy (Kroemer et al., 2005). Later in 2008, Kroemer and Levine characterized ACD morphologically (by transmission electron microscopy) as a cell death process occurring in the absence of chromatin condensation but characterized by large-scale sequestration of cytoplasmic components into autophagosomes, imparting a characteristic vacuolated appearance to the cell.

ACD is mainly a morphological phenomenon, and there currently is no conclusive evidence that a specific mechanism of autophagic death exists (Tsujimoto & Shimizu, 2005; Yorimitsu & Klionsky, 2005). It is difficult to define the pathophysiological role of ACD; hence, the attempt to hypothesize the reason for this mode of cell death remains elusive. Pinpointing the exact function of autophagy in programmed cell death is not only challenging but also equally complicated due to the simultaneous occurrence of caspase-dependent apoptosis, often occurring in the context of autophagy. Kinetically speaking, it can be anticipated that caspase-mediated proteolytic degradation would occur faster than self-digestion by autophagy. Accordingly, the cell would be experiencing a predominant extent of apoptosis in spite of extensive autophagy (Debnath, Baehrecke, & Kroemer, 2005). However, the cell displays a highly multifaceted crosstalk between the apoptotic and ACD mechanism(s); which can promote antagonism, synergism, or mutually independent pathways of induction that are context dependent. It is difficult to conceptualize how on one hand ACD is part of the cell death mechanism, while multiple studies also emphasize a protective role of ACD since autophagic inhibition does not stop cell death but may even promote death. Whether ACD is primarily a mediator of the death mechanism, an innocent bystander, or a double-edged sword in cell survival/death processes remains to be determined. It is also possible that this duality of functions depends on the temporal induction of autophagy and the context in which this pathway is induced or suppressed. It is clear that a complete understanding of the autophagic process as well as its mediators and determinants of expression represents an evolving story that will only become clarified with additional research.

3. AUTOPHAGY IN TUMOR INITIATION AND DEVELOPMENT

Autophagy is believed to play an essential role in tumor initiation and development (Chen & Debnath, 2010; Liang & Jung, 2010). When base-line levels of autophagy fluctuation were compared, the amount of proteolysis or autophagic degradation in cancer cells was less than that of their normal counterparts (Gunn, Clark, Knowles, Hopgood, & Ballard, 1977; Kisen et al., 1993). This differential expression suggests a direct connection between tumorigenesis and decreased levels of autophagy. Intriguingly, many oncogenes and tumor suppressor genes affect autophagic pathways (Maiuri et al., 2009), and the deregulation of the autophagic process contributes to malignant transformation. For example, many tumor suppressor proteins such as p53, phosphatase and tensin homolog (PTEN), death-associated protein kinase (DAPK), tuberous sclerosis 1 (TSC1), and TSC2 that provide constitutive input signals to activate autophagy are mutated in multiple cancers.

PTEN, a dual lipid/protein phosphatase, dephosphorylates PIP3 to PIP2, preventing inhibition of autophagy by the PI3K/Akt/mTOR pathway. In human tumors, PTEN is mutated, resulting in activation of Akt that suppresses autophagy (Maehama, 2007; Yin & Shen, 2008) and also participates in increased protein translation, cell growth, and cell proliferation, which is a contributor to tumorigenesis (Hafner et al., 2007; Horn et al., 2008; LoPiccolo, Blumenthal, Bernstein, & Dennis, 2008; Vivanco & Sawyers, 2002). Additionally, p53 plays a divergent role in the regulation of autophagy. Within the nucleus, p53 can act as an autophagy-inducing transcription factor through AMPK and TSC1/TSC2 dependent activation (Tasdemir et al., 2008). In contrast, cytoplasmic p53 exerts an autophagy-inhibitory function, and its degradation is actually required for the induction of autophagy. Although the relationship between autophagy and p53 is complicated, it is clear that p53 mutation(s) cause alterations in p53-mediated autophagy that leads to cancer development. Additionally, another target of p53 that is present in nucleus, DRAM (damage-regulated autophagy modulator), has been shown to positively regulate autophagy. DRAM is essential for p53-mediated autophagy and apoptosis in response to DNA-damaging agents, and the overexpression of DRAM is sufficient to activate autophagy without affecting apoptosis (Crighton et al., 2006). The fact that DRAM is also deleted in multiple types of cancer underscores its importance and highlights the possibility that autophagy might play a fundamentally important role in cancer.

The death-associated protein kinase (DAPK), a cytoskeleton-associated calmodulin-regulated serine/threonine protein kinase, has been shown to possess multiple tumor- and metastasis-suppressor functions (Bialik & Kimchi, 2006; Eisenberg-Lerner & Kimchi, 2009). It is often decreased or lost in many human cancers, and ectopic expression is associated with p53-mediated apoptosis and decreased cell migration and invasion. Moreover, DAPK expression has been shown to suppress formation of metastatic foci in Lewis lung carcinoma in mice (Inbal, Bialik, Sabanay, Shani, & Kimchi, 2002). Recently, DAPK and DAPK-related protein kinase-1 (DRP-1) have been found to activate autophagy in MCF-7 and HeLa cells. Expression of these genes triggered membrane blebbing and ACD and conversely inhibition of DAPKs resulted in decreased autophagy (Moretti, Yang, Kim, & Lu, 2007). Metabolic stress results in a decline in ATP:ADP ratios and induction of the tumor suppressor *LKB-I* (*STK-I*), which is serine threonine kinase that is upregulated in

the LKB-I/AMPK/mTOR pathway. It phosphorylates the a subunit at Thr172 (Shaw et al., 2004) and activates AMPK. AMPK either activates TSC2 and the regulatory-associated protein raptor by promoting phosphorylation of TSC2 or directly phosphorylating raptor, leading to inhibition of mTOR and autophagy induction (Corradetti, Inoki, Bardeesy, DePinho, & Guan, 2004). Moreover, the LKB-I/AMPK/mTOR axis activates p27, a cyclin-dependent kinase inhibitor, inducing cell cycle arrest for energy conservation (Liang et al., 2007).

