ANTIOXIDANTS & REDOX SIGNALING
Volume 11, Number 4, 2009

DOI: 10.1089/ars.2008.2270

Forum Review Article

Regulation of Autophagy by Reactive Oxygen Species (ROS): Implications for Cancer Progression and Treatment

Meghan B. Azad,[1,2] Yongqiang Chen,[1] and Spencer B. Gibson[1,2,3]

Abstract

Reactive oxygen species (ROS) have been identified as signaling molecules in various pathways regulating both cell survival and cell death. Autophagy, a self-digestion process that degrades intracellular structures in response to stress, such as nutrient starvation, is also involved in both cell survival and cell death. Alterations in both ROS and autophagy regulation contribute to cancer initiation and progression, and both are targets for developing therapies to induce cell death selectively in cancer cells. Many stimuli that induce ROS generation also induce autophagy, including nutrient starvation, mitochondrial toxins, hypoxia, and oxidative stress. Some of these stimuli are under clinical investigation as cancer treatments, such as 2-methoxyestrodial and arsenic trioxide. Recently, it was demonstrated that ROS can induce autophagy through several distinct mechanisms involving Atg4, catalase, and the mitochondrial electron transport chain (mETC). This leads to both cell-survival and cell-death responses and could be selective toward cancer cells. In this review, we give an overview of the roles ROS and autophagy play in cell survival and cell death, and their importance to cancer. Furthermore, we describe how autophagy is mediated by ROS and the implications of this regulation to cancer treatments. *Antioxid. Redox Signal.* 11, 777–790.

The development of rationally targeted cancer therapeutics requires the characterization of cancer-specific signaling pathways. It is widely recognized that reactive oxygen species (ROS) play an important role in cancer initiation and progression (46). Recently, ROS have been identified as signaling molecules in various pathways regulating cell survival and cell death (116). Similarly, the autophagy pathway of cellular digestion is involved in cancer progression and functions to promote both cell survival and cell death (44). In addition, autophagy is regulated by ROS (98). In this review, the importance of ROS in the regulation of autophagy and how this relates to cancer progression and treatment are discussed.

Reactive Oxygen Species

ROS are highly reactive species formed by the incomplete one-electron reduction of oxygen, including molecules or ions such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), nitric oxide (NO), peroxynitrite ($ONOO^-$), and nitrogen dioxide radical (NO_2) (18) (Fig. 1). The major endogenous source of cellular ROS is the mitochondrial electron-transport chain (mETC), where continuous electron leakage to O_2 occurs during aerobic respiration, generating O_2^- (2). Only moderately reactive itself, O_2^- is the substrate for superoxide dismutase (SOD) enzymes that generate H_2O_2, ultimately yielding the highly toxic OH^- in the presence of reduced iron (Fe^{2+}) or copper (Cu^+) through the Fenton reaction. Besides the mETC, low levels of ROS are produced by membrane-localized NADPH oxidase (Nox) enzymes (65), peroxisomes (100), and the cytochrome p450 system (43). Exogenous sources of ROS also exist, including UV and ionizing radiation (92, 107), inflammatory cytokines (79), chemical irritants such as tobacco (111), environmental toxins such as paraquat (22), and various pharmaceutical agents (3) (Fig. 1).

[1]Manitoba Institute of Cell Biology; [2]Biochemistry and Medical Genetics, Faculty of Medicine, University of Manitoba; and [3]CancerCare Manitoba, Winnipeg, Manitoba, Canada.

