Review

Diet, Autophagy, and Cancer: A Review

Keith Singletary[1] and John Milner[2]

[1]Department of Food Science and Human Nutrition, University of Illinois, Urbana, Illinois and [2]Nutritional Science Research Group, Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland

Abstract

A host of dietary factors can influence various cellular processes and thereby potentially influence overall cancer risk and tumor behavior. In many cases, these factors suppress cancer by stimulating programmed cell death. However, death not only can follow the well-characterized type I apoptotic pathway but also can proceed by nonapoptotic modes such as type II (macroautophagy-related) and type III (necrosis) or combinations thereof. In contrast to apoptosis, the induction of macroautophagy may contribute to either the survival or death of cells in response to a stressor. This review highlights current knowledge and gaps in our understanding of the interactions among bioactive food constituents, autophagy, and cancer. Whereas a variety of food components including vitamin D, selenium, curcumin, resveratrol, and genistein have been shown to stimulate autophagy vacuolization, it is often difficult to determine if this is a protumorigenic or antitumorigenic response. Additional studies are needed to examine dose and duration of exposures and tissue specificity in response to bioactive food components in transgenic and knockout models to resolve the physiologic implications of early changes in the autophagy process. (Cancer Epidemiol Biomarkers Prev 2008;17(7):1596–610)

Introduction

A wealth of evidence links diet habits and the accompanying nutritional status with cancer risk and tumor behavior (1-3). Characterizing how essential and nonessential dietary constituents can modulate single or multiple hallmark processes of carcinogenesis (4) is fundamental to identifying more effective mechanistic-based strategies for cancer prevention and therapy. To date, numerous structurally diverse biologically active food components have been reported to interfere with intercellular and intracellular signaling pathways that may activate critical cellular proto-oncogenes or inactivate tumor suppressor genes (3, 4). Consequently, these altered pathways can modify growth autonomy, proliferation, angiogenesis, and metastasis and dysregulate cellular processes. Furthermore, a host of dietary factors can stimulate programmed cell death (5). In this regard, it is now apparent that the demise of cancer cells not only can follow the well-characterized type I apoptotic pathway but also can proceed by stimulation of nonapoptotic modes such as type II (macroautophagy-related) and type III (necrosis) or combinations thereof (6-8). However, in contrast to apoptosis, the induction of macroautophagy (hereafter referred to as autophagy) may contribute to either cell survival or death in response to a stressor. As described in more detail below, autophagy is a degradative process by which cytoplasmic constituents of cells are engulfed within a cytoplasmic vacuole and delivered to the lysosome for degradation. Paradoxically, depending on the circumstances, this process of ''self-consumption'' may be involved in both health and disease progression (9-13). This review will highlight the current knowledge and gaps in our understanding about the ingestion of bioactive food constituents, autophagy, and cancer and will suggest issues and opportunities for future study.

Functions and Detection of Autophagy

In eukaryotic cells, two major strategies for protein degradation exist: the ubiquitin-proteasome system for disposal of short-lived proteins and the lysosomal system for degradation of intracellular and extracellular proteins. As a part of the lysosomal system, autophagy is an evolutionarily conserved cellular strategy to engulf and degrade long-lived cytosolic proteins and organelles to provide substrates for energy metabolism and to recycle amino acids, fatty acids, and nucleotides for the biosynthetic needs of the cell. Typically, these metabolic changes are constitutively active at low levels in cells and also may occur in the face of nutrient deprivation, growth factor withdrawal, or other stressors (14, 15).

The initiation stage of autophagy involves formation of a double-membrane vacuole, the autophagosome, which nonselectively sequesters as cargo cytoplasmic proteins, mitochondria, endoplasmic reticulum, and ribosomes (Fig. 1). These components, as well as endocytosed materials, are transported during the maturation stage of autophagy for eventual degradation by lysosomal hydrolases following fusion with the lysosome. The resulting degraded cargo components can then be returned to the cytoplasm by permeases in the lysosomal membrane for reuse. A similar process of ''self eating,''

Received 12/19/07; revised 3/28/08; accepted 4/18/08.

Requests for reprints: Keith Singletary, Department of Food science and Human Nutrition, University of Illinois, 905 South Goodwin Avenue, Urbana, IL 61801. Phone: 217-244-4497; Fax: 217-265-0925. E-mail: kws@illinois.edu

doi:10.1158/1055-9965.EPI-07-2917

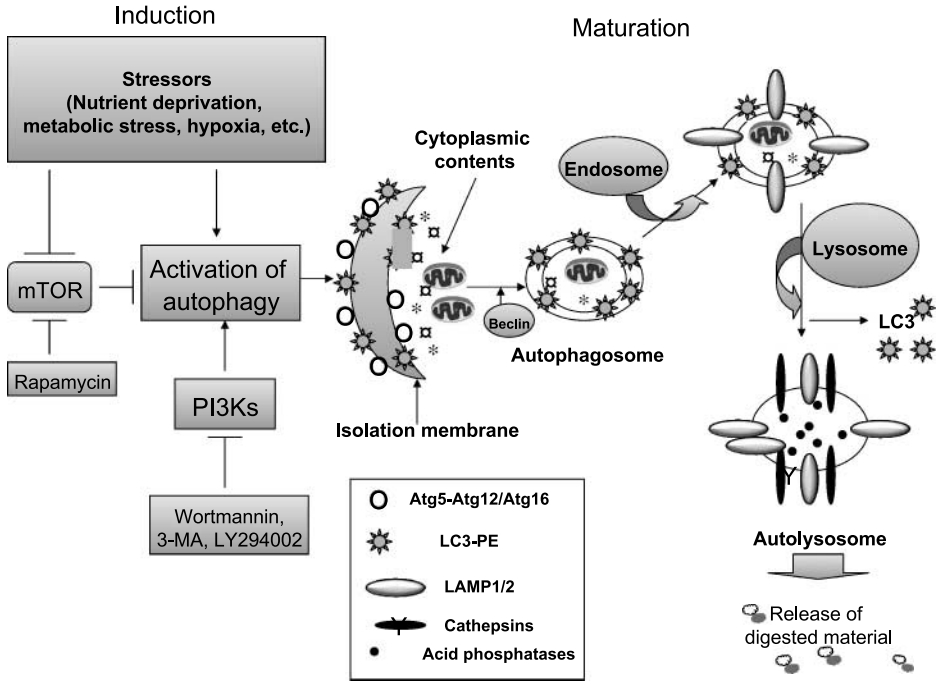

Figure 1. Steps in the formation of autolysosome during autophagy. (Modified from ref. 214).

referred to as chaperone-mediated autophagy, involves selectively delivering soluble cytosolic proteins that contain a chaperone-mediated autophagy targeting motif for incorporation into lysosomes (16). In this case, a chaperone-cochaperone complex transports the specific substrate to the lysosomal membrane for translocation into the lysosome following binding to lysosomal-associated membrane protein 2A. A third process of self-cannibalization is microautophagy, whereby cytoplasmic constituents are engulfed by invaginations of the lysosomal membrane and subsequently degraded (17). The engulfment of peroxisomes and mitochondria by autophagosomes for transport to lysosomal degradation is referred to as pexophagy and mitophagy, respectively.

Autophagy can serve a variety of functions. It is a means by which cells can respond to metabolic stress or adapt to changing environment. Thus, it is a basal housekeeping process present in most cells, sometimes referred to as a garbage disposal mechanism (18). Its purpose is to dispose of defective organelles and macromolecular structures, as well as cytosolic components such as damaged and aggregate-prone proteins. It has cell-specific developmental functions such as elimination of the ribosomes and mitochondria from erythrocytes following removal of the nucleus. Autophagy also functions during initial postpartum survival of the neonate, normal tissue remodeling, embryogenesis, and counteracting age-related physiologic changes such as those due to oxidative stress. It can contribute during infections to presentation of foreign antigens for recognition by the immune system and to killing of bacteria ingested by cells. Furthermore, autophagy has been associated with development of pathologies such as neurodegenerative disease and carcinogenesis (10, 11, 19-24).

As a type of programmed cell death, autophagy is morphologically distinct from apoptosis and necrosis. Autophagic cell death is identified by extensive inclusion of cytoplasm and organelles within autophagosomes and localization of microtubule-associated light chain 3 (LC3) protein into autophagosomal membranes. There is little, if any, caspase activation and DNA fragmentation, and the cytoskeleton initially remains intact. This is in contrast to apoptosis during which there is chromosome condensation, DNA fragmentation, usually caspase activation, and membrane rearrangement and blebbing. In apoptosis and autophagy, the remains of cells are disposed of by phagocytosis without an inflammatory response, in contrast to the massive cellular disintegration and subsequent inflammation accompanying necrotic programmed cell death (8, 12, 25). Morphologic features of more than one type of cell death program can be observed in the same cell (25-28).

The occurrence of autophagy can be confirmed by several methodologies (17). Foremost is the appearance of autophagic vacuoles. These vacuoles may be identified by transmission electron microscopy, which is considered one of the gold standards used to determine double-membrane vacuole structures that engulf cytoplasmic components (such as endoplasmic reticulum and ribosomes) and organelles (such as mitochondria). Additional confirmation of autophagy can include immunohistochemical evidence (29) that there is punctuate localization of the LC3 protein within the membranes of the putative autophagic vacuoles. LC3 protein is normally dispersed diffusely throughout the cytoplasm in healthy cells and accumulates within the autophagosomal membrane in a form covalently linked to phosphatidylethanolamine during stimulation of autophagic vacuolization (30). The degradation rate of long-lived proteins in response to a treatment may also be used as an indicator that autophagy is triggered (17, 31).

Induction and Regulation of Autophagy

The canonical up-regulator of autophagy is nutrient deprivation and starvation (32). Thus, autophagy is sensitive to levels of nutrients such as amino acids and glucose; hormones such as insulin and glucagon; and growth factors and cytokines such as insulin-like growth factor I, TNFα, and interleukin-3 (33). A coordinated response to nutrient (particularly amino acid) deprivation is regulated in mammals largely by cellular ''nutrient sensors,'' a major example of which is the target of rapamycin (TOR) kinase (Fig. 2).

The mammalian TOR (mTOR) kinase is a repressor of autophagy and is an important controller of cell growth and proliferation. Therefore, it is not surprising that it is regulated by and communicates with multiple signaling pathways (34-40). mTOR is known to be activated by the Akt/protein kinase B pathway. Total calories and the intake of fatty acids and amino acids have been reported to influence mTOR and Akt/protein kinase B expression or activity (34). Akt has been identified as a proto-oncogene that is up-regulated in various cancers (35). Its activation as an oncogene is often a consequence of mutational activation of class I phosphatidylinositol 3-kinase (PI3K) that catalyzes production of phosphatidylinositol 3,4,5-trisphosphate from phosphatidylinositol-4,5-bisphosphate. In contrast, PI3K activity can be opposed by the tumor suppressor phosphatase and tensin homologue deleted from chromosome 10, which dephosphorylates phosphatidylinositol 3,4,5-trisphosphate, subsequently decreasing Akt activity. The activating effect of Akt on mTOR activity is mediated by Akt kinase inhibitory phosphorylation of the tumor suppressor tuberous sclerosis complex 1/2. Tuberous sclerosis complex 1/2 acts as an inhibitor of mTOR and as a gateway by which other signaling pathways influence mTOR activity. Alternatively, the mTOR kinase can be activated by Akt independently of tuberous sclerosis complex 1/2 and can be regulated by mitogen-activated protein kinases. Recently, another mTOR complex (complex 2, mTORC2) has been characterized. This mTORC2 is rapamycin insensitive and activates Akt by phosphorylation at Ser^{473}, thus contributing another route for regulation of autophagy (35, 36).