Conversely, oncogenes including *Akt*, mTOR, Bcl-2, and FLICE-like inhibitory protein (FLIP) inhibit autophagic processes indicating that elevated autophagy signaling may contribute to tumor suppression (Lee et al., 2009; Morselli et al., 2009). In most cancers, the PI3K–Akt axis undergoes mutation in either upstream or downstream regulators (Shaw & Cantley, 2006). Thus, downstream activation of mTOR favors cell growth stimulation and inhibition of autophagy. Constitutive activation of Akt inhibits autophagy *in vitro* and *in vivo* in Bax and Bak double mutants. Monoallelic knockout of *beclin1* or biallelic knockout of *atg5* in mice promotes genomic alterations due to activation of Akt (Karantza-Wadsworth et al., 2007; Mathew, Karantza-Wadsworth, & White, 2007; Mathew, Kongara, et al., 2007). The BH3 receptor domain of Bcl-2 and the multidomain anti-apoptotic proteins bind with the amphipathic BH3 helix of Beclin1 (Oberstein, Jeffrey, & Shi, 2007), promoting its sequestration and blocking its interaction with Vps34. Overexpressed Bcl-2 along with deletion in one allele of beclin1 accelerates tumor growth *in vivo* (Degenhardt et al., 2006). Notably, the Bcl2 gene is overexpressed in a majority of cancers (Levine, Sinha, & Kroemer, 2008; Pattingre & Levine, 2006), and knockdown or gene silencing of Bcl-2 through antisense oligonucleotides or siRNA heteroduplexes, respectively, in MCF-7 cells results in induction of autophagy (Akar et al., 2008).

Identification of *beclin1* as a haploinsufficient tumor suppressor frequently containing deletion of one allele in a large proportion of human breast, ovarian, and prostate cancers provided initial evidence for a potential direct link between autophagy and cancer. Notably, overexpression of Beclin1 in MCF-7 breast carcinoma cells induced autophagy and restricted proliferation and clonigenicity *in vitro*, and it also inhibited tumorigenesis in nude mice (Liang et al., 1999). The contribution of the allelic deletion of *Beclin1* to carcinogenesis is supported by the observation that *beclin1*+/− mice have an increased incidence of lung cancer, hepatocellular carcinoma (HCC), and lymphoma (Yue, Jin, Yang, Levine, & Heintz, 2003; Qu et al., 2003). Furthermore, allelic loss of *beclin1* in HCC causes accumulation of p62/SQSTM (Mathew et al., 2009). ER chaperones and damaged mitochondria result in an elevated production of ROS (reactive oxygen species). Generation of ROS promotes a cascade of events, including increased oxidative stress, DNA damage, and chromosomal instability, which ultimately lead to inhibition of the NF-kB pathway and development of HCC. Consequently, tumor-suppressive or -supportive properties of Beclin1-interacting molecules including UVRAG, Bif-1, and Rubicon have been documented (Funderbur et al., 2010). UVRAG activates Beclin1 and induces autophagosome formation. Ectopic expression of UVRAG suppresses the proliferation and tumorigenicity of HCT116 tumor cells and sensitizes these cells to undergo self-directed autophagy even without starvation treatment (Liang et al., 2006). Monoallelic loss of

UVRAG is observed in various colon cancer cells and tissues. Nonsense mutations of *UVRAG* are observed in colon and gastric cancers resulting in inactivation of autophagy (Ionov, Nowak, Perucho, Markowitz, & Cowell, 2004; Kim et al., 2008). Moreover, Bif 1 (also known as Endophilin B1) interacts with Beclin1 through UVRAG (Takahashi et al., 2007). Downregulation of *Bif 1* is frequently found in different types of cancer, and loss of *Bif 1* leads to suppression of autophagy by decreasing Vps34 kinase activity, which promotes colon adenocarcinoma formation (Coppola et al., 2008). Moreover, *Bif* $1^{-/-}$ mice are cancer prone (Takahashi et al., 2007). By contrast, Rubicon, a newly identified Beclin1-interacting protein, reduces Vps34 lipid kinase activity and downregulates autophagy (Matsunaga et al., 2009; Zhong et al., 2009) with aberrant expression in multiple types of cancer. Thus, Beclin1 acts as a nodal point and activates PI3K III, revealing its mandatory role in autophagy and tumorigenesis (Table 2.1).

Additional autophagic genes involved at different stages of autophagy have been identified as contributors to the oncogenic and tumor-suppressive signaling pathways of cancer (Table 2.1). For instance, Atg7 contributes to the maintenance of HSCs (hematopoietic stem cells) (Mortensen et al., 2011). *Atg7*-deficient LSK ($Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$) cells show defects in HSC functions with impairment of production of both lymphoid and myeloid progenitors in lethally irradiated mice. Similarly, suppression of *Atg5* and *Atg16L1* genes leads to tissue injury in intestinal Paneth cells culminating in Crohn's death, a known risk factor for colorectal cancer in humans (Cadwell et al., 2008). Moreover, mice in which *Atg5* and liver-specific *Atg*$7^{-/-}$ have been deleted develop liver cancer and display mitochondrial swelling, p62 accumulation, and oxidative stress and genomic damage responses in isolated hepatocytes (Takamura et al., 2011). Amino acid depletion, which hinders metabolism, occurs during the survival period of neonatal mice deficient in *Atg5*. Moreover, mice with *Atg4C* deficiency have increased tendency for carcinogen-induced tumorigenesis (Marino et al., 2007).

Cellular senescence is a state of stable dynamic cell cycle arrest that limits the proliferation of damaged cells and has been regarded as a tumor suppressor mechanism. A recent study showed that autophagy was activated during senescence, and its activation was correlated with negative feedback in the PI3K–mTOR pathway (Young et al., 2009). A subset of autophagy-related genes is upregulated during senescence. For instance, overexpression of ULK3/Atg3-induced autophagy and senescence contributed to tumor suppression. Furthermore, inhibition of autophagy delayed the senescence phenotype, including senescence-associated secreted factor.