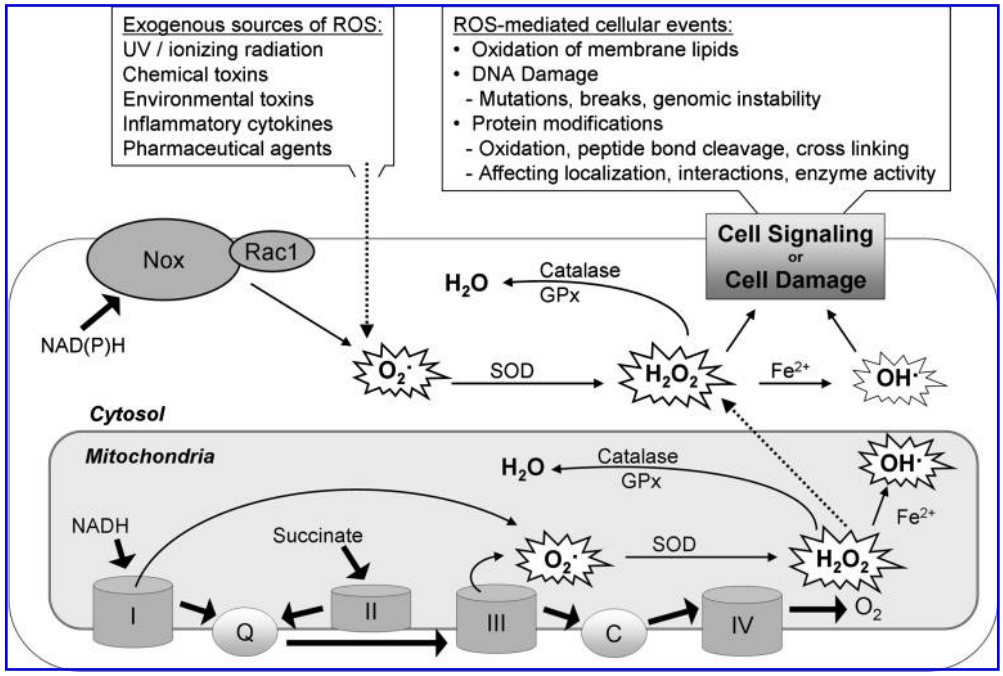

FIG. 1. Generation and effect of cellular ROS. Cellular ROS can originate from exogenous sources (listed), or can be generated within the cell. The main source of intracellular ROS is the mitochondrial electron-transport chain (mETC), the site of aerobic respiration. Four components of the mETC are depicted (enzyme complexes I–IV), and the flow of electrons is indicated (*thick black arrows*). Continuous electron leakage occurs at complexes I and III, resulting in incomplete one-electron reduction of oxygen to form superoxide anion ($O_2\cdot$). Superoxide also can be generated in the cytoplasm by the membrane-associated NADPH oxidase (Nox) complex, which is dependent on the small GTPase, Rac1. Superoxide dismutase (SOD) enzymes catalyze the conversion of $O_2\cdot$ to hydrogen peroxide (H_2O_2), which in turn can be converted to the highly toxic hydroxyl radical (OH^-) in the presence of reduced iron (Fe^{2+}) or copper (Cu^+) through the Fenton reaction. Alternatively, H_2O_2 can be reduced to water and oxygen in a reaction catalyzed by the antioxidant enzymes catalase or glutathione peroxidase (GPx). ROS can cause cell damage or participate in cell signaling by various mechanisms. Notably, H_2O_2 is relatively long lived and can diffuse across lipid membranes to act as a signaling molecule.

At low levels, ROS participate in cellular signaling. However, at high levels, ROS can cause irreversible oxidative damage to lipids, proteins, and DNA, interfering with vital cellular functions (37). Complex antioxidant defense mechanisms have evolved to protect cells from oxidative injury, including enzymatic and nonenzymatic systems (Fig. 1). Antioxidant enzymes include catalase, SOD, glutathione peroxidase (GPx), and peroxiredoxin III (PrxIII) (5, 18). The nonenzymatic system includes vitamins C, E, and B_2, coenzyme Q_{10}, glutathione, and β-carotene (5). These ROS-scavenging systems are required to maintain cellular redox balance: when the amount of ROS exceeds the capacity of the antioxidant machinery, oxidative stress occurs (116).