Another sensor of cellular bioenergetics, specifically in response to energy depletion, is the AMP-activated protein kinase (AMPK). AMPK is an activator of tuberous sclerosis complex 1/2, which, as a suppressor of mTOR, increases autophagy. Decreased energy availability leading to an increased intracellular AMP/ATP ratio results in activation of AMPK, in part, via the upstream kinase activities of the tumor suppressor LKB1 and Ca^{2+}/calmodulin kinase kinase β (41-44). AMPK has also been implicated in an ''energy checkpoint'' due to its role in phosphorylating p53 and inducing cell cycle arrest following energy deprivation (44). There are newly identified cellular sensors of energetic status, such as PAS-kinase, which have not yet been evaluated for their contributions to regulation of autophagy (45). A hypothesis particularly relevant to the growing tumor is that metabolic stress precipitates autophagy, especially when apoptosis is defective (10, 25, 46-49). This inability of a cancer cell to adapt efficiently to metabolic stress could be exploited by strategies that stimulate metabolic catastrophe and cell death (50, 51).

There is also emerging evidence that other signal transduction pathways may regulate autophagy in a cell-specific manner. Such pathways include those associated with p53, Ras/mitogen-activated protein kinase/extracellular signal–regulated kinase (ERK) kinase (MEK)/ERK1/2, RIP1 kinase, c-Jun NH_2-terminal kinase, death-associated protein kinase, and insulin-like growth factor I

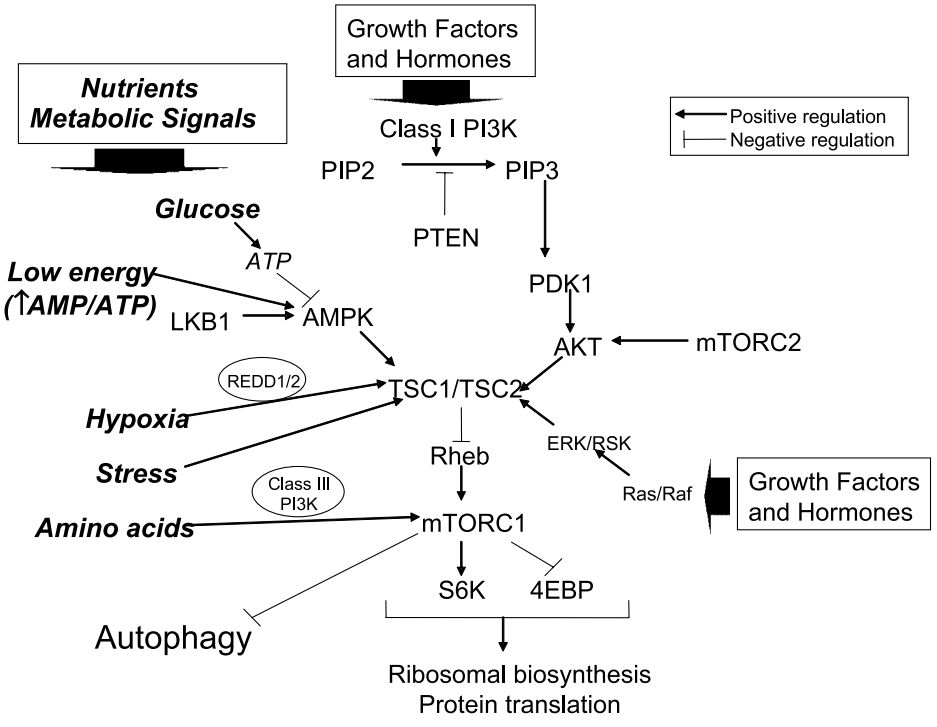

Figure 2. Signaling events associated with induction of autophagy.

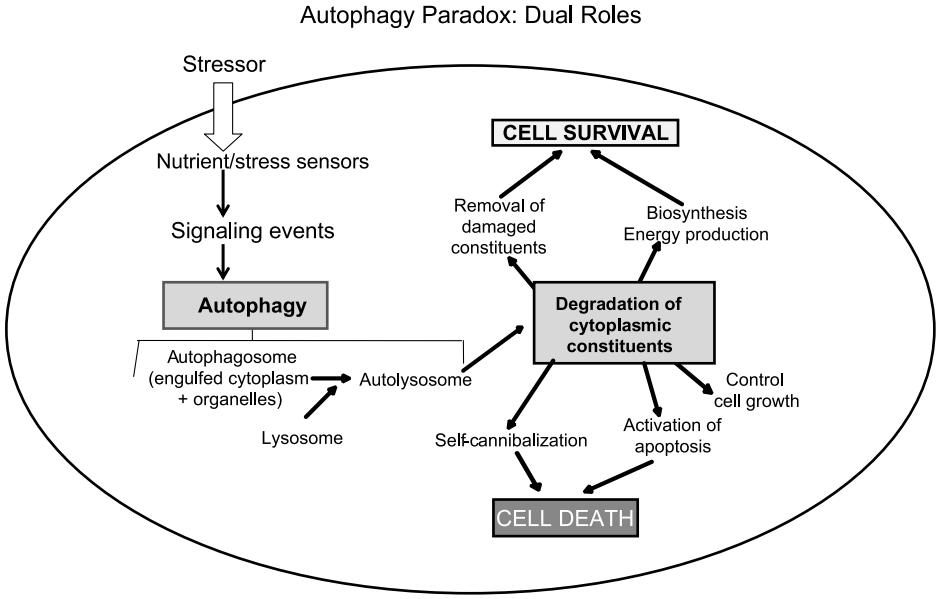

Figure 3. Dual roles of autophagy in cell survival and death. (Modified from ref. 22).

(52-64). Recently, in pancreatic cancer cells protein kinase Cδ and transglutaminase 2 signaling have been reported to inhibit autophagy (65, 66).

Thus nutrient status and metabolic stressors can influence the autophagic response via signaling networks that often, but not exclusively, converge at mTOR and that include both oncogene- and tumor suppressor gene–regulated intermediate steps.

The stressor-induced intracellular triggers that initiate and escalate the autophagy program have not been clearly identified (46, 58, 67). Mitochondrial damage or membrane alterations of mitochondria and lysosomes have been suggested as initiators of autophagy, and recently there has been considerable interest in the role of endoplasmic reticulum stress as an early event (68-77). Likewise, endoplasmic reticulum and cytosolic Ca^{2+} homeostases, as well as the generation of reactive oxygen species (ROS), have been proposed as being necessary for the autophagic process to occur (16, 78-87). For example, starvation-induced ROS occurring in Chinese hamster ovary cells act as signaling molecules that stimulate autophagy, in part, by posttranslational modification of redox-sensitive proteases (26, 80, 81). Others, using a mouse L929 cell model, have provided evidence that selective autophagic degradation of the ROS scavenger catalase leads to accumulation of ROS, loss of plasma membrane integrity, and cell death (87). Thus, ROS generation may contribute to an autophagic response through a variety of mechanisms.

Many of the genes controlling the formation of the autophagic vacuole and its delivery to the lysosome have been characterized in yeast (88). A number of the homologous human autophagy (*Atg*) genes have been identified (10, 83). For example, Atg8 or LC3 in mammals functions as a ubiquitin-like protein during the early stages of autophagosome formation. Several autophagy gene products have been identified as partnering with oncogenes and tumor suppressor genes in response to cellular stressors. The major example of this is the discovery that the complex that regulates formation of LC3 in the autophagosomal membrane is composed of the putative tumor suppressor and autophagy-related protein Beclin 1 (Atg6) and the class III PI3K (Vps34 kinase; ref. 89). Beclin 1 was identified from a yeast two-hybrid screen (90) as a novel Bcl-2–interacting coil-coil protein, and the *beclin 1* gene was subsequently mapped to a tumor susceptibility locus on human chromosome 17q21, which is monallelically deleted in 75% of ovarian cancers, 50% of breast cancers, and 40% of prostate cancers. Heterozygous disruption of the *beclin 1* gene in mice is associated with accelerated development of hepatitis B virus–induced premalignant lesions, increased lymph, lung and liver malignancies, increased mammary gland proliferation, and decreased autophagy *in vivo* (91-93). Furthermore, in addition to interacting with the antiapoptotic protein Bcl-2, Beclin 1 recently was found to bind to proteins that can regulate cell death decisions, including the tumor suppressor UVRAG as well as Ambra1 and Bif-1 (10, 24, 94-96). It is likely that genes will continue to be identified that have multiple cellular functions, including those for autophagy regulation, as recently reported for the NAD-dependent deacetylase Sirt1 (97).

Autophagy in Cancer Suppression and Cancer Survival

The role of autophagy in health and disease has been described as a double-edged sword (9) and as a Doctor Jekyll and Mr. Hyde phenomenon (13) because of its paradoxical and controversial involvement in both cell survival and cell death (Fig. 3), either independently or in concert with apoptosis and/or necrosis. The orchestration of this ''dance'' toward either autophagy-associated survival or autophagy-associated death of cancer cells is dependent on the stage of carcinogenesis, the cell/tissue context, the molecular characteristics of the target cell, and the nature (as well the magnitude and the duration) of the stressor (98).

Autophagy and Cancer Survival. Autophagy as a means to maintain macromolecular synthesis and ATP production can be regarded as an important survival mechanism in normal and cancer cells. This may occur under a variety of conditions, such as when cells are limited in their supply of glucose and nutrients, when growth factor signaling is compromised, or when there is a precipitous increase in metabolic needs (10). Its contribution as a survival strategy is particularly apparent in apoptotic-deficient cells that are deprived of a growth factor such as interleukin-3 (49). As a survival strategy, it has even been proposed that a low level of autophagy might actually be needed for tumor initiation (25). According to this concept, nascent clusters of transformed cells might use autophagy as a temporary survival strategy before recruitment of neovasculature. Solid tumors can take advantage of autophagy to survive when confronted with metabolic stress such as during hypoxic conditions due to poor vascularization. In fact, it has been hypothesized that overactivated Ras signaling, a characteristic of various cancers, may trigger autophagy as a survival tactic of cancer cells within the tumor interior until neoangiogenesis can deliver nutrient support (67).

Additional evidence for the prosurvival activity of autophagy comes from studies in which *Atg* genes are knocked down and a subsequent improvement in other forms of programmed cell death is observed. For example, the silencing of autophagy genes such as *beclin 1* or *Atg5* has been reported to enhance starvation- or stress-induced apoptotic cell death in HeLa cells, suggesting that autophagy is delaying death mediated by other death strategies and that there is considerable cross talk between type I and type II cell death (99). It should also be noted that autophagic removal of damaged organelles and proteins, such as those produced during oxidative-stress conditions, may be yet another protective strategy for cancer cells to avoid exacerbating DNA damage and consequent genomic instability (48). In this regard, autophagy is also stimulated in tumors during exposure to ionizing radiation and chemotherapeutic agents (28, 100-105). Likewise, it has been suggested that autophagy can protect cancer cells from the fatal consequences of excessive necrosis and inflammation that follow the accumulation of cellular garbage (48, 106).

Autophagy and Cancer Suppression. In contrast, there is evidence that autophagy is associated with cancer suppression (108). Certainly, the excessive degradation of intracellular constituents is a self-limiting property of autophagy that may ultimately lead to cell death (102). However, there is evidence that, perhaps under certain conditions, autophagy is acting fundamentally as a type II programmed cell death activity that is important for tumor suppression, rather than a protracted and overactivated cell survival response turned fatal. Defective apoptosis is an abnormal cellular condition in which the consequences of faulty autophagy can be particularly apparent. For example, silencing of autophagy genes by RNA interference in apoptotic-deficient cells treated with DNA-damaging agents blocked the expected cell death. Furthermore, the failure of autophagy in the context of defective apoptosis may create an environment favorable to impaired genomic integrity and the development of a mutator phenotype because of deficient clearance of damaged cellular macromolecules (18, 109, 110). A consequence of inhibiting caspases necessary for functional apoptosis in L929 mouse fibroblasts is the selective degradation of catalase, accumulation of ROS, loss of plasma membrane integrity, and, ultimately, death associated with autophagy. These changes, however, were prevented by suppression of autophagy using chemical inhibitors of autophagy or knockdown of autophagy proteins (21, 56). This response to caspase inhibition has not been reported to occur in other cells and needs to be explored.