4. AUTOPHAGY IN TUMOR PROGRESSION AND METASTASIS

Tumor metastasis is a complex, multistep process by which tumor cells from a primary site migrate to and colonize at distant organ sites (Fig. 2.2; Das et al., 2012). This process involves multiple, discrete steps including invasion of tumor cells from the primary tumor site, intravasation and survival in the blood stream, extravasation at a distant site, and finally colonization of disseminated tumor cells (DTCs) at distant sites (Bingle, Brown, & Lewis, 2002; Das et al., 2012). In primary tumors, inflammatory cells infiltrate tumor sites in response to necrosis resulting from hypoxia and metabolic stress (Degenhardt et al., 2006;

Jin & White, 2007). Protective autophagy, promoted by hypoxia and metabolic stress, inhibits inflammation at primary sites that is required for initiation of metastasis. Interestingly, autophagy reduces necrosis and subsequent macrophage infiltration thereby decreasing primary tumor growth, and genetic inhibition of autophagy can cause cell death, tissue damage, and chronic inflammation (Mathew, Karantza-Wadsworth, et al., 2007; Mathew, Kongara, et al., 2007). Another important function of autophagy is clearance of cellular debris accumulated as unfolded protein, damaged organelles, and high-cargo receptor p62 in response to metabolic stress during tumor progression. When autophagy is defective, cellular toxic substances are not degraded which cause ROS production followed by DNA damage and chromosomal instability that initiate metastasis (Mathew et al., 2009). Additionally, autophagy activates a proinflammatory immune response by enhancing the release of immunostimulatory molecules including high-mobility group box protein-1 (HMGB-1) from dying tumor cells which mediate antitumor immunity (Fig. 2.3; Thorburn et al., 2008).

Apart from autophagy's antimetastatic properties, it can also have an opposite effect enhancing the metastatic potential of tumor cells. It is well established that death ligand-induced apoptosis, particularly TNF-related apoptosis inducing ligand (TRAIL), plays a critical role in regulating the suppression of metastasis by T-cells and NK cells (Wang, 2008). But recently it was demonstrated that protective autophagy is upregulated in TRAIL-resistant cancer cells, which enhances viability and survival of tumor cells during metastasis (Fig. 2.3; Han et al., 2008; Herrero-Martin et al., 2009).

Another important property of metastatic cancer cells is resistance to anoikis, apoptosis associated with lack of proper extracellular matrix attachment (ECM) (Taddei, Giannoni, Fiaschi, & Chiarugi, 2012). Aberrant activation of growth factor pathways including Ras/MAPK and PI3K/Akt pathways is a common mechanism utilized by cancer cells to evade anoikis. Although autophagy-mediated cell death was initially recognized in association with anoikis, a recent study also supports the protective nature of autophagy in anoikis (Kenific, Thorburn, & Debnath, 2010). Fung, Lock, Gao, Salas, and Debnath (2008) demonstrated autophagy induced by detachment from the substratum or inhibition of the b1-integrin receptor, and inhibition of autophagy by knockdown of *atg* genes enhanced detachment-induced cell death. Anoikis resistance in tumor cells is protective and facilitates survival and expansion of metastatic cells. Although the detailed mechanisms of protective autophagy in anoikis resistance remain largely unknown, a recent study indicates that PERK, an ER kinase, facilitates survival of ECM-detached cells by promoting autophagy and antioxidant activity induced through ROS generation, which may provide fitness to cells without ECM contact during later stages of cancer dissemination and metastasis (Fig. 2.3; Avivar-Valderas et al., 2011).

Alterations in cellular metabolism, an important hallmark of cancer, are employed by neoplastic cells to adapt to specific growth requirements during cancer progression (Hanahan & Weinberg, 2011; Mizushima & Klionsky, 2007). Cancer cells specifically consume glucose through anaerobic glycolysis during hypoxic conditions, which is known as the "Warburg effect," with high levels of glycolytic intermediates and lactate as reported in human colon and gastric cancers (Hirayama et al., 2009). In addition, cancer cells also

display increased glutamine utilization. Autophagy induced under stressful conditions causes high degradation and recycling of proteins providing substrates for energy and carbon/nitrogen sources for biomass production required for rapidly proliferating cancer cells (Mizushima & Klionsky, 2007). Similarly, intracellular fat storage provides acetyl-CoA for mitochondria to support the TCA cycle through lipophagy to meet the elevated metabolic demands of deregulated tumor cell growth (Rabinowitz & White, 2010). Another important property of autophagy is preservation and maintenance of organelle function, especially mitochondria that are required for cell growth during tumor progression, whereas it prevents tumor growth by reducing tissue damage and necrosis during cancer initiation (Twig et al., 2008). Damaged mitochondria are the major site of ROS production in cells, and mitochondria-selective autophagy, "mitophagy," clears depolarized mitochondria and maintains cellular homeostasis (Wu et al., 2009). Recent studies indicate that Ras-expressing cells have upregulated basal autophagy that is required to maintain the pool of functional mitochondria necessary to support growth of aggressive tumors (Guo et al., 2011).

As tumors grow with defects in apoptosis and following long-term metabolic stress conditions, they may require autophagy to survive in nutrient-limited and low-oxygen conditions, especially in the central area of the tumor, which is often poorly vascularized. Survival through autophagy is a key process enabling long-term tumor cell viability and eventual regrowth and tumor recurrence. Accordingly, induction of autophagy allows cancer cells to survive in low-nutrient and low-oxygen conditions through activation of HIF-1 (hypoxia-inducible factor) and AMPK (5′-AMP-activated protein kinase) (Eisenberg-Lerner & Kimchi, 2009; Mathew, Karantza-Wadsworth, et al., 2007; Mathew, Kongara, et al., 2007). For instance, the oncogenic gene astrocyte elevated gene-1 (Emdad et al., 2009; Kang et al., 2005; Su et al., 2002) was associated with protective autophagy through AMPK/mTOR-dependent pathway and inhibition of the protective autophagy by ATG-5 knockdown provided therapeutic benefits (Fig. 2.4; Bhutia, Dash, et al., 2010; Bhutia, Kegelman, et al., 2010). HIF-1 and AMPK are components of a concerted cellular response to maintain energy homeostasis in oxygen- and nutrient-limited tumor microenvironments.