ROS in survival signaling

At low levels, ROS participate in vital cellular signaling (Fig. 2). ROS can act as second messengers in the regulation of various cellular processes by oxidation of cysteine residues on target molecules, including kinases and phosphatases (21), redox-sensitive transcription factors (104), cell-cycle regulators (93), and cell membrane lipids (88). ROS-induced modification of protein targets can include cleavage of peptide bonds, protein "cross linking," release of iron (Fe) from nonheme iron enzymes, and oxidation of amino acid side chains (71). These modifications can alter enzyme activity, cellular localization, and protein–protein interactions.

One important ROS-mediated survival factor is nuclear factor kappa B (NF-κB), a transcription factor involved in proliferation and anti-apoptotic signaling (49) (Fig. 2B). NF-κB is composed of the DNA-binding subunits p50 and p65 complexed with the inhibitory IκB molecule, which is degraded on phosphorylation. NF-κB activation requires degradation of IκB and phosphorylation of p65, which facilitates nuclear translocation and binding to κB motifs of target genes (97). IκB phosphorylation is induced by IκB kinases (IKK), which are activated by several tyrosine kinases, such NF-κB-inducing kinase (NIK). H_2O_2 induces NIK activation, leading to NF-κB activation (68), and Rac1-dependent generation of ROS mediates IKK activation, also leading to NF-κB activation (96). In addition, H_2O_2 has been shown to activate Syk kinase, which phosphorylates IκB, causing dissociation, phosphorylation, and nuclear translocation of p65 (109). Several lines of evidence have suggested that reactive oxygen intermediates serve as messengers for most if not all NF-κB stimuli (49). Activation of NF-κB ultimately leads to increased expression of antiapoptotic proteins such as Bcl-2 family members and inhibitor of apoptosis proteins (IAP) family members (97).

ROS also participate significantly in hypoxia (low oxygen)-induced signaling pathways (Fig. 2B). Hypoxia occurs during physiologic processes, such as development, and in pathologic conditions, such as tumorigenesis and ischemia. Under these conditions, gene expression is altered to facilitate adaptation and survival in hypoxia (89). The master regulator of this response is the transcription factor hypoxia-inducible factor α (HIF-1α). Under normoxic conditions, HIF-1α is rapidly hydroxylated by prolyl 4-hydroxylases (PHDs) and targeted for proteasomal degradation. During hypoxia, PHDs are inhibited, promoting stabilization of HIF-1α (89). Recently