Even in apoptosis-competent cells, autophagy can be an essential contributor to the demise of the cell (69, 111, 112). In fact, overexpression of the autophagy gene *Atg1* is sufficient to induce death in apoptosis-proficient *Drosophila* (113). In addition, autophagy, via its capacity to increase cellular ATP production, has been reported to be necessary for the clearance of apoptotic cell corpses during embryonic development (114). This requirement for autophagy genes in the process of engulfment of cell corpses is due to autophagy-dependent generation of ''eat-me'' and ''come-get-me'' signals (114, 115). This is reminiscent of published reports describing how interference with either autophagy or apoptosis during *in vitro* human mammary acini formation can lead to luminal filling that resembles hyperplastic, precancerous breast lesions (51, 116).

Additional evidence for a cancer suppressive function of autophagy comes from three chemically induced animal tumor models, including an *Atg* knockout mouse. Gozuacik and Kimchi (107) discuss early studies in a chemically induced liver cancer model whereby preneoplastic lesions and carcinomas of the liver exhibited lower autophagic activity compared with nonneoplastic liver counterparts. During conditions of nutrient stress, a variety of transformed cell lines exhibit lower protein degradation rates compared with nontransformed control cells (107). One liver cancer scenario proposed by Schwarze and Seglen (117, 118) is that down-regulation of autophagy in preneoplastic hepatocytes provides a survival advantage by preventing excessive protein loss during nutrient starvation or fluctuations in nutrient levels and, thus, ensuring adequate cellular reserves for continued outgrowth during subsequent periods of neoplastic progression. In a chemically induced rodent pancreatic cancer model, Rez et al. (119) and Toth et al. (120) report that the autophagic capacity of preneoplastic lesions is higher compared with pancreatic lesions that become more malignant. In another tumor model in which *Atg4* knockout mice were treated with the carcinogen methylcholanthrene, fibrosarcoma incidence is dramatically higher and latency much shorter compared with wild-type counterparts (121). This was attributed to the lower capacity of these Atg4-null mice to launch a proper autophagic response to carcinogen-induced stress conditions.

In humans, a role for autophagy in tumorigenesis is suggested by the association of monoallelic loss of *beclin 1* in cancers of the ovary, breast, and prostate (93). Furthermore, autophagic structures and beclin-1 expression have been detected in a variety of surgically resected human tumor specimens (108, 122). Collectively, these data provide evidence that autophagy indeed can function as a primary contributor to cancer suppression

but may be especially important in response to specific stressors or under specific conditions, such as when apoptosis is compromised. These are issues that deserve to be more fully explored. Likewise, the role of autophagy during the temporal development of malignancy could be characterized in well-defined animal tumor models. Such information will be critical to collect before the design of strategies using the induction or suppression of autophagy for inhibiting carcinogenesis (123).

Autophagy in Cancer Suppression and Survival: Possible Regulators. The cellular factors, genes, and signaling pathways that control whether autophagy contributes to cancer inhibition or survival and how autophagy interacts with other cell death programs are not clearly defined. One hypothesis is that the decision about whether autophagy contributes to survival or death is dependent on the rate or magnitude of autophagic degradation and the specificity of targeting of engulfed components (such as mitochondria and endoplasmic reticulum; ref. 47). In light of the observation that prolonged cellular autophagy and substantial depletion of cell mass can be reversed on resupply of nutrients, it can be argued that autophagic cell death may not simply be a matter of crossing a quantitative threshold of self-digestion (10).

Several signaling networks (46, 58), some involving autophagy-related genes, have also been identified that can act as ''switches'' to regulate cell death decisions (Fig. 4). It has been proposed that sphingolipid metabolism, and particularly the generation of specific ceramide and sphingosine metabolites, may play a central role in mediating the contribution of autophagy to cell death or survival. For example, exogenous, and presumably endogenous, C2-ceramide and C2-dihydroceramides are capable of inducing autophagy and cancer cell death (124, 125). On the other hand, sphingosine-1-phosphate, often considered to be a tumor promoter, can also induce autophagy, but with a different outcome, namely cell survival (126). Thus, a novel and intriguing model has been proposed in which sphingolipid metabolism serves as a ''rheostat'' system, with C2-ceramide promoting autophagy-associated cell death and sphingosine-1-phosphate triggering autophagy-associated cell survival (127). The elucidation of these models and of the interconnections among all these different trigger events warrants further attention, especially *in vivo*.

The relative levels of Bcl-2 and Beclin-1 and their subsequent binding in cells have been suggested as another rheostat that determines whether oncogenesis is inhibited or stimulated (128, 129). This Beclin 1-Bcl-2 interaction involves the participation of other genes such as the tumor suppressor UVRAG and likely additional autophagy genes and other Bcl-2 family proteins (95, 130, 131). In *Drosophila*, Atg1 overexpression is sufficient not only to induce autophagy but also to kill apoptosis-proficient cells (113). The role of Atg1 in autophagy has now expanded to include participation in signaling pathways that control cell growth and modulate TOR activity (113, 132). The cross talk between apoptosis and autophagy may be an important regulator of cell survival and cell death. For example, Atg5 has emerged as another autophagy-related protein that participates in cross talk with the apoptotic machinery. Atg5 originally was characterized as a binding partner with Atg12 in yeast, forming a complex that regulates the processing of LC3 (Atg8) and autophagosome formation. It was recently established that Atg5 can be cleaved by calpain, following a death stimulus, into a fragment that then promotes mitochondria-mediated apoptosis (133-135). This apoptotic function of Atg5 is independent of autophagy. Whether these newly identified properties of Atg1 and Atg5 extend to mammals and affect tumorigenesis is unknown. These findings, however, underscore the growing recognition that autophagy can be an important, independent regulator of cell death, with a role in carcinogenesis that needs to be better characterized. The continuing discovery that dysregulation of other genes, such as *c-Myc*, *Ras*, phosphatase and tensin homologue deleted from chromosome 10 (*PTEN*), *PI3K, Akt*, and death-associated protein kinase (*DAPK*),

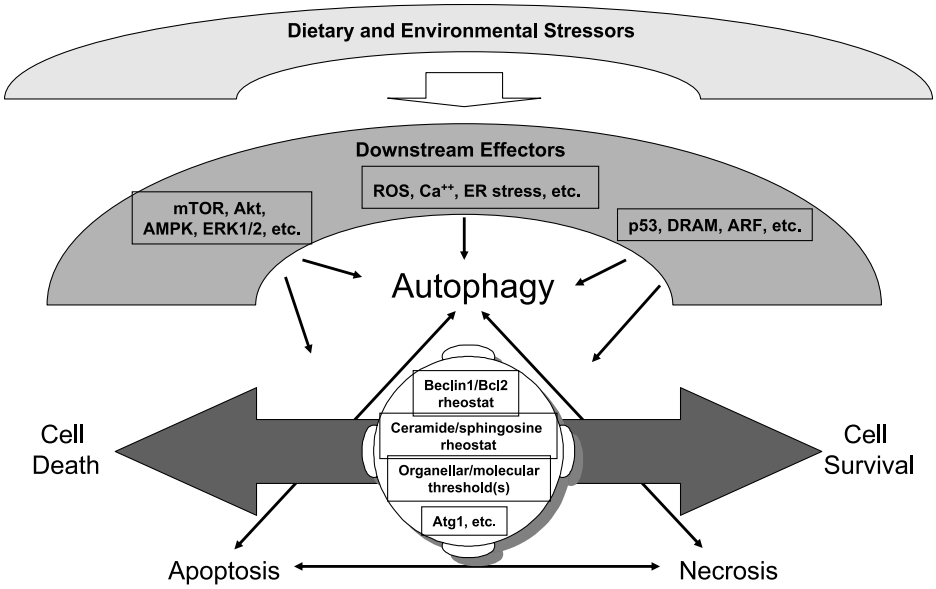

Figure 4. Role of autophagy in cell survival and death decisions. (Modified from ref. 138).

links the loss of autophagic capacity with the development of cancer (107) underscores this point.

It should be emphasized that it is too simplistic to view the decision of a cancer or normal cell to engage autophagy or apoptosis and thus engage cell survival or suicide pathways as a simple ''switch'' process. These two programmed cell death pathways share common upstream signals, which can lead, on one hand, to the concurrent occurrence of autophagy and apoptosis or, on the other hand, to mutually exclusive cell death responses (136). Furthermore, it is now apparent that the promotion of cell death or survival depends additionally on input from other compensatory autophagic pathways such as chaperone-mediated autophagy that may be triggered when macroautophagy is compromised (137). Thus, cell type, the nature and magnitude of stressors, the intracellular triggers and signaling pathways activated, the metabolic status of the cell, the integrity of autophagic and apoptotic machinery, and the function of key oncogenes and tumor suppressor genes all likely contribute to this complex continuum of cellular responses of autophagy or apoptosis that govern cell death or survival (Fig. 4). Several recent reviews provide insights into how these complex events may be integrated (58, 136, 138).

Induction of Autophagy and Cancer Cell Death by Chemotherapeutic Drugs and Dietary Factors

Modulation of Autophagy by Drugs and Natural Products. Induction of cancer cell death by autophagy is recognized as a mechanism of cytotoxicity resulting from treatment with a variety of drugs and natural products. An example of this is the antiestrogen tamoxifen, a drug that is currently in use clinically not only for its therapeutic benefits but also as an important tool in breast cancer chemoprevention strategies. Tamoxifen at a dose of 1 μmol/L has been reported to induce autophagy in human MCF-7 breast cancer cells, a response that was necessary for irreversible cell death to occur (101, 139-142), although there is a recent report indicating a prosurvival action of autophagy in tamoxifen-treated cells (143). Interestingly, tamoxifen-induced autophagic vacuolization seems to be mediated by sphingolipid metabolites, including ceramide (124). Arsenic trioxide (As_2O_3) can stimulate autophagy-associated and apoptotic cell death in malignant glioma and leukemia cells, changes that are accompanied by up-regulation of Beclin 1 (144, 145). The tyrosine kinase inhibitor imatinib (Gleevec), plant alkaloid voacamine, Chinese herbal diterpenoid oritonin, vitamin D analogue EB1089, and natural quinonoid plumbagin are examples of other structurally diverse cytotoxic cancer agents that work, in part, via induction of autophagic cell death (146-151). Perhaps the most well-known stimulator of autophagy is the drug rapamycin, the prototypical inhibitor of mTORC1. Rapamycin is now currently under active investigation for use in several cancer therapies (152). DNA-damaging agents such as camptothecin, etoposide, and temolozamide can also stimulate autophagy; however, this induction of autophagy may actually play a role in delaying cell death and contributing to drug resistance (153, 154). For example, in malignant glioma cell lines, temolozamide and etoposide produced autophagy-associated surges in ATP production that protected cells from mitotic catastrophe (153). In addition, in MCF-7 breast cancer cells treated with camptothecin, both autophagy and apoptosis are stimulated. However, inhibition of the autophagy response to camptothecin by knockdown of Beclin 1 actually accelerated the latent apoptotic cascade that also is a camptothecin response. Interestingly, this autophagy-associated unmasking of apoptosis occurred only in noninvasive cell lines (154). In addition, the synthetic derivative of retinoic acid, fenretinide, stimulated autophagy and death in breast cancer cells, whereas in malignant glioma cells fenretinide treatment provided an autophagy-associated survival advantage (155, 156). Thus, manipulating autophagy may be a means to enhance therapeutic response to chemotherapeutic drugs, although the tumor-specific and malignancy-dependent sensitivity of such modulations needs to be better characterized.

Modulation of Autophagy by Dietary Factors

Caloric Restriction. The influence of diet-induced autophagy in cancer prevention is poorly understood. Considerable evidence obtained with yeast and metazoan cultures documents that both nutrient and growth factor deprivation are strong inducers of autophagy. Such findings have sparked interest in exploring the importance of autophagy in mediating the antiaging and anticancer effects of dietary/caloric restriction (157). *In vitro* data and a limited number of animal studies suggest that the mechanism by which caloric restriction may extend the life span is by stimulating autophagy along with reducing insulin and glucose levels and decreasing growth hormone-insulin-like growth factor-I axis signaling (157, 158). In this regard, ketone bodies (such as β-hydroxybutyrate), which are elevated during prolonged starvation in humans, have been shown to induce autophagy (159). This benefit from increased autophagy also may be due to the improved housekeeping function of lysosomal degradation in removing damaged organelles and cellular proteins following lifelong exposure to oxidative stress, inflammation, and other cellular stressors. In light of the recognition that diet/caloric restriction is a potent suppressor of carcinogenesis (160), the contributions of autophagy and related signaling pathways, such as mTOR (161), to this cancer-protective effect need to be clarified.