5. AUTOPHAGY IN TUMOR DORMANCY

Tumor dormancy is a protracted stage in tumor progression in which tumors remain occult and asymptomatic for extended periods of time. This state can be present as one of the earliest stages in tumor development, as well as in the in micrometastasis stage, and can occur when minimal residual disease remains after surgical removal or treatment of primary tumors (Almog, 2010). Clinically, the dormant tumor cells are not easily detected representing a major problem in breast cancer, ovarian cancer, and other malignancies. Apart from analysis of autopsies of trauma victims and clinical data accumulating from patients with late recurrence or relapse, recently DTCs and circulating tumor cells (CTCs) in cancer patients provide data relative to the frequency and prevalence of tumor dormancy. Tumor dormancy can result from angiogenesis arrest, a balance between apoptosis and cell proliferation, cell cycle arrest, and immune surveillance (Pantel, Alix-Panabières, & Riethdorf, 2009). Recently, the role of autophagy in tumor dormancy has been recognized (Fig. 2.3). Tumor cells in dormant conditions are not efficiently associated with extracellular matrix and stimulate autophagy for survival and maintenance of dormancy. Impaired b1-

integrin signaling, a known inducer of autophagy, has been shown to promote dormancy in MMTV-PyMT breast cancer model (White et al., 2004). In breast cancer metastases to bone, disseminated cells displayed Src-mediated TRAIL resistance and remained dormant in the bone marrow for extended periods of time (Zhang et al., 2009). As mentioned earlier, autophagy can protect cells from TRAIL-induced apoptosis. Based on this consideration, it is speculated that dormancy in the bone marrow could induce protective autophagy and support survival of dormant cells (Kenific et al., 2010). A direct link between autophagy and tumor dormancy was recently demonstrated in ovarian cancer cells. The tumor suppressor aplasia Ras homolog member I (ARHI) induced autophagic death in ovarian tumor cells *in vitro*. However, in xenografted tumors in mice ARHI-induced autophagic death was switched to dormant tumor cell survival in the context of the tumor microenvironment suggesting that autophagy may be a prerequisite for tumor dormancy (Lu et al., 2008). Future studies focused on defining the precise mechanism by which protective autophagy is associated with tumor dormancy is warranted and holds potential for defining new therapeutic strategies for treating cancer.

6. AUTOPHAGY IN CANCER INITIATING/STEM CELLS

The “stem cell hypothesis” embodies the concept of tumor cell heterogeneity, in which only tumor-initiating cells in the heterogeneous tumor population are capable of proliferating and differentiating into new tumor-producing cells. Stem cells form the apex of the tumor hierarchy and retain the capability to replicate and grow into a tumor *in vivo*. It has been argued that cancer initiating or tumor-initiating stem cells (CSCs) do not arise from normal stem cells (Clevers, 2011). They can arise from subpopulation of cancer cells, including cancer initiating stem cells, cancer progenitor cells, or differentiated cancer cells. The phenotype with respect to the self-renewal and differentiating capacity of CSCs depends upon tumor type and is quite predictable (Zhou et al., 2009). Cancer initiating/stem cells have now been identified and isolated from tumors of the hematopoietic system, skin, breast, brain, prostate, colon, head and neck, and pancreas, and reduced CSC numbers can initiate tumor growth in xenograft models (Fu et al., 2009). This sub-population within the tumor evades therapy, persists, and initiates recurrence thereby enhancing malignant spread of the disease. Several pathways over-expressed in different types of cancers including Wnt/Notch/Hedgehog have been identified and shown to be critical to the self-renewal behavior of CSCs. Moreover, CSCs show resistance to apoptosis; they have high expression of ATP-binding cassette transporters, and display enhanced DNA repair capacity making therapy extremely difficult. Apart from its functions in cancer, autophagy plays a seminal role in maintaining and modulating growth and survival of cancer initiating/stem cells.

Autophagy is downregulated in specific CSCs as compared to the remaining portion of cancer cells. For example, brain CSCs ($CD133^+$) display decreased expression of autophagy-related proteins and are more resistant to temozolomide as compared to putative brain cancer $CD133^-$ nonstem cells (Fu et al., 2009). In contrast, a recent study showed that autophagy in CSCs provides a protective effect to current cancer therapeutics, and inhibition of protective autophagy improves therapeutic response. For example, radiation-induced autophagy in glioma CSCs and the $CD133^+$ cells exhibited a larger degree of autophagy compared with the $CD133^-$ cells. Moreover, the $CD133^+$ cells expressed higher levels of

LC3-II, Atg5, and Atg12, and inhibition of autophagy sensitized these cells to g-radiation (Lomonaco et al., 2009). Similarly, therapy with tyrosine kinase inhibitors is associated with drug resistance in chronic myeloid leukemia (CML) CSCs, and autophagy is one of the mechanisms involved in this protective response. Suppression of autophagy using either pharmacological inhibitors or RNA interference of essential autophagy genes results in nearly complete elimination of phenotypically and functionally defined CML CSCs (Bellodi et al., 2009). However, these contradicting reports need to be reconciled by further experimentation. Understanding the role of autophagy in CSCs holds significant promise for enhancing cancer therapies (Fig. 2.3).