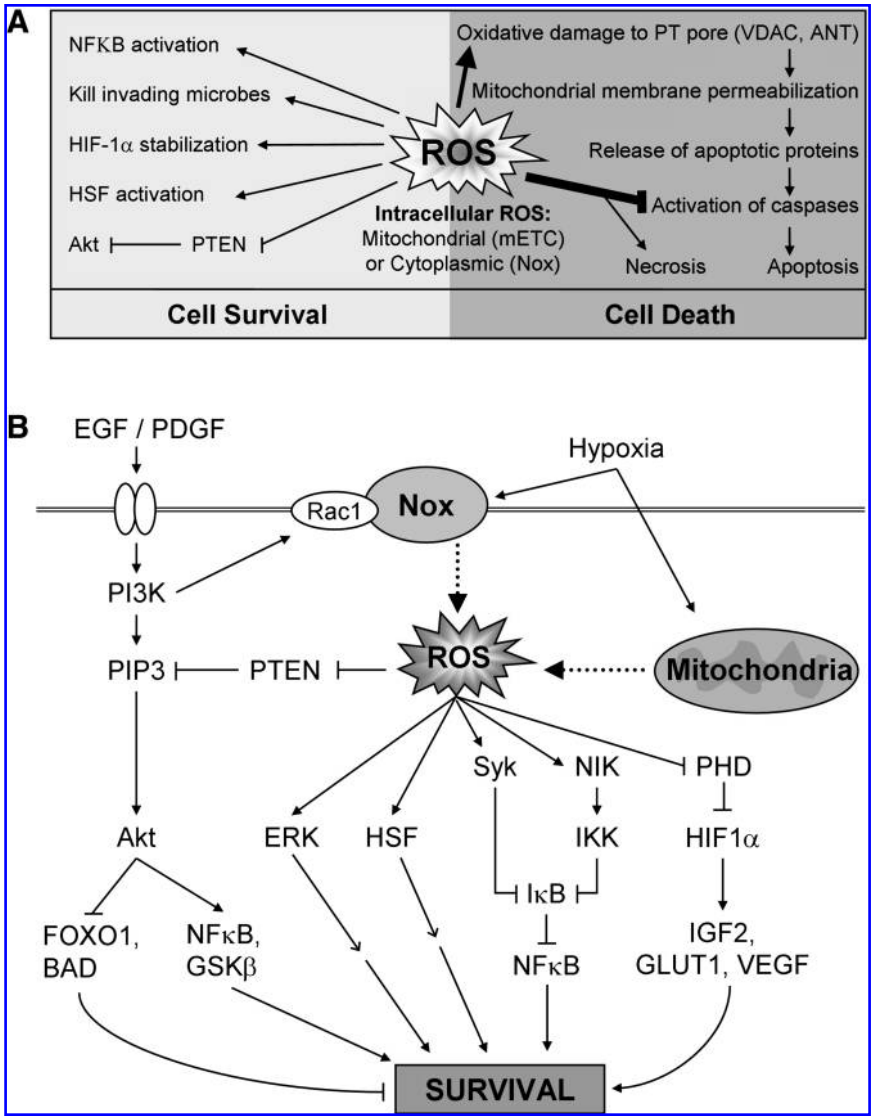

FIG. 2. Dual role of ROS in cell survival and cell death. (**A**) Intracellular ROS can be produced in the mitochondria (by the mETC) or in the cytoplasm (by Nox) (see Fig. 1). ROS mediate both cell survival (*left panel*) and cell death (*right panel*), depending partially on the level of ROS (represented here by line thickness). ROS-mediated survival occurs by direct or indirect activation of prosurvival transcription factors such as nuclear factor kappa B (NF-κB), heat-shock factors (HSF), and hypoxia-inducible factor alpha (HIF-1α). These pathways are illustrated in detail in (**B**). ROS also are used to kill invading microbes in the innate immune response and can inactivate protein phosphatases such as PTEN to activate survival and antiapoptotic pathways such as the Akt pathway. ROS-mediated cell death can occur by caspase-dependent apoptosis as depicted, or by necrosis when excessive ROS inactivate caspases through oxidation. (**B**) ROS participate in survival signaling by several pathways. Growth-factor signaling triggers Nox-dependent ROS production through PI3K and Rac1, leading to oxidation of PTEN and activation of the Akt pathway, with ultimate activation of survival factors (NF-κB, GSKβ) and inhibition of pro-death molecules (FOXO1, BAD). Nox-derived ROS also are required for activation of heat-shock factors, ERK phosphorylation, and activation of NF-κB through degradation of IκB. Hypoxia triggers both cytoplasmic and mitochondrial ROS production, required for stabilization of HIF-1α through inhibition of PHD. HIF-1 prosurvival targets include IGF2, GLUT1, and VEGF.

it was shown that mitochondrial ROS participate in hypoxic inhibition of PHDs: during hypoxia, a ROS burst is generated at complex III of the mitochondrial electron-transport chain (mETC), and the resulting efflux of H_2O_2 inhibits PHD activity, leading to increased HIF-1α stability and the ensuing adaptive responses through increased HIF-1 transcriptional activity (60). In particular, HIF-1 activation leads to increased glycolysis, angiogenesis, and survival signaling. These responses are achieved through enhanced transcription of HIF-responsive genes such as GLUT-1, vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF-2), respectively (89).