Nonnutrient Bioactive Constituents. Adding specific bioactive dietary constituents present in natural products to cell cultures can also induce autophagy, in addition to that classically observed following nutrient deprivation (Table 1). Whereas these findings are intriguing, the relatively high concentrations used make the physiologic relevance unclear. Nonetheless, a remarkable variety of dietary constituents have been reported to stimulate autophagy, although subsequent effects on carcinogenesis are not necessarily uniform. The effects of these diverse compounds will be discussed based on structural classification.

Flavonoids and Polyphenolics. Several members of the flavonoid family of phytochemicals can stimulate autophagic vacuolization. For example, in ovarian cancer cells, treatment with the isoflavonoid genistein at a pharmacologic dose of 50 μmol/L resulted in apoptosis

Table 1. Induction of autophagy by nutrition and diet-related bioactive food constituents

Treatment	Cell/tissue	Dose	Mechanism	Reference
Avicin D triterpenoid saponins	Bone, breast, lung, ovarian, prostate cancers	2 μg/mL	AMPK, TSC2	(190)
B-group triterpenoid soyasaponins	Colon cancer	100 ppm	Akt, ERK1/2	(182, 183)
Benzyl isothiocyanate	Breast cancer	2.5-10 μmol/L	ROS	(193)
Caloric restriction			Hormones, growth factors	(157, 158)
Curcumin	Brain cancer	40 μmol/L	Akt/mTOR/S6 kinase, ERK1/2	(180, 181)
Genistein	Ovarian cancer	50 μmol/L	Akt	(162)
Ketones				(159)
Naringen	Hepatocytes	100 μmol/L	AMPK, PP2A	(173, 174)
Prenylated flavones	Breast cancer	25-50 μmol/L		(163)
Prenylated flavones	Prostate cancer	100-200 μmol/L		(164)
Prostaglandin J2	Prostate cancer		PPARγ	(201)
Quercetin	Colon cancer	20 μmol/L		(164)
Resveratrol	Ovarian cancer	50 μmol/L	Akt, mTOR, glycolysis	(176, 177)
Resveratrol	Colorectal cancer	100 μmol/L	Vps34	(178)
Resveratrol	Salivary gland cancer	50-100 μmol/L		(175)
Resveratrol	Lung cancer	50-100 μmol/L		(175)
Sodium butyrate	Cervical cancer	50 mmol/L		(203)
Sodium selenite	Brain cancer	7 μmol/L		(200)
Sulforaphane	Prostate cancer	40 μmol/L	Mitochondria/mitophagy	(191)
Tocotrienols	Pancreatic cells	20 μmol/L		(197, 198)
Vitamin C	Glial cells	100 μmol/L		(194)
Vitamin C	Lung cancer	250-500 μmol/L		(195)
Vitamin D3	Head and neck cancers	100 nmol/L	$p19^{INK4D}$	(196)
Vitamin K2	Liver cancer	10-50 μmol/L		(199)

Abbreviations: TSC2, tuberous sclerosis complex 2; PP2A, protein phosphatase 2A; PPARγ, peroxisome proliferator–activated receptor γ.

and a caspase-independent cell death that exhibited hallmarks of autophagy (punctuate localization of LC3) and was accompanied by inhibition of Akt activation and glucose uptake (162). At doses of 25 and 50 μmol/L, again relatively high exposures compared with what occurs in humans eating considerable amounts of soy products, cytoplasmic vacuoles that stained positive for the autophagic marker monodansylcadaverine were detected. Natural prenylated flavones isolated from *Artocarpus elasticus* were antiproliferative toward both estrogen receptor–positive and estrogen receptor–negative human breast cancer cells (163). Likewise, prenylflavonoids from hop (*Humulus lupulus* L.) at doses of 100 to 200 μmol/L induced autophagic cell death in human prostate cancer cells (164).The food polyphenol quercetin was shown to induce apoptosis and, at a dose of 20 μmol/L, to stimulate autophagic vacuolization specifically in Ha-Ras–transformed human Caco2 colon cancer cells. Quercetin also suppressed cell viability and anchorage-independent growth (165). In contrast, Caco2 cells with wild-type Ras or transformed with Ki-Ras resisted quercetin-induced autophagic vacuolization. This report is noteworthy because, considered together with other reports of autophagy in transformed and nontransformed cells, it suggests that specific oncogene-associated characteristics may enhance cellular sensitivity to autophagy (166-172). Unquestionably, this proposal deserves further investigation. In hepatocytes, the flavonoid naringin at a dose of 100 μmol/L counteracted the autophagy-suppressive action of okadaic acid, possibly due, in part, to its effects on AMPK and protein phosphatase 2A (173, 174).

The natural bioflavonoid resveratrol, which is present in grapes, nuts, and red wine, has been reported to induce both apoptosis and autophagy in cancer cell lines. For example, in lung cancer and salivary gland adenocarcinoma cell lines, resveratrol at doses of 50 to 100 μmol/L reversibly inhibited proliferation and induced autophagy. Furthermore, resveratrol induced the accumulation of the estrogen receptor coactivator PELP1 in autophagosomes, providing a link between the actions of estrogen receptor and autophagy (175). In A2780 ovarian cancer cells, resveratrol exhibited antineoplastic activity and caused mitochondrial release of cytochrome *c*, apoptosome formation, and caspase activation. Yet, death in cells treated with resveratrol at a dose of 50 μmol/L was apoptosome and caspase independent and was accompanied by a pronounced formation of monodansylcadaverine-positive autophagocytic granules containing extensively degraded organelles (176). It was subsequently determined in these cells that the mechanism for resveratrol-induced autophagocytosis involved inhibition of glucose uptake and glycolysis that was associated with down-regulation of phospho-Akt and mTOR (177). Thus, the relative contribution of each pathway to resveratrol-induced cell death seemed to be dose dependent. Furthermore, the length of exposure to resveratrol may also determine its cytotoxic potential. For example, in human colorectal cancer cells, resveratrol at a dose of 100 μmol/L rapidly and reversibly stimulated autophagy as part of a prosurvival stress response. However, chronic exposure to this phytochemical ultimately led to autophagy- and caspase-dependent cell death (178). In contrast, in other types of cancer cells such as U937 leukemia cells, resveratrol has been observed to induce only apoptosis (179). The relative importance of autophagy and apoptosis in these cell-specific differences needs to be characterized not only for resveratrol but also for many bioactive food constituents.

Curcumin, a polyphenolic constituent of the spice turmeric, has been reported to have numerous anticancer actions. Curcumin treatment at a dose of 40 μmol/L stimulated G_2-M arrest and nonapoptotic autophagic death in malignant glioma cells, phenomena that were

associated with inhibition of the Akt/mTOR/p70S6 kinase pathway and activation of the ERK1/2 pathway (180, 181). Furthermore, there was evidence that curcumin was able to inhibit the growth of these malignant gliomas *in vivo*. Compared with tumors from control mice, tumors from curcumin-treated mice exhibited signs of autophagy as measured by substantially increased expression of LC-II.

Triterpenoids. Triterpenoids are compounds structurally related to steroids that are present in a variety of plants including ginseng, legumes, and oats. In human colon cancer cells, treatment with 100 ppm of triterpenoid B-group soyasaponins was reported to suppress proliferation and cause pronounced accumulation of autophagosomes (182). Furthermore, the induction of autophagy by the saponins was not observed in nonneoplastic colon cells, however. This action of the triterpenoid soyasaponins was accompanied by inactivation of Akt kinase and was dependent on the activation of the MEK/ERK1/2 pathway (183). This requirement for ERK1/2 activation in the induction of autophagic cell death by curcumin and soyasaponin triterpenoids seems to be paradoxical because this mitogen-activated protein kinase has frequently been depicted as a stimulator of cell proliferation and tumorigenesis (184). Yet, a similar requirement for Raf-1/MEK/ERK1/2 activity in the drug- and starvation-dependent induction of autophagy has been observed in human colon cancer cells. In this case, ERK1/2 activity was important in stimulating cross talk with trimeric G-proteins, specifically the GTPase-activating activity of Gα-interacting protein (185-187). Opposing actions of cancer-preventive dietary agents on ERK1/2 activity have been reported by others as well (188) and are likely to depend on the tissue context, intensity of stimulation, transient or sustained nature of the activation, and even intracellular localization of downstream effectors (189). Another plant triterpenoid saponin, avicin D, at a dose of 2 μg/mL triggered autophagic cell death in tumor cells, which was mediated, in part, by the activation of AMPK (190).

Isothiocyanates. Isothiocyanates are a family of compounds derived from the hydrolysis of sulfur-containing glycosides in cruciferous plants. Sulforaphane (40 μmol/L), an isothiocyanate constituent of cruciferous vegetables, induced autophagy in human prostate cancer cells before the appearance of apoptosis (191). Interestingly, the response to sulforaphane actually led to an inhibition of cytochrome *c* release, presumably due to sequestration of mitochondria in autophagosomes, and thus delayed the activation of the intrinsic caspase cascade and apoptosis. Such information suggests that induction of autophagy represented a cell survival mechanism against sulforaphane-induced apoptosis (191). This proautophagic, antiapoptotic action of sulforaphane is similar to that observed when human colon cancer cells were exposed to the nonsteroidal anti-inflammatory drug sulindac sulfide (192). In contrast to sulforaphane, the cruciferous vegetable constituent benzyl isothiocyanate at doses between 2.5 and 10 μmol/L suppressed proliferation of human breast cancer cells and caused cell cycle arrest at G_2-M, caspase-dependent apoptosis, ROS formation, and accumulation of autophagosmes (193). Nontransformed breast cells were more resistant to this cytotoxic effect of benzyl isothiocyanate. It was not determined whether benzyl isothiocyanate–induced autophagy inhibited apoptotic cell death in these breast cancer cells, as was observed for sulforaphane-treated prostate cancer cells (191).

Nutrients

Vitamins and Trace Mineral Elements. In human astrocyte glial cells, incubation with vitamin C (ascorbate) at a dose of 100 μmol/L was able to maintain physiologic levels of this vitamin within the cells (~1-2 mmol/L) and stimulate autophagy, in part, by stabilizing the acidic intralysosomal pH and maximizing lysosomal hydrolase activity (194). It will be of considerable interest to determine if this effect of vitamin C can suppress the growth of and induce autophagy in malignant counterparts of these glial cells. Ascorbate also was recently reported to inhibit non–small-cell lung cancer growth by caspase-independent apoptosis and autophagy and to synergistically enhance adenoviral vector–mediated transfer of the tumor suppressor gene *101F6* (195). Non–small-cell lung cancer H1299 and H322 cells treated with ascorbate at doses of 500 and 250 μmol/L, respectively, exhibited significant accumulation of acidic vacuolar organelles and enhanced punctate LC3 localization, which was accompanied by increased intracellular H_2O_2 production. Inhibition of the autophagic/lysosomal pathway in these non–small-cell lung cancer cells by 3-methyladenine actually resulted in greater cell viability, suggesting that ascorbate was acting to induce an autophagy-associated cell death.

Human SCC25 head and neck squamous carcinoma cells treated with 100 nmol/L 1,25 vitamin D_3 (but not retinoic acid) stimulated autophagic cell death, but only in cells deficient in expression of the cyclin-dependent kinase inhibitors $p19^{INK4D}$ and $p27^{KIP1}$ (196). In contrast, vitamin D_3 did not induce autophagy in U937 leukemia cells with knockdown of $p19^{INK4D}$, suggesting that this effect of $p19^{INK4D}$ deficiency on autophagy induction is cancer cell specific.