7. AUTOPHAGY IN CANCER THERAPY

Because cancer cells often display defective autophagic capacities, induction of ACD is viewed as a tumor suppressor mechanism. Induction of autophagic death, “type II programmed cell death,” could be a useful therapeutic approach for apoptosis-resistant cancer cells and could provide a complementary approach along with apoptosis in promoting cancer cell death. On other hand, autophagy has been shown to provide resistance to therapy-mediated tumor cell death. When tumor cells induce protective autophagy, inhibition of autophagy may provide a way of sensitizing tumor cells to therapy by activating apoptosis. ACD by anticancer drugs may occur depending on cell type and genetic background. Based on the type of treatment, different signaling pathways can be activated in the same cell and produce varied types of autophagy. Understanding whether autophagy will be “protective” or “toxic” is a key area for further development and will define whether it is appropriate to block or promote autophagy in specific cancer contexts (Chen & Karantza, 2011; Kondo, Kanzawa, Sawaya, & Kondo, 2005; White & DiPaola, 2009).

7.1. Stimulation of autophagic cell death

Therapeutic induction of ACD through overstimulation of autophagy remains an important approach for tumor cell elimination. A number of studies have reported that ACD is activated in cancer cells derived from tissues such as breast, colon, prostate, and brain, in response to various anticancer therapies. The consequence of promoting autophagy depends on multiple factors, including extent of induction, duration, and cellular context. Several chemotherapeutic drugs (alkylating agents, actinomycin D, arsenic trioxide), radiation and photodynamic therapy, hormonal therapies (tamoxifen and vitamin D analogs), cytokines (IFN-g), gene therapies (p53, *mda*-7/IL-24, and $p27^{Kip1}$), and natural compounds (resveratrol and plant lectins) have been shown to trigger ACD in various cancer cells *in vitro* (Chen & Karantza, 2011). Accumulating evidence indicates that autophagic death contributes to *in vivo* antitumor effects. For instance, a natural BH3-mimetic, small-molecule inhibitor of Bcl2, (−)-gossypol, shows potent antitumor activity in ongoing phase II and III clinical trials for human prostate cancer. The antitumor activity by (−)-gossypol is mediated through induction of both apoptosis and autophagic death (Lian et al., 2011). ACD can occur independently or it can act synergistically or assist apoptotic cell death (Kondo et al., 2005; Maiuri et al., 2009). Interestingly, combining two therapies that trigger autophagy by targeting different pathways increased sensitivity to ACD, an alternative form of

programmed cell death to promote synergistic cancer inhibitory effects (White & DiPaola, 2009). But, autophagy appears to serve as a death program primarily when the apoptotic machinery is defective, as observed in most tumors. One major drawback in using autophagy-promoting drugs may involve unwanted paradoxical effects by actually protecting tumors against cell death triggered by simultaneous anticancer therapies or by nutrient deprivation in the tumor environment (Chen & Karantza, 2011).

7.2. Inhibition of protective autophagy

Autophagy, which is decreased in cancer cells as compared to normal cells, can provide a target for enhancing cancer therapy. Although Beclin1 is a haploinsufficient tumor suppressor, deletion of the remaining Beclin1 *in vitro* induces growth arrest in cancer cells (Wirawan et al., 2010). Similarly, elimination of Atg5 induces growth arrest in cancer cells (Yousefi et al., 2006). An *in vivo* study revealed that transplantation of Bcr–Abl-expressing hematopoietic cells depleted of Atg3 to lethally irradiated mice failed to induce leukemia based on ablation of autophagy (Altman et al., 2010). This suggests that a certain level of autophagy is required for tumor growth, and autophagic inhibitors could have relevant anticancer effects even when applied alone. However, it is more likely that autophagy inhibitors will be most effective when used in combination with cytotoxic drugs that activate a protective autophagy to permit cancer cell survival upon treatment. Accordingly, it has been demonstrated that melanoma differentiation-associated gene-7/Interleukin-24 (MDA-7/IL-24), a member of IL-10 gene family, shows nearly ubiquitous antitumor properties *in vitro* and *in vivo* through induction of cancer-specific apoptosis (Dash et al., 2010; Fisher, 2005). A recent report indicates that the apoptosis potential of MDA-7/IL-24 increases by inhibiting protective autophagy with 3-methyladenosine (3-MA) in prostate cancer cells (Bhutia, Dash, et al., 2010; Bhutia, Kegelman, et al., 2010). Similarly, inhibition of autophagy by 3-MA or Atg7 knockdown induced apoptosis in colon cancer cells treated with 5-FU (Li, Hou, Faried, Tsutsumi, & Kuwano, 2010). Inhibition of protective autophagy was shown to sensitize resistant cells to TRAIL-mediated apoptosis in apoptosis-defective leukemic and colon cancer cell lines (Han et al., 2008). Additionally, protective autophagy was accompanied with Ginsenoside F2-induced apoptosis in breast cancer stem cells, and treatment with chloriquine (CQ), an autophagy inhibitor, enhanced Ginsenoside F2-mediated cell death (Mai et al., 2012).

Autophagy also plays an important role in chemoresistance of cancer to some therapeutic agents that typically induce an apoptotic response (Carew, Nawrocki, & Cleveland, 2007; Carew, Nawrocki, Kahue, et al., 2007). Although autophagy has been proposed as a “magic bullet” in fighting apoptosis-resistant cancers (Gozuacik & Kimchi, 2004), a more recent study demonstrated that rapamycin-induced autophagy could protect various tumor cells against apoptosis induced by general apoptotic stimuli (Ravikumar, Berger, Vacher, O’Kane, & Rubinsztein, 2006). Recent reports highlight that treatment of estrogen-receptor-positive breast cancer cells with the anti-estrogen tamoxifen, combined with a histone deacetylase inhibitor, maintains a subpopulation of cells with elevated autophagy that display a remarkable resistance to apoptosis. These apoptosis-resistant cells only become apoptotic after inhibition of autophagy (Thomas, Thurn, Biçaku, Marchion, & Münster, 2011).