Although the majority of intracellular ROS are generated by the mitochondria as a by-product of aerobic metabolism, it is now recognized that specific enzymes, the NADPH oxidases (Nox 1–5) generate ROS in a carefully regulated manner, contributing to various signaling pathways (65). The Nox enzyme complex is membrane bound and generates superoxide by transferring electrons from NADPH to molecular oxygen, with secondary production of H_2O_2 and other ROS (Fig. 1). The first Nox was identified in neutrophils and macrophages, in which Nox-produced ROS contribute to in innate immunity by killing microbes. However, Nox homologues have now been identified in a variety of other cell types, in which Nox-produced ROS participate in signal transduction for many prosurvival functions (65) (Fig. 2B). For example, Nox-derived ROS are a necessary component of the stress-induced protective signaling pathway that leads to the activation of heat-shock factors (HSFs) and transcription of heat-shock proteins (HSPs) (83). In addition, several growth factors critical for cell survival and proliferation are known to signal through ROS-dependent mechanisms involving Nox: both the epidermal growth factor (EGF) receptor and the platelet-derived growth factor (PDGF) receptor signal through Nox-mediated generation of H_2O_2 (10, 105). Ligand-induced receptor dimerization activates phosphatidylinositol 3-kinase (PI3K), leading to activation of Rac1, a Rho-like small GTPase in the Ras superfamily. A regulatory subunit of the Nox complex, Rac1 activates Nox to produce superoxide and ultimately H_2O_2 for downstream signaling. One of the

downstream signaling pathways that is activated by Rac1-dependent/Nox-generated ROS is the ERK signaling pathway contributing to cell survival (94). A more recent study demonstrated that hypoxia also can activate Nox to produce ROS, but the affect on HIF-1α stability was not addressed in this case (77).

H_2O_2 propagates signal transduction by inactivating tyrosine phosphatases such as PTEN (phosphatase and tensin homologue) (Fig. 2B). Inactivation occurs through oxidation of catalytic cysteine residues, leading to disulfide bond formation at the active site (21). The role of PTEN is to reverse the reaction catalyzed by PI3K, that is, to dephosphorylate phosphatidylinositol (3,4,5)-triphosphate (PIP3) to the precursor PIP2. On ROS-mediated inactivation of PTEN, PIP3 accumulates, functioning to recruit Akt (protein kinase B, PKB) and phosphoinositide-dependent protein kinase 1 (PDK1) at the membrane. PDK1 phosphorylates Akt, which then acts on numerous targets to promote survival by activating antiapoptotic substrates such as NF-κB and GSK-β and inhibiting proapoptotic substrates such as FOXO1 and BAD (75).

ROS and cell death

Whereas low levels of ROS are important for cellular function and survival signaling, excessive ROS can lead to cell death through several mechanisms, including apoptosis, necrosis, and autophagy (see later sections) (101, 114) (Fig. 2A). Apoptosis is an orderly form of cell death that occurs through two major pathways, initiated either by death-receptor ligation (extrinsic pathway) or by release of apoptotic proteins from the mitochondria, including cytochrome *c*, Smac/Diablo, and Endo G (intrinsic pathway) (31). Both pathways culminate in the activation of caspases (cysteine proteases), triggering a proteolytic cascade that ultimately "disassembles" the cell. This strictly regulated process requires energy in the form of ATP, and is characterized by nuclear condensation, cell shrinkage, DNA cleavage, and formation of apoptotic bodies, which are recognized and removed by phagocytic cells. Notably, apoptosis occurs without inducing inflammation, thereby minimizing damage to nearby cells and tissue (31).