Tocotrienols compose a collection of natural vitamin E compounds with reported anticancer actions. In activated rat pancreatic stellate cells, identified as the cell type responsible for pancreatic fibrosis, exposure to tocotrienols at a dose of 20 μmol/L resulted in apoptosis and autophagy, mitochondrial membrane depolarization, ROS formation, and mitophagy (197, 198). It is noteworthy that this death-promoting action of tocotrienols was observed in these ''transformed'' activated stellate cells but not in their quiescent or terminally differentiated counterparts.

Vitamin K2 belongs to a group of structurally related fat-soluble compounds (menaquinones) present in plant and animal tissues and bacteria. Cholangiocellular carcinoma cells treated with vitamin K2 doses of 10 to 50 μmol/L evidenced profound accumulation of autophagic vacuoles and cell growth inhibition with limited evidence of apoptosis (199). In contrast, vitamin K2–treated HL-60 leukemia cells evidenced a stronger apoptotic response associated with blockage of cell growth. The reason for these cell-specific differences is unknown.

Recently, the dietary trace element selenium was reported to induce autophagy in malignant gliomas. Specifically, 7 μmol/L sodium selenite caused autophagic cell death that was preceded by superoxide-mediated

mitochondrial damage and mitophagy (200). It has been proposed that antioxidants collectively may share a common capacity to induce autophagy because vitamin E, catechin, and epigallocatechin-3-gallate also have been reported to trigger this type of programmed cell death (32). However, cellular changes in other pathways may also be involved, and thus this generalization may be premature.

Fatty Acids and Metabolites. Adding 10 μmol/L 15-deoxy-Δ12,14-prostaglandin J2, a metabolite of dietary ω-3 fatty acids, inhibited proliferation and induced autophagic cell death of prostate cancer cells, in part, by activating peroxisome proliferator–activated receptor γ (201). It was proposed, therefore, that modulation of 15-deoxy-Δ12,14-prostaglandin J2 by increasing the proportion of ω-3 in the diet may be a prostate cancer–preventive or therapeutic strategy. Lastly, the short-chain fatty acid sodium butyrate, a product of microbial fermentation of soluble dietary fiber in the human colon and a histone deacetylase inhibitor, induced (at millimolar concentrations) both mitochondria-mediated apoptosis and caspase-independent autophagic cell death in human cervical cancer cells (202). Although these concentrations can occur in the gastrointestinal tract, the physiologic relevance of these findings in the cervix needs to be clarified.

It is important to note that although autophagic vacuole formation has been reported to occur in response to various bioactive food constituents, the appearance of autophagosomes in itself does not necessarily indicate that cell death is occurring. The example of sulforaphane clearly underscores this point. Thus, studies examining the induction of autophagy in response to a dietary factor need to establish that autophagy is indeed contributing to cytotoxicity. Furthermore, to assess relevance to cancer prevention in humans, more physiologically relevant doses of dietary bioactive constituents should be evaluated *in vitro*. Moreover, agent-induced autophagy should be shown *in vivo*, as reported for curcumin by Aoki et al. (180).

Issues in Measuring Autophagy and Future Research Opportunities

In light of the growing recognition that autophagy plays an important role in health and disease, there will be a continued interest in examining the effect of nutrients and bioactive dietary factors on the relationship between autophagy and cancer. Several issues need to be underscored about the measurement and ultimate role of autophagy in response to a cellular stressor. First, as previously stated, the appearance of autophagic vacuolization is not, in and of itself, evidence that autophagy is stimulated. Increased accumulation of autophagosomes may not only be the consequence of increased initiation of vacuoles but may also simply be a secondary outcome of failure or blockage of fusion of the autophagosome with the lysosome. This situation can be experimentally induced by treatment of cells with the drug bafilomycin A and actually can lead to impediment of delivery of vacuolar cargo to the lysosome for degradation by lysosomal hydrolases. This is also illustrated by the recent observation that the carcinogen lindane induced extensive vacuolization of Sertoli cells by disrupting autophagosome maturation into functional autolysosomes (203). Establishing how the flux, in other words both autophagosome formation and its delivery to the lysosomal compartment, changes in response to a stimulus or stressor is important. In addition, establishing that the vacuoles observed in stressed cells are indeed autophagosomes is clearly essential. Transmission electron microscopy is considered one of the gold standards used to determine that double-membrane vacuole structures are formed, which engulf cytoplasmic components (such as endoplasmic reticulum and ribosomes) and organelles (such as mitochondria). It is recommended that transmission electron microscopy evidence be supported with additional immunohistochemical evidence that there is punctate localization of the LC3 protein within the membranes of these putative autophagic vacuoles (17, 29, 31). In this regard, the construction of green fluorescent protein–tagged LC3 and its introduction into cells can considerably improve visualization of LC3 localization (204). There are circumstances, however, in which caution needs to be exercised in the interpretation of LC3 localization (205). Monitoring LC3 conversion from its parent form (LC3 I) to its lipidated LC3 II form (which accompanies autophagic vacuole formation) in cellular extracts by Western blotting can also be done, although some care must be exercised in the interpretation of the results (17, 206).

Quantifying the degradation rate of long-lived proteins in response to a treatment has routinely been used as an indicator that autophagy has been triggered (17, 31). Extensive guidelines for the use and interpretation of autophagy assays have recently been published by a consortium of autophagy researchers (207). They emphasize that multiple assays be selected based on the specific experimental objectives and hypotheses to be examined. This should provide valuable assistance to scientists intending to show a relationship among dietary factors, autophagy, and carcinogenesis.

As discussed above, the appearance of autophagosomes is not always an indication of death because autophagic vacuolization can be an indicator of a survival or death strategy in stressed cells. Autophagy is reversible provided that the nucleus and mitochondria are intact. Thus, several procedures can be used, at least *in vitro*, to determine whether the autophagic response is associated with enhanced or suppressed cell killing. Decreases in cell viability may be determined by propidium iodide staining and should be confirmed by a clonogenic assay. Autophagy is known to be inhibited by the PI3K inhibitors wortmannin, LY294002, and 3-methyladenine. Therefore, whether autophagy is contributing to cell death or survival can be partly assessed by determining if death is accelerated or delayed following cotreatment with these autophagy inhibitors. Some care is needed in interpreting data from studies using 3-methyladenine because it is not solely an inhibitor of the autophagy-activating class III PI3K.

Furthermore, the involvement of autophagy in cell death can be confirmed by using strategies to silence or knock out genes essential for the formation of the autophagosome and then evaluating the subsequent propensity for cell death. *Atg5, Atg7*, and *Beclin1* are examples of such genes that have been silenced in various studies (17). However, it has been cautioned that more than a single autophagy gene needs to be knocked out or

knocked down before a conclusive role of autophagy in specific cell death scenarios can be ascertained (136). A detailed and thorough discussion has been published of experimental strategies that can be used to characterize the role of autophagy in cell death *in vitro* before undertaking the evaluation of its regulation *in vivo* (208).

Despite numerous studies that have characterized the molecular mechanisms of autophagy *in vitro*, additional issues related to the physiologic relevance of autophagy and cancer deserve attention. There continues to be a considerable lack of physiologically relevant *in vivo* information on the role of autophagy in neoplastic development starting from the initiation of a normal cell and ultimately progressing to malignancy. Some insights into this issue have been provided (as mentioned above) by data from four tumor models: chemically induced rodent fibrosarcomas, liver tumors and pancreatic cancers, as well as Beclin 1 haploinsufficient mice. However, the relationship between autophagic capacity and stage of carcinogenesis for other common cancers has not yet been determined in preclinical models. As described above, the autophagy-deficient *beclin*$^{+/-}$ mouse exhibited increased susceptibility to several neoplasms and preneoplastic conditions (91, 92). This and other models with compromised *Atg* gene status can, therefore, provide an unusual opportunity to more conclusively study how dietary bioactive constituents modify autophagy in the context of *in vivo* carcinogenesis, particularly in comparison with their normal counterparts.

A list of mouse phenotypes resulting from mutations in autophagy genes has been described (10). Furthermore, the generation of the green fluorescent protein-LC3–expressing transgenic mouse allows investigators to characterize the *in vivo* effects of dietary agents and carcinogens on the stimulation and control of autophagy in a variety of tissues (204). Clearly, further studies are needed to characterize in other established tumor models the role of autophagy in carcinogenesis both in specific target tissues and during multiple stages of tumor development including tumor angiogenesis (209).

In this regard, there also are relatively few studies that have compared autophagic activity in normal or nonneoplastic cells with those that are neoplastic or malignant, especially in response to a variety of cancer inhibitory agents. Those reports describing the different protein degradation capacities of nontransformed and transformed cells are one example where this difference has been characterized (107). Sensitivity to ROS-induced autophagy has been reported to differ between nontransformed and cancer cells (210, 211). In addition, Ellington et al. (182) determined that normal human colon cells exhibited no induction of autophagy compared with the substantial induction observed in human colon cancer cells after treatment with triterpenoid soyasaponins.

Kuo et al. (151) reported that the drug plumbagin suppressed human breast cancer xenograft growth in mice without evidence of organ toxicities, although the induction of autophagy in noncancerous tissues was not reported. Differentiated human colon cancer cells exhibited decreased autophagy compared with their undifferentiated counterparts (212). Recently, the effect of autophagy on cell survival during endoplasmic reticulum–induced stress was determined to be dependent on the transformation status of the cell (70). On the other hand, the tyrosine kinase inhibitor imatinib induced autophagic vacuole formation effectively in both neoplastic and nonneoplastic cultures of neuronal cells (146). These different sensitivities to autophagy induction in normal versus cancerous tissues and as a function of differentiation will be important to be more thoroughly characterized before dietary modulation of autophagy induction in cancer prevention strategies can be considered.

In summary, autophagy is a cellular response to multiple factors influencing cellular homeostasis, including therapeutic drugs and the presence or absence of food constituents. Undeniably, cancer researchers need to clarify what tips the balance of autophagy between prosurvival and prodeath responses (213). Whereas data about bioactive food components are intriguing, the induction or suppression of autophagy as a modifier of carcinogenesis needs to be examined in terms of dose and duration of exposures both *in vitro* and *in vivo*. It is encouraging that the tools for these studies are increasingly available so that the molecular contributions of autophagy-related signaling pathways and the physiologic effect of autophagy on *in vivo* carcinogenesis can be examined.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Popkin B. Understanding global nutrition dynamics as a step towards controlling cancer incidence. Nat Rev Cancer 2007;7:61–7.
2. Thomasset S, Berry D, Garcea G, Marczylo T, Steward W, Gescher A. Dietary polyphenolic phytochemicals—promising cancer chemopreventive agents in humans? A review of their clinical properties. Int J Cancer 2007;120:451–8.
3. Singletary K, Jackson S, Milner J, Non-nutritive components in foods as modifiers of the cancer process. In: Bendich A. and Deckelbaum R, editors. Preventive nutrition, the comprehensive guide for health professionals. Totowa (NJ): Humana Press; 2005. p. 55–88.
4. Hanahan D, Weinberg R. The hallmarks of cancer. Cell 2000;100:57–70.
5. Martin K. Targeting apoptosis with dietary bioactive agents. Exp Biol Med 2006;231:117–29.
6. Ricci M, Zong W. Chemotherapeutic approaches for targeting cell death pathways. Oncologist 2006;11:342–57.
7. Leist M, Jaattela M. Four deaths and a funeral: from caspases to alternative mechanisms. Nat Rev Mol Cell Biol 2001;2:1–10.
8. Lockshin R, Zakeri Z. Apoptosis, autophagy, and more. Int J Biochem Cell Biol 2004;36:2405–19.
9. Shintani T, Klionsky D. Autophagy in health and disease: a double-edged sword. Science 2004;306:990–5.
10. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008;132:27–42.
11. Mizushima N, Levine B, Cuervo A, Klionsky D. Autophagy fights disease through self digestion. Nature 2008;451:1069–75.
12. Krysko D, VandenBerghe T, D'Herdek A, Vandensbeele P. Apoptosis and necrosis: detection, discrimination and phagocytosis. Methods 2008;44:205–21.
13. Eskelinen E. Doctor Jekyll and Mr. Hyde: autophagy can promote both cell survival and cell death. Cell Death Differ 2005;12:1468–72.
14. Heymann D. Autophagy: a protective mechanism in responses to stress and inflammation. Curr Opin Investig Drugs 2006;7:443–50.
15. Lum J, DeBernardinis R, Thompson C. Autophagy in metazoans: cell survival in the land of plenty. Nat Rev Mol Cell Biol 2005;6:439–48.
16. Kaushik S, Cuervo A. Autophagy as a cell repair mechanism: activation of chaperone-mediated autophagy during oxidative stress. Mol Aspects Med 2006;27:444–54.
17. Klonisky D, Cuervo A, Seglen P. Methods for monitoring autophagy from yeast to man. Autophagy 2007;3:1–26.