The potential to inhibit autophagy and sensitize tumor cells to metabolic stress is another promising approach for cancer therapy. Many current cancer therapies including angiogenesis, growth factor, and receptor inhibitors when combined with autophagy inhibition produced synergistic anticancer effects (Corcelle, Puustinen, & Jäättelä, 2009; Moretti et al., 2007; White & DiPaola, 2009). In addition, autophagy is also involved in removing damaged and potentially dangerous organelles from the cell. Therefore, combining organelle-damaging drugs, such as sigma-2 receptor agonists, with an autophagy inhibitor might be an effective means of anticancer therapy (Ostenfeld et al., 2008). It is likely that ER stress inducers, including thapsigargin and tunicamycin, that trigger cell death in cancer cells will increase cell killing when autophagy is inhibited (Carew, Nawrocki, & Cleveland, 2007; Carew, Nawrocki, Kahue, et al., 2007). Protein turnover by lysosomal degradation through the autophagy pathway is functionally complementary and linked with ubiquitin proteasome protein degradation. Consequently, targeting both proteasome- and autophagy-mediated protein degradations might be an effective antitumor approach for highly metabolically active tumor cells (Ding, Ni, Gao, Hou, et al., 2007; Ding, Ni, Gao, Yoshimori, et al., 2007). The proteasome inhibitor bortezomib has the approval of the US Food and Drug Administration and has demonstrated potent efficiency in treating multiple myeloma (Roccaro et al., 2006).

The metastasis prone state of tumor cells may be particularly susceptible to autophagy inhibition as cells in isolation are expected to be more reliant on autophagy, although this possibility remains to be confirmed. In this regard, chloroquine (CQ), which inhibits lysosome acidification and thereby autophagy, in conjunction with alkylating agents, displayed remarkable efficacy in inhibiting tumor growth in mice as well as in clinical studies (Høyer-Hansen & Jäättelä, 2008; Moretti et al., 2007; White & DiPaola, 2009). Synergy between CQ and the HDAC inhibitor SAHA in killing imatinib refractory chronic myeloid leukemia cells also supports a protective role for autophagy, reinforcing the therapeutic use of autophagy inhibitors in cancer therapy (Carew, Nawrocki, & Cleveland, 2007; Carew, Nawrocki, Kahue, et al., 2007). Similarly, the synergy between CQ and the PI3K–mTOR inhibitor NVP-BEZ235 induced apoptosis in glioma xenografts (Fan et al., 2010). Likewise, CQ enhanced cyclophosphamide-induced tumor cell death in a Myc-induced murine lymphoma similar to that shown by shRNA knockdown of *Atg5*, and it delayed the time-to-tumor recurrence (Amaravadi et al., 2007). These studies documented that CQ, or its analog hydroxychloroquine, when used as autophagy inhibitors in combination with proapoptotic drugs, increases twofold the median survival of cancer patients (Carew, Nawrocki, & Cleveland, 2007; Carew, Nawrocki, Kahue, et al., 2007; Fimia et al., 2007; Garber, 2011; Savarino, Lucia, Giordano, & Cauda, 2006; Sotelo, Briceño, & López-González, 2006).

One underlying concern is that autophagy inhibitors approved for cancer patients might actually act as promoters of tumor development. However, the tumor-promoting effect of autophagy inhibitors, which depends on necrotic cell lysis that follows the inflammatory response, could prevent this undesirable effect upon cotreatment with immunosuppressive drugs (Chen & Karantza, 2011; Høyer-Hansen & Jäättelä, 2008).

8. CONCLUSIONS

Although the multiple roles of autophagy in cancer require further clarification, it is obvious that autophagy is directly involved in many important physiological processes such as metabolism, response to stress, and cell death pathways in cancer cells. Both tumor suppressor genes and oncogenes are implicated in autophagy regulation, thereby linking autophagy directly to cancer development and progression. Interestingly, autophagy also limits necrosis and inflammation and may restrict the invasion and dissemination of tumor cells from a primary site, thereby inhibiting a critical and early event in metastasis (Fig. 2.3). In contrast, autophagy may paradoxically promote metastasis at later stages by protecting detached and stressed tumor cells as they travel through blood vessel and establish new colonies at distant sites (Fig. 2.3). Accordingly, it is suggested that autophagy might elicit disparate effects in tumors at different stages of progression. Therapy-stimulated accumulation of autophagosomes is by itself not sufficient to draw any conclusions whether autophagy has a lethal or protective function. Accordingly, the role of autophagy in cancer raises a number of intriguing questions. Does autophagy play any direct or indirect role in cancer development and progression? If it does, what is its exact contribution in cancer development and progression? Does autophagy regulate cancer stem cell development, and if it does is its pattern of regulation different from that in normal stem cells? Are there any genetic and cellular physiologic conditions that direct when and how autophagy facilitates cancer cells to survive or causes them to die? Can autophagy be exploited as a means of enhancing cancer therapies? Considering the potential seminal roles of autophagy in both normal and abnormal cellular physiology it is important to unravel its complex regulation. This information will be crucial if one is to exploit autophagy in the future as a potential therapeutic for advanced cancers and potentially other proliferative diseases.

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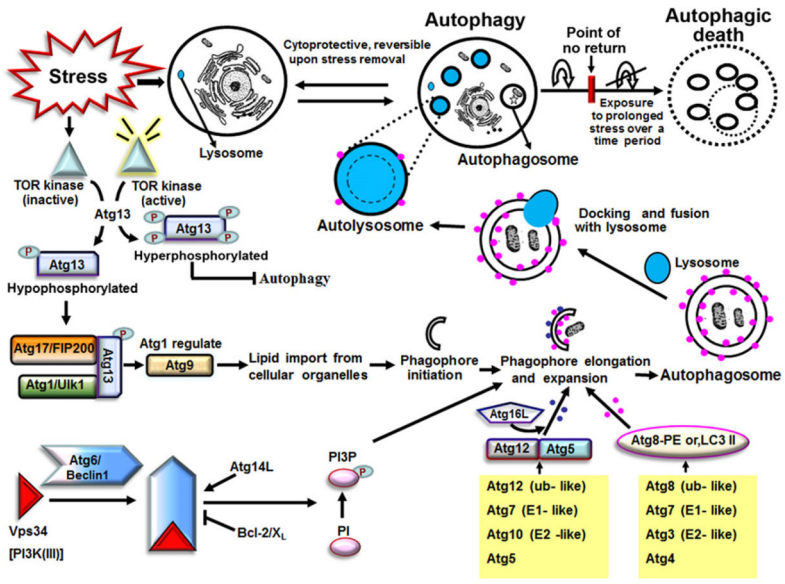

Figure 2.1.

Molecular events in the autophagy pathway. A stress response, such as nutrient withdrawal, causes cells to initiate autophagy. The stress sensor TOR kinase remains inactivated in low-nutrient condition and maintains hypophosphorylated Atg13. Atg1/Ulk1 interacts with Atg13 and Atg17 and regulates transmembrane protein Atg9 involved in lipid import from cellular organelles to act as a “phagophore” formation initiator. Next, Vps34/Beclin1 converts PI to PI3P followed by Atg5–Atg12 conjugation and interaction with Atg16L resulting in multimerization at the phagophore and formation of nascent curvature; coupled to these changes, LC3 processing helps elongation and expansion. Random or selective cargoes are targeted for degradation, followed by formation of a complete double-membrane ring called an “autophagosome.” Lysosomes dock and fuse with the autophagosome, forming an “autolysosome” where degraded cargoes generate amino and fatty acids to be transported back into the cytoplasmic pool. Autophagy acts as a primary response promoting cell viability and serving a cytoprotective role and upon stress removal the cell resumes normal function. However, extreme stress pushes the cell to cross the point of no return and commits it toward autophagic cell death (type II programmed cell death, PCD).

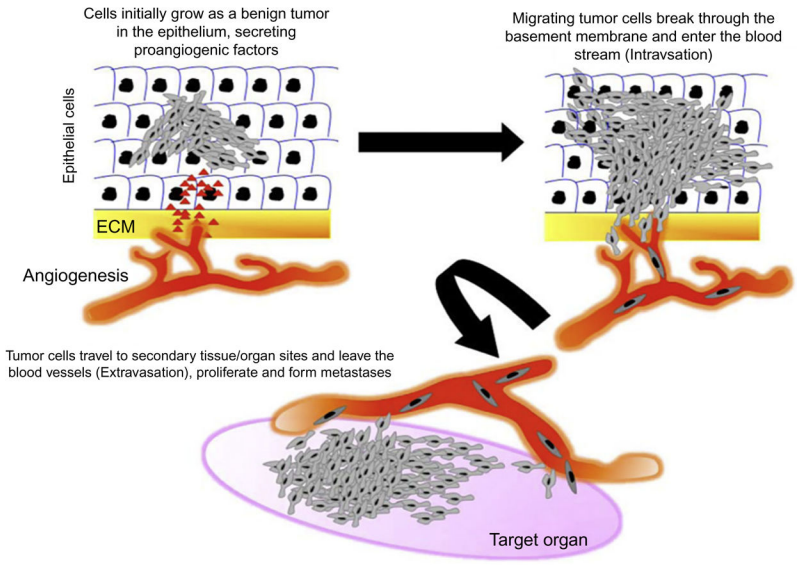

Figure 2.2.

Model of the primary events involved in the metastatic cascade. The metastatic process is complex and involves numerous changes in cellular phenotype resulting from both genetic and epigenetic modifications of the cancer genome. The process is initiated by the spread of cancer cells from a primary tumor site to other regions in the body. Cells initiate growth as primary tumors in the epithelium and with genetic and epigenetic modifications, subsets of tumor cells develop metastatic properties allowing them to degrade the basal layer and invade the blood stream (Intravasation). A small percentage of tumor cells escape into blood vessels (Extravasation), survive in the bloodstream, adhere to new target organ sites, and ultimately form secondary tumors (metastases) in distant organ or tissue sites. A key component of both the primary and secondary expansion of the tumor and metastases is the development of a new supply of blood vessels, that is, angiogenesis. *Taken from* Das et al. (2012).

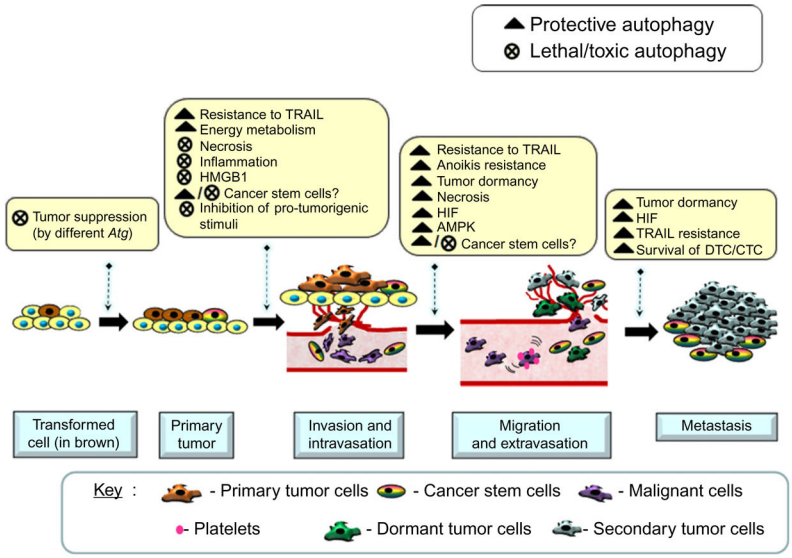

Figure 2.3.