18. Mathew R, White E. Why sick cells produce tumors. Autophagy 2007;3:502–5.
19. Kundu A, Thompson C. Autophagy: basic principles and relevance to disease. Annu Rev Pathol 2008;3:427–55.
20. Mizushima N. The pleitropic role of autophagy: from protein metabolism to bacteriocide. Cell Death Differ 2005;12:1535–41.
21. Levine B, Yuan J. Autophagy in cell death: an innocent convict? J Clin Invest 2005;115:2679–88.
22. Levine B. Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. Cell 2005;120:159–62.
23. Kuma A, Hatano M, Matsui M, et al. The role of autophagy during early neonatal starvation period. Nature 2004;432:1032–6.
24. Fimia M, Stoykova A, Romagnoli A, et al. Ambra1 regulates autophagy and development of the nervous system. Nature 2007; 447:1121–5.
25. Edinger A, Thompson C. Death by design: apoptosis, necrosis and autophagy. Curr Opin Cell Biol 2004;16:663–9.
26. Liu Z, Lenardo M. Reactive oxygen species regulate autophagy through redox-sensitive proteases. Dev Cell 2007;12:484–5.
27. Gonzalez-Polo R, Boya P, Pauleau A, et al. The apoptosis/autophagy paradox: autophagic vacuolization before apoptotic death. J Cell Sci 2005;118:3091–102.
28. Paglin S, Hollister T, Deloherty T, et al. A novel response of cancer cells to radiation involves autophagy and formation of acidic vesicles. Cancer Res 2001;61:439–44.
29. Martinet W, DeMeyer G, Andries L, Herman A, Kockx M. Detection of autophagy in tissue by standard immunohistochemistry: possibilities and limitations. Autophagy 2006;2:55–7.
30. Tanida I, Ueno T, Kominami E. LC3 conjugation system in mammaliam autophagy. Int J Biochem Cell Biol 2004;36:2503–18.
31. Mizushima N. Methods for measuring autophagy. Int J Biochem Cell Biol 2004;36:2491–502.
32. Kadowski M, Karim M, Carpi A, Miotto G. Nutrient control of macroautophagy in mammaliam cells. Mol Aspects Med 2006;27: 426–43.
33. Tsujimoto Y, Shimizu S. Another way to die: autophagic programmed cell death. Cell Death Differ 2005;12:1528–34.
34. Fingar D, Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. Oncogene 2004;23:3151–71.
35. Bhaskar P, Hay N. The two TORCs and Akt. Dev Cell 2007;12: 487–502.
36. Sarbassov D, Guertin D, Ali S, Sabatini D. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 2005; 307:1098–101.
37. Feng Z, Zhang H, Levine A, Jin S. The coordinate regulation of the p53 and mTOR pathways in cells. Proc Natl Acad Sci U S A 2005;102: 8204–9.
38. Dann S, Selvaraj A, Thomas G. mTOR complex1-6K1 signaling: at the crossroads of obesity, diabetes and cancer. Trends Mol Med 2007;13: 252–9.
39. Feng Z, Hu W, De Stanchina E, et al. The regulation of AMPKβ1, TSC2, and PTEN expression by p53: stress, cell and tissue specificity, and the role of these gene products in modulating the IGF-1-Akt-mTOR pathways. Cancer Res 2007;67:3043–53.
40. Pattingre S, Espert L, Biard-Piechaczyk M, Codogno P. Regulation of macroautophagy by mTOR and Beclin1 complexes. Biochimie 2008; 90:313–23.
41. Liang J, Shao S, Xu Z, et al. The energy sensing LKB1-AMPK pathway regulates p27kip1 phosphorylation mediating the decision to enter autophagy or apoptosis. Nat Cell Biol 2007;9:218–24.
42. Hoyer-Hansen M, Jaattela M. AMP-activated protein kinase: a universal regulator of autophagy? Autophagy 2007;3:381–3.
43. Hardie G, Hawley S, Scott J. AMP-activated protein kinase—development of the energy sensor concept. J Physiol 2006;574(Pt 1): 7–15.
44. Hardie D. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nat Rev Mol Cell Biol 2007;8:774–81.
45. Lindsley J, Rutter J. Nutrient sensing and metabolic decisions. Comp Biochem Physiol B Biochem Mol Biol 2004;139:543–59.
46. Degenhardt K, Mathew R, Beaudoin B, et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 2006;10:51–64.
47. Kundu M, Thompson C. Macroautophagy versus mitochondrial autophagy: a question of fate? Cell Death Differ 2005;12:1484–9.
48. Jin S, White E. Role of autophagy in cancer; management of metabolic stress. Autophagy 2007;3:28–31.
49. Lum J, Bauer D, Knog M, et al. Growth factor regulation of autophagy and cell survival in the absence of apoptosis. Cell 2005;120:237–48.
50. Jin S, DiPaola R, Mathew R, White E. Metabolic catastrophe as a means to cancer cell death. J Cell Sci 2007;120:379–83.
51. Nelson D, White E. Exploiting different ways to die. Genes Dev 2004; 18:1223–6.
52. Bialik S, Kimchi A. The death-associated protein kinases: structure, function and beyond. Annu Rev Biochem 2006;75:189–210.
53. Inbal B, Bialik S, Sabanay I, Shani G, Kimchi A. DAP kinase and DRP-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death. J Cell Biol 2002;157:455–68.
54. Petiot A, Pattingre S, Arico S, Meley D, Codogno P. Diversity of signaling controls of macroautophagy in mammalian cells. Cell Struct Funct 2002;27:431–41.
55. Botti J, Djavaheri-Mergny M, Pilatte Y, Codogno P. Autophagy signaling and the cogwheels of cancer. Autophagy 2006;2:67–73.
56. Yu L, Alva A, Su H, et al. Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. Science 2004;304:1500–02.
57. Corcelle E, Djerbi N, Mari M, et al. Control of autophagy maturation step by the MAPK ERK and p38. Autophagy 2007;3:57–9.
58. Moretti L, Cha Y, Niermann K, Lu B. Switch between apoptosis and autophagy: radiation-induced endoplasmic reticulum stress? Cell Cycle 2007;6:793–8.
59. Codogno P, Meijer A. Autophagy and signaling: their role in cell survival and cell death. Cell Death Differ 2005;12:1509–18.
60. Hoyer-Hansen M, Bastholm L, Szyniarowski P, et al. Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-β, and Bcl-2. Mol Cell 2007;25:193–205.
61. Levine A, Feng Z, Mak T, You H, Jin S. Coordination and communication between p53 and IGF-1-Akt-Tor signal transduction pathways. Genes Dev 2006;20:267–75.
62. Crighton D, Wilkinson S, Ryan K. DRAM links autophagy to p53 and programmed cell death. Autophagy 2007;3:72–4.
63. Crighton D, O'Prey J, Bell H, Ryan K. p73 regulates DRAM-indiependent autophagy that does not contribute to programmed cell death. Cell Death Differ 2007;10:1–9.
64. Green D, Chipuk J. p53 and metabolism: inside the TIGAR. Cell 2006; 126:30–2.
65. Akar U, Ozpolat B, Mehta K, Fok J, Kondo Y, Lopez-Berestein G. Tissue transglutaminase inhibits autophagy in pancreatic cancer cells. Mol Cancer Res 2007;5:241–9.
66. Ozpolat B, Akar U, Lopez-Berrestein G. PKCδ and tissue transglutaminase are novel inhibitors of autophagy in pancreatic cancer cells. Autophagy 2007;3:480–3.
67. Kang C, Avery L. To be or not to be, the level of autophagy is the question. Autophagy 2008;4:82–4.
68. Criollo A, Maluri M, Tasdemir E, et al. Regulation of autophagy by the inositol triphosphate receptor. Cell Death Differ 2007;14:1029–39.
69. Reef S, Zalckvar E, Shifman O, et al. A short mitochondrial form of $p10^{ARF}$ induces autophagy and caspase-independent cell death. Mol Cell 2006;22:463–75.
70. Ding W, Ni H, Gao W, et al. Differential effects of endoplasmic reticulum stress-induced autophagy on cell survival. J Biol Chem 2007;282:4702–10.
71. Bernales S, McDonald K, Walter P. Autophagy counterbalances endoplasmic reticulum stress during the unfolded protein response. PLoS Biol 2005;4:2311–24.
72. Ogata M, Hino S, Saito A, et al. Autophagy is activated for cell survival after endoplasmic reticulum stress. Mol Cell Biol 2006;26: 9220–31.
73. Yorimitsu T, Nair U, Yang Z, Klionsky D. Endoplasmic reticulum stress triggers autophagy. J Biol Chem 2006;281:30299–304.
74. Rodriguez-Enriquez S, He L, Lemasters J. Role of mitochondrial permeability transition pores in mitochondrial autophagy. Int J Biochem Cell Biol 2004;36:2463–72.
75. Jin S. Autophagy, mitochondrial quality control and oncogenesis. Autophagy 2006;2:80–4.
76. Tardy C, Codogno P, Autefage H, Levade T, Andrieu-Abadie N. Lysosomes and lysosomal proteins in cancer cell death (new players of an old struggle). Biochim Biophys Acta 2006;1765:101–25.
77. Kroemer G, Jaattela M. Lysosomes and autophagy in cell death control. Nat Rev Cancer 2005;5:886–97.
78. Swerdlow S, Distelhorst C. Bcl-2-regulated calcium signals as common mediators of both apoptosis and autophagy. Dev Cell 2007;12:1788–99.
79. Pinton P, Rizzuto R. Bcl-2 and Ca^{2+} homeostasis in the endoplasmic reticulum. Cell Death Differ 2006;13:1409–18.
80. Scherz-Shouval R, Snvets E, Fass E, Shorer H, Gil L, Elazar Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. EMBO J 2007;26:1749–60.
81. Scherz-Shouval R, Shvets E, Elazar Z. Oxidation as a post-translational modification that regulates autophagy. Autophagy 2007;3: 371–3.
82. Kiffin R, Bandyopadhyay U, Cuervo A. Oxidative stress and autophagy. Antioxid Redox Signal 2006;8:152–62.