Role of autophagy at different stages of cancer. During the initial phase of cancer development, autophagy-related cell death has been regarded as a primary mechanism for tumor suppression. Moreover, autophagy also restricts necrosis and inflammation thus limiting invasion and dissemination of tumor cells from a primary site, resulting in restriction of metastasis at a premature step. Moreover, lethal (toxic) autophagy directly causes the release of immunomodulatory factors such as HMGB-1 from dead tumor cells, which activates immune response and restricts metastasis by inhibiting protumorigenic responses. On the other hand, altered energy metabolism and TRAIL-resistant phenomena of tumor cells are maintained through protective autophagy during tumor progression. Similarly, autophagy may promote metastasis by enhancing tumor cell fitness in response to environmental stresses, such as anoikis during metastatic progression. Protective autophagy is involved in maintaining dormant tumor cells and promoting their survival under stressful conditions. The exact role of autophagy in cancer stem cells is unclear in tumor progression. Finally, tumor cells maintain protective autophagy through activation of HIF-1 (hypoxia-inducible factors) and AMPK (5′-AMP-activated protein kinase) in apoptosis deficient and long-term metabolic stress conditions, in a full-blown cancer.

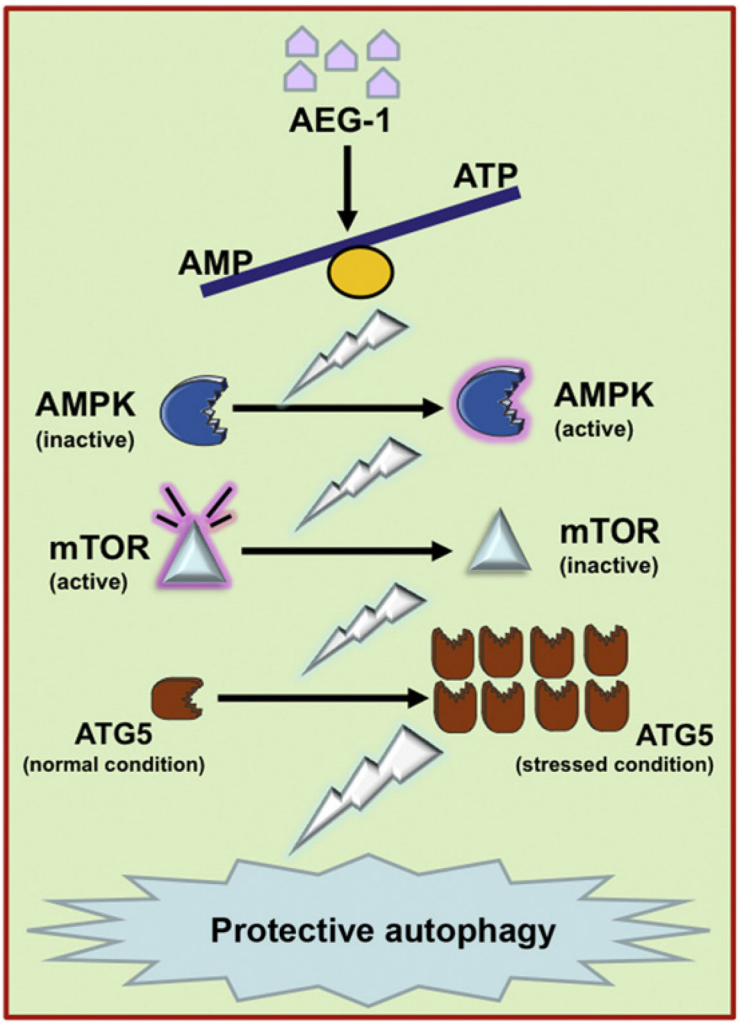

Figure 2.4.

Astrocyte elevated gene-1 (AEG-1) and protective autophagy. Model illustrating the possible molecular mechanism of AEG-1-mediated protective autophagy, which promotes escape from apoptosis and resistance to chemotherapy. *Taken from* Bhutia, Kegelman, et al. (2010).

Table 2.1

Autophagy genes in cancer

Autophagy process	Autophagy gene	Cancer	Mechanism	Autophagy in cancer	References
Induction	UKL3	Tumorigenesis	Oncogene-induced cell senescence	Lethal	Young et al. (2009)
Nucleation	Beclin1	Tumorigenesis	Highly mutated in human breast, ovarian, and prostate tumors; haploinsufficient tumor suppressor	Lethal	Liang et al. (1999)
	UVRAG	Tumorigenesis	Mutations detected in human colorectal, breast, and gastric tumors; haploinsufficient tumor suppressor	Lethal	Ionov et al. (2004) and Kim et al. (2008)
	Bif-1	Tumorigenesis	*Bif-1*$^{-/-}$ mice are cancer prone; decreased	Lethal	Coppola et al. (2008)
	Ambra1	Tumorigenesis	*Ambra1*$^{-/-}$ mice have severe neural tube defects	Lethal	Garber (2011)
Elongation	Atg12–Atg5	Tumorigenesis	Atg5 frameshift mutations in gastric cancers	Lethal	Cadwell et al. (2008)
	Atg4C	Tumorigenesis	*Atg4C*$^{-/-}$ mice develop fibrosarcomas in response to carcinogen treatment	Lethal	Marino et al. (2007)
	Atg7	Tumorigenesis	Defects in hematopoietic stem cell functions,liver-specific *Atg7*$^{-/-}$ mice develop liver cancer	Lethal	Takamura et al. (2011)
	Atg16	Tumorigenesis	Mutations detected in Crohn's disease	Lethal	Cadwell et al. (2008)
Maturation	Rab7	Tumorigenesis	*Rab7*$^{-/-}$ aberrant expression in human leukemia	Protective	Liang and Jung (2010)
Cargo selection	p62	Tumorigenesis	ROS accumulation through NF-kB induction leads to tumorigenesis in autophagy-deficient conditions	Lethal	Mathew et al. (2009)
Unknown	Unknown	Metastasis	Resistance to TRAIL, energy metabolism, necrosis, inflammation, HMGB	Lethal/protective (?)	-
Unknown	Unknown	Tumor dormancy	Resistance to TRAIL, Ras homolog member I induction	Protective	-
Unknown	Unknown	Cancer stem cells	Unknown	Lethal/protective (?)	-