83. Cullinan S, Diehl A. Coordination of ER and oxidative stress signaling: the PERK/Nrf2 signaling pathway. Int J Biochem Cell Biol 2006;38:317–32.
84. Djavaheri-Mergny M, Amelotti M, Mathieu J, Besancon F, Bauvy C, Codogno P. Regulation of autophagy by NF-κB transcription factor and reactive oxygen species. Autophagy 2007;3:390–2.
85. Djavaheri-Mergny M, Amelotti M, Mathiou J, et al. NF-κB activation represses tumor necrosis factor-α-induced autophagy. J Biol Chem 2006;281:30373–82.
86. Xu Y, Kim S, Li Y, Han J. Autophagy contributes to caspase-independent macrophage cell death. J Biol Chem 2006;281:19179–86.
87. Yu L, Wa F, Dutta S, et al. Autophagic programmed cell death by selective catalase degradation. Proc Natl Acad Sci U S A 2006;103: 4952–7.
88. Mizushima N, Klionsky D. Protein turnover via autophagy: implications for metabolism. Annu Rev Nutr 2007;27:19–39.
89. Furuya N, Yu J, Byfield M, Pattingre S, Levine B. The evolutionarily conserved domain of beclin 1 is required for Vps34 binding, autophagy and tumor suppressor function. Autophagy 2005;1:46–52.
90. Liang X, Kleemen L, Jiang H, et al. Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. J Virol 1998;72:8586–96.
91. Liang X, Jackson S, Seaman M, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 1999;402:672–6.
92. Qu X, Yu J, Bjagat G, et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest 2003;112:1809–20.
93. Yue Z, Jin S, Yang C, Levine A, Henitz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. Proc Natl Acad Sci U S A 2003;100:15077–82.
94. Liang C, Feng P, Ku B, et al. Autophagic and tumour suppressor activity of a novel Beclin 1-binding protein UVRAG. Nat Cell Biol 2006;8:688–99.
95. Liang C, Feng P, Ku B, Oh B, Jung J. UVRAG, a new player in autophagy and tumor cell growth. Autophagy 2007;3:69–71.
96. Takahashi Y, Coppola D, Matsushita N, et al. Bif-I interacts with Beclin I through UVRAG and regulates autography and tumorigenesis. Nat Cell Biol 2007;9:1142–51.
97. Lee I, Cao L, Mostosiavsky R, et al. A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. Proc Natl Acad Sci U S A 2008;105:3374–9.
98. Ogier-Denis E, Codogno P. Autophagy: a barrier or an adaptive response to cancer. Biochim Biophys Acta 2003;1603:113–28.
99. Boya P, Gonzalez-Polo R, Casares N, et al. Inhibition of macroautophagy triggers apoptosis. Mol Cell Biol 2005;25:1025–40.
100. Moretti L, Attia A, Kim K, Lu B. Cross talk between Bak/Bax and mTOR signaling regulates radiation-induced autophagy. Autophagy 2007;3:142–4.
101. Kondo Y, Kanzawa T, Sawaya R, Kondo S. The role of autophagy in cancer development and response to therapy. Nat Rev Cancer 2005;5: 726–34.
102. Rubinsztein D, Gestwicki J, Murphy L, Klionsky D. Potential therapeutic applications of autophagy. Nat Rev Drug Discov 2006;6:312.
103. Kondo Y, Kondo S. Autophagy and cancer therapy. Autophagy 2006; 2:85–90.
104. Pouyssegur J, Dayan F, Mazure N. Hypoxia signaling in cancer and approaches to enforce tumour regression. Nature 2006;44:437–43.
105. Arsham A, Neufeld T. Thinking globally and acting locally with TOR. Curr Opin Cell Biol 2006;18:589–97.
106. Mathew R, Karantza-Wadsworth V, White E. Role of autophagy in cancer. Nat Rev Cancer 2007;7:961–7.
107. Gozuacik A, Kimchi A. Autophagy as a cell death and tumor suppressor mechanism. Oncogene 2004;23:2891–906.
108. Alva A, Gultekin S, Baehrecke E. Autophagy in human tumors: cell survival or death? Cell Death Differ 2004;11:1046–8.
109. Mathew R, Kongara S, Beaudoin B, et al. Autophagy suppresses tumor progression by limiting chromosomal instability. Genes Dev 2007;21:1367–81.
110. Karantza-Wadsworth V, Patel S, Kravchuk O, et al. Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. Genes Dev 2007;21:1621–35.
111. Pyo J, Jang M, Kwon Y, et al. Essential roles of Atg5 and FADD in autophagic cell death. J Biol Chem 2005;280:20722–9.
112. Guillon-Munos A, VanBemmelen M, Clarke P. Autophagy can be a killer even in apoptosis-competent cells. Autophagy 2006;2:140–2.
113. Scott R, Juhasz G, Neufeld T. Direct induction of autophagy by Atg1 inhibits cell growth and induces apoptotic cell death. Curr Biol 2007; 17:1–11.
114. Qu X, Zou Z, Sun Q, et al. Autophagy gene-dependent clearance of apoptotic cells during embryonic development. Cell 2007;128: 931–46.
115. Yoshimori T. Autophagy: paying Charon's toll. Cell 2007;128:833–6.
116. Mills K, Reginato M, Debnath J, Queenan B, Brugge J. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is required for induction of autophagy during lumen filling *in vitro*. Proc Natl Acad Sci U S A 2004;101:3438–43.
117. Schwarze P, Seglen P. Reduced autophagic activity, improved protein balance and enhanced *in vitro* survival of hepatocytes isolated from carcinogen-treated rats. Exp Cell Res 1985;157:15–28.
118. Seglen P, Schwarze P, Saeter G. Changes in cellular ploidy and autophagic responsiveness during rat liver carcinogenesis. Toxicol Pathol 1986;14:342–8.
119. Rez G, Toth S, Palfia Z. Cellular autophagic capacity is highly increased in azaserine-induced premalignant atypical acinar nodule cells. Carcinogenesis 1999;20:1893–8.
120. Toth S, Nagy K, Palfia Z, Rez G. Cellular autophagic capacity changes during azaserine-induced tumour progression in the rat pancreas. Cell Tissue Res 2002;309:409–16.
121. Marino G, Salvador-Montoliu N, Fueyo A, Knecht E, Mizushima N, Lopez-Otin C. Tissue-specific autophagy alterations and increased tumorigenesis in mice deficient in Atg4C/autophagin-3. J Biol Chem 2007;282:18573–83.
122. Ahn C, Jeong E, Lee J, et al. Expression of beclin1, an autophagy-related protein in gastric and colorectal cancers. APMIS 2007;115: 1344–9.
123. Hippert M, O'Toole P, Thorburn A. Autophagy in cancer: good, bad, or both? Cancer Res 2006;66:9349–51.
124. Scarlatti F, Bauvy C, Ventruti A, et al. Ceramide-mediated macroautophagy involves inhibition of protein kinase B and up-regulation of Beclin 1. J Biol Chem 2004;279:18384–91.
125. Zheng W, Kollmeyer J, Symolon H, et al. Ceramides and other bioactive sphingolipid backbones in health and disease: lipidomic analysis, metabolism, and roles in membrane structure, dynamics, signaling and autophagy. Biochim Biophys Acta 2007;1758:1864–84.
126. Lavieu G, Scarlatti F, Sala G, et al. Regulation of autophagy by sphingosine kinase 1 and its role in cell survival during nutrient starvation. J Biol Chem 2006;281:8518–27.
127. Lavieu G, Scarlatti F, Sala G, et al. Is autophagy the key mechanism by which the sphingolipid rheostat controls the cell fate decision? Autophagy 2007;3:45–7.
128. Pattingre S, Tassa A, Qu X, et al. Bcl-2 antiapoptotic proteins inhibit Beclin1-dependent autophagy. Cell 2005;122:927–39.
129. Pattingre S, Levine B. Bcl-2 inhibition of autophagy: a new route to cancer? Cancer Res 2006;66:2885–8.
130. Shimizu S, Kanaseki T, Mizushima N, Arakawa-Kobayashi S, Thompson C, Tsujimoto Y. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol 2004;6:1221–8.
131. Maiuri M, LeToumelin G, Criollo A, et al. Functional and physical interaction between Bcl-XL and a BH-3like domain of Beclin-1. EMBO J 2007;26:2527–39.
132. Stephan J, Herman P. The regulation of autophagy in eukaryotic cells: do all roads pass through Atg1? Autophagy 2006;2:146–8.
133. Yousefi S, PerozzoR, Schmid I, et al. Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis. Nat Cell Biol 2006;8:1124–32.
134. Codogno P, Meijer A. Atg5: more than an autophagy factor. Nat Cell Biol 2006;8:1045–7.
135. Luo S, Rubinsztein D. Atg5 and Bcl-2 provide novel insights into the interplay between apoptosis and autophagy. Cell Death Differ 2007; 14:1247–50.
136. Maiuri M, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: cross talk between autophagy and apoptosis. Nat Rev Mol Cell Biol 2007;8:741–52.
137. Wang Y, Singh R, Massey A, et al. Loss of macroautophagy promotes or prevents fibroblast apoptosis depending on the death stimulus. J Biol Chem 2008;283:4766–77.
138. Thorburn A. Apoptosis and autophagy: regulator connections between two supposedly different processes. Apoptosis 2008;13:1–9.
139. Bursch W, Ellinger A, Kienzl H, et al. Active cell death induced by the anti-estrogens tamoxifen and ICI164 384 in human mammary carcinoma cells (MCF-7) in culture: the role of autophagy. Carcinogenesis 1996;17:1595–607.
140. Bursch W, Hochegger K, Torok L, Marian B, Ellinger A, Hermann R. Autophagic and apoptotic types of programmed cell death exhibit different fates of cytoskeletal filaments. J Cell Sci 2000;113:1189–98.
141. Petrovski G, Zahuczky G, Katona K, et al. Clearance of dying autophagic cells of different origin by professional and non-professional phagocytes. Cell Death Differ 2007;14:1117–28.
142. Bilir A, Altinoz M, Erkan M, Ozmen V, Aydiner A. Autophagy and nuclear changes in FM3A breast tumor cells after epirubicin, medroxyprogesterone and tamoxifen treatment *in vitro*. Pathobiology 2001;69:120–6.

143. Qadir M, Kwok B, Dragowska W, et al. Macroautophagy inhibition sensitizes TAM-resistant breast cancer cells and enhances mitochondrial depolarization. Breast Cancer Res Treat. In press 2008.
144. Kanzawa T, Kondo Y, Ito H, Kondo S, Germano I. Induction of autophagic cell death in malignant glioma cells by arsenic trioxide. Cancer Res 2003;63:2103–8.
145. Qian W, Liu J, Jin J, Ni W, Xu W. Arsenic trioxide induces not only apoptosis but also autophagic cell death in leukemia cell lines via up-regulation of Beclin-1. Leuk Res 2007;31:329–39.
146. Ertmer A, Huber V, Gilch S, et al. The anticancer drug imatinib induces cellular autophagy. Leukemia 2007;21:936–42.
147. Cui Q, Tashiro S, Onodera S, Minami M, Ikejima T. Autophagy preceded apoptosis in oridonin-treated human breast cancer MCF-7 cells. Biol Pharm Bull 2007;30:859–64.
148. Hoyer-Hansen M, Bastholm L, Mathiasen I, Elling F, Jaattela M. Vitamin D analog EB1089 triggers dramatic lysosomal changes and Beclin 1-mediated autophagic cell death. Cell Death Differ 2005;12: 1297–309.
149. DeMasters G, Di X, Newsham I, Shiu R, Gewirtz D. Potentiation of radiation sensitivity in breast tumor cells by vitamin D3 analogue, EB 1089, through promotion of autophagy and interference with proliferative activity. Mol Cancer Ther 2006;5:2786–97.
150. Meschini S, Condello M, Marra M, Formisano G, Federici E, Arancia G. Autophagy-mediated chemosensitizing effect of the plant alkaloid voacamine on multidrug resistant cells. Toxicol In Vitro 2007;21:197–203.
151. Kuo P, Hsu Y, Cho C. Plumbagin induces G_2-M arrest and autophagy by inhibiting the AKT/mammalian target of rapamycin pathway in breast cancer cells. Mol Cancer Ther 2006;5:3209–21.
152. Tsang C, Qi H, Liu L, Zheng X. Targeting mammaliam target of rapamycin (mTOR) for health and diseases. Drug Discov Today 2007; 12:112–24.
153. Katayama M, Kawaguchi T, Berger M, Pieper R. DNA damaging agent-induced autophagy produces a cytoprotective adenosine triphosphate surge in malignant glioma cells. Cell Death Differ 2007;14:548–58.
154. Abedin M, Wang D, McDonnell M, Lehmann U, Kelekar A. Autophagy delays apoptotic death in breast cancer cells following DNA damage. Cell Death Differ 2007;14:500–10.
155. Tiwari M, Bajpai V, Sahasrabuddhe A, et al. Inhibition of *N*-(4-hydroxyphenyl)retinamide-induced autophagy at a low doses enhances cell death in malignant glioma cells. Carcinogenesis 2008;29:600–9.
156. Fazi B, Bursch W, Fimia G, et al. Fenretinide induces autophagic cell death in caspase-defective breast cancer cells. Autophagy 2008;4:1–7.
157. Donati A. The involvement of macroautophagy in aging and anti-aging interventions. Mol Aspects Med 2006;27:455–70.
158. Bergamini E, Cavallini G, Donati A, Gori Z. The role of autophagy in aging: its essential part in the anti-aging mechanism of caloric restriction. Ann N Y Acad Sci 2007;1114:69–78.
159. Finn P, Dice J. Ketone bodies stimulate chaperone-mediated autophagy. J Biol Chem 2005;280:25864–70.
160. Sell C. Caloric restriction and insulin-like growth factors in aging and cancer. Horm Metab Res 2003;35:705–11.
161. Kopelovich L, Fay J, Sigman C, Crowell J. The mammalian target of rapamycin pathway as a potential target for cancer chemoprevention. Cancer Epidemiol Biomark Prev 2007;16:1330–40.
162. Gossner G, Choi M, Tan L, et al. Genistein-induced apoptosis and autophagocytosis in ovarian cancer cells. Gynecol Oncol 2007;105: 23–30.
163. Pedro M, Lourenco C, Cidade H, Kijjoa A, Pinto M, Nascimento M. Effects of natural prenylated flavones in the phenotypical ER(+) MCF-7 and ER(−) MDA-MB-231 human breast cancer cells. Toxicol Lett 2006;164:24–36.
164. Delmulle L, Berghe K, Keukeleire D, Vandenabeele P. Treatment of PC-3 and DU145 prostate cancer cells by prenylflavonoids from hop (*Humulus lupulus* L.) induces a caspase-independent form of cell death. Phytother Res 2008;22:197–203.
165. Psahoulia F, Moumtzi S, Roberts M, Sasazuki T, Shirasawa S, Pintzas A. Quercetin mediates preferential degradation of oncogenic Ras and causes autophagy in Ha-RAS-transformed human colon cancer cells. Carcinogenesis 2007;28:1021–31.
166. Chi S, Kitanaka C, Noguchi K, et al. Oncogenic Ras triggers cell suicide through the activation of a caspase-independent dell death program in human cancer cells. Oncogene 1999;18:2281–90.
167. Kitanaka C, Kato K, Ijiri R, et al. Increased Ras expression and caspase-independent neuroblastoma cell death: possible mechanism of spontaneous neuroblastoma regression. J Natl Cancer Inst 2002;94: 358–68.
168. Tsuneoka M, Umata T, Kimura H, et al. C-myc induces autophagy in rat 3Y1 fibroblast cells. Cell Struct Funct 2003;28:195–204.
169. Kaul A, Overmeyer J, Maltese W. Activated Ras induces cytoplasmic vacuolization and non-apoptotic death in glioblastoma cells via novel effector pathways. Cell Signal 2007;19:1034–43.
170. Li D, Cui Q, Chen S, et al. Inactivation of ras and changes of mitochondrial membrane potential contributes to oridonin-induced autophagy in A431 cells. J Pharmacol Sci 2007;105:22–33.
171. Cui Q, Tashiro S, Onodera S, Minami M, Ikejima T. Oridonin-induced autophagy in human cervical HeLa cells through Ras JNK, p38 regulation. J Pharmacol Sci 2007;105:317–25.
172. Berry D, Baehrecke E. Growth arrest and autophagy are required for salivary gland cell degradation in Drosophila. Cell 2007;131:1137–48.
173. Blankson H, Holen I, Seglen P. Disruption of the cytokeratin cytoskeleton and inhibition of hepatocytic autophagy by okadaic acid. Exp Cell Res 1995;218:522–30.
174. Samari H, Moller M, Holden L, Asmyhr T, Seglen P. Stimulation of hepatocytic AMP-activated protein kinase by okadaic acid and other autophagy suppressive toxins. Biochem J 2005;386:237–44.
175. Ohshiro K, Rayala S, Kondo S, et al. Identifying the estrogen receptor coactivator PELP1 in autophagosomes. Cancer Res 2007;67:8164–71.
176. Opipari A, Tan L, Boitano A, Sorenson D, Aurora A, Liu J. Resveratrol-induced autophagocytosis in ovarian cancer cells. Cancer Res 2004;64:696–703.
177. Kueck A, Opiari A, Griffith K, et al. Resveratrol inhibits glucose metabolism in human ovarian cancer cells. Gynecol Oncol 2007;107: 450–7.
178. Trincheri N, Follo C, Nicotra G, Peracchio C, Castino R, Isidoro C. Resveratrol-induced apoptosis depends on the lipid kinase activity of Vps34 and on the formation of autophagosomes. Carcinogenesis 2008;29:381–9.
179. Park J, Choi Y, Suh S, et al. Bcl-2 overexpression attenuates resveratrol-induced apoptosis in U937 cells by inhibition of caspase-3 activity. Carcinogenesis 2001;22:1633–9.
180. Aoki H, Takada Y, Sawaya R, Aggarwal B, Kondo Y. Evidence that curcumin suppresses the growth of malignant gliomas *in vitro* and *in vivo* through induction of autophagy: role of Akt and ERK signaling pathways. Mol Pharmacol 2007;72:29–39.
181. Shingima N, Yokoyama T, Kondo T, Kondo S. Roles of Akt/mTOR/p70S6K and ERK1/2 signaling pathways in curcumin-induced autophagy. Autophagy 2007;3:635–7.
182. Ellington A, Berhow M, Singletary K. Induction of macroautophagy in human colon cancer cells by soybean B-group triterpenoid saponins. Carcinogenesis 2005;26:159–67.
183. Ellington A, Berhow M, Singletary K. Inhibition of Akt signaling and enhanced ERK1/2 activity are involved in induction of macroautophagy by triterpenoid B-group soyasaponins in colon cancer cells. Carcinogenesis 2006;27:298–306.
184. Wallace E, Lyssikatos J, Yeh T, Winkler J, Koch K. Progress towards therapeutic small molecule MEK inhibitors for use in cancer therapy. Curr Top Med Chem 2005;5:215–29.
185. Ogier-Denis E, Pattingre S, El Benna J, Codogno P. Erk1/2-dependent phosphorylation of Gα-interacting protein stimulates its GTPase accelerating activity and autophagy in human colon cancer cells. J Biol Chem 2000;275:39090–5.
186. Pattingre S, Bauvy C, Codogno P. Amino acids interfere with the ERK1/2-dependent control of macroautophagy by controlling the activation of Raf-1 in human colon cancer HT-29 cells. J Biol Chem 2003;278:16667–74.
187. Pattingre S, De Vries L, Bauvy C, et al. The G-protein regulator AGS3 controls an early event during macroautophagy in human intestinal HT-29 cells. J Biol Chem 2003;278:20995–1002.
188. Agarwal R. Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents. Biochem Pharmacol 2000;60:1051–9.
189. Gohla A, Klement K, Nurnberg B. The heterotrimeric G protein (Gi3) regulates hepatic autophagy downstream of the insulin receptor. Autophagy 2007;3:393–5.
190. Xu Z, Liang J, Haridas V, et al. A plant triterpenoid, avicin D, induces autophagy by AMP-activated protein kinase activation. Cell Death Differ 2007;14:1948–57.
191. Herman-Antosiewicz A, Johnson D, Singh S. Sulforaphane causes autophagy to inhibit release of cytochrome *c* and apoptosis in human prostate cancer cells. Cancer Res 2006;66:5828–35.
192. Bauvy C, Gane P, Arico S, Codogno P, Ogier-Denis E. Autophagy delays sulindac-induced apoptosis in the human intestinal colon cancer cell line HT-29. Exp Cell Res 2001;268:139–49.
193. Xiao D, Vogel V, Singh S. Benzyl isothiocyanate-induced apoptosis in human breast cancer cells is initiated by reactive oxygen species and regulated by Bax and Bak. Mol Cancer Ther 2006;5:2931–45.
194. Martin A, Joseph J, Cuervo A. Stimulatory effect of vitamin C on autophagy in glial cells. J Neurochem 2002;82:538–49.
195. Ohtani S, Iwamaru A, Deng W, et al. Tumor suppressor 101F6 and

ascorbate synergistically and selectively inhibit non-small cell lung cancer growth by caspase-independent apoptosis and autophagy. Cancer Res 2007;67:6293–303.
196. Tavera-Mendoza, Wang T, Lallemant B, et al. Convergence of vitamin D and retinoic acid signaling at a common hormone response element. EMBO Rep 2006;7:180–5.
197. Vaquero E, Rickmann M, Molero X. Tocotrienols: balancing the mitochondrial cross talk between apoptosis and autophagy. Autophagy 2007;6:652–4.
198. Rickmann M, Vaquero E, Malagelada J, Molero X. Tocotrienols induce apoptosis and autophagy in rat pancreatic stellate cells through mitochondrial death pathway. Gastroenterol 2007;132:2518–32.
199. Enomoto M, Tsuchida A, Miyazawa K, et al. Vitamin K2-induced cell growth inhibition via autophagy formation in cholangiocellular carcinoma cell lines. Int J Mol Med 2007;20:801–8.
200. Kim E, Sohn S, Kwon H, et al. Sodium selenite induces superoxide-mediated mitochondrial damage and subsequent autophagic cell death in malignant glioma cells. Cancer Res 2007;67:6314–24.
201. Butler R, Mitchell S, Tindall D, Young C. Nonapoptotic cell death associated with S-phase arrest of prostate cancer cells via the peroxisome proliferators-activated receptor γ ligand, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2. Cell Growth Different 2000;11:49–61.
202. Shao Y, Gao Z, Marks P, Jiang X. Apoptotic and autophagic death induced by histone deacetylase inhibitors. Proc Natl Acad Sci U S A 2004;101:18030–5.
203. Corcello E, Nebout M, Bekri S, et al. Disruption of autophagy at the maturation step by the carcinogen lindane is associated with the sustained mitogen-activated protein kinase/extracellular signal-regulated kinase activity. Cancer Res 2006;66:6861–70.
204. Mizushima N, Yamamoto A, Matsui M, Yoshimori T, Ohsumi Y. *In vivo* analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Mol Biol Cell 2004;15:1101–11.
205. Kuma A, Matsui M, Mizushima N. LC3, an autophagosome marker, can be incorporated into protein aggregates independent of autophagy: caution in the interpretation of LC3 localization. Autophagy 2007;3:323–8.
206. Mizushima N, Yoshimori T. How to interpret LC3 immunoblotting. Autophagy 2007;3:542–5.
207. Klionsky D, Abeliovich M, Agostinis P, et al. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. Autophagy 2008;4:151–75.
208. Thorburn A. Studying autophagy's relationship to cell death. Autophagy 2008;4:1–4.
209. Ramakrishnan S, Nguyen T, Subramanian S, Kelekar A. Autophagy and angiogenesis inhibition. Autophagy 2007;3:512–5.
210. Chen Y, McMillan-Ward E, Kong J, Israels S, Gibson S. Mitochondrial electron-transport-chain inhibitors of complexes I and II induce autophagic cell death mediated by reactive oxygen species. J Cell Sci 2007;120:4155–66.
211. Chen Y, McMillan-Ward E, Kong J, Israels S, Gibson S. Oxidative stress induces autophagic cell death independent of apoptosis in transformed and cancer cells. Cell Death Differ 2008;15:171–82.
212. Houri J, Ogier-Denis E, DeStefanis D, et al. Differentiation-dependent autophagy controls the fate of newly synthesized N-linked glycoproteins in the colon adenocarcinoma HT-29 cell line. Biochem J 1995;309:521–7.
213. Levine B. Unraveling the role of autophagy in cancer. Autophagy 2006;2:65–6.
214. Kir Kegaard K, Taylor MP, Jackson WT. Cellular autogrpahy: surrender, avoidance and subversion by microorganisms. Nat Rev Microbiol 2004;21:301–14.