High levels of ROS can induce apoptosis by triggering the opening of the mitochondrial permeability transition (PT) pore (101) (Fig. 2A). Opening of the PT pore is a key event in the intrinsic apoptosis pathway, leading to mitochondrial membrane rupture and release of apoptotic proteins (31). The PT pore complex consists of two components, both susceptible to oxidative damage by ROS: the inner membrane component (adenine nucleotide translocase, ANT) and the outer membrane component (voltage-dependent ion channel, VDAC) (23, 72). ROS production has been associated with apoptotic cell death in numerous pathologic conditions such as stroke, inflammation, and ischemia (101). Furthermore, many agents that induce apoptosis are known to generate oxidative stress: ionizing and UV irradiation induce apoptosis through generation of OH^- and H_2O_2, whereas chemotherapeutics such as arsenic trioxide and buthionine sulfoximine deplete cells of the antioxidant enzyme glutathione peroxidase, causing accumulation of ROS, leading to cell death (32).

In contrast to apoptosis ("programmed cell death"), necrosis is regarded a "passive" form of cell death, typically initiated by toxic insults or severe stress conditions. Whereas apoptosis requires ATP, necrosis results from ATP depletion during acute cellular dysfunction. Necrotic cells swell and then lyse, releasing their contents into the extracellular space, causing inflammation and damage to surrounding tissue (31). In many cases, apoptosis and necrosis may occur sequentially or simultaneously in the same tissue. It has been postulated that the switch from apoptotic to necrotic cell death involves not only a decrease in cellular ATP, but also a burst in intracellular ROS (80). For example, Hampton and Orrenius (42) found that low concentrations of H_2O_2 induced apoptosis in Jurkat cells, whereas at higher concentrations, the cells died by necrosis. Thus, it appears that although moderate levels of ROS can trigger apoptosis, excessive ROS leads to necrosis. This is likely due to the sensitivity of caspases to oxidative inactivation at their active cysteine group: at high levels of oxidative stress, the cell cannot maintain a reducing environment, and caspases will not function (76). Thus, apoptosis is inhibited, and cell death must proceed through necrosis (Fig. 2A).

Taken together, low levels of ROS generally participate in survival signaling, whereas excessive ROS contribute to cell death (Fig. 2). In addition to the amount of ROS, the site of ROS generation may affect cell fate: Deshpande and colleagues (27) observed an intracellular ROS burst in endothelial cells after stimulation with tumor necrosis factor-α (TNFα) (27). They showed that Nox-derived ROS in the cytoplasm led to protective responses, whereas mitochondria-derived ROS promote TNF-α–induced apoptosis. Thus, ROS could participate in cell-survival or cell-death pathways, depending on both the amount and site of ROS generation.

ROS and cancer

The role of ROS in tumor initiation and development is complicated and not fully understood. As described later, high levels of ROS may contribute to carcinogenesis, and ROS-mediated signaling can promote tumor cell survival, proliferation, and metastasis (116). However, excessive ROS leads to cell death, and therefore, various cancer-treatment strategies aim to enhance ROS production for tumor cell killing (3). Additionally, modulation of antioxidant systems has been adopted as a novel therapeutic approach; however, contradictory results have failed to clarify whether antioxidant mechanisms should be enhanced or inhibited during cancer treatment (84). Clearly, cellular redox balance is intricately involved in cancer progression, and more research is required to characterize this association fully.

ROS-mediated DNA damage has long been associated with carcinogenesis and malignant transformation (Fig. 3): hydroxyl radicals react with DNA and chromatin proteins, resulting in mutations, strand breaks, genomic instability, and altered gene expression (84). Mitochondrial DNA (mtDNA) is especially vulnerable because of its close proximity to the site of ROS generation, as well as its lack of introns and limited DNA repair. mtDNA mutations are frequently detected in cancer cells and are likely to result in mETC malfunction, further amplifying the generation of ROS (15).

Cancer cells, both *in vivo* and *in vitro*, characteristically have higher ROS levels compared with normal cells (87) (Fig. 3). This may be due to increased metabolic activity, mitochondrial malfunction, infiltration of inflammatory cells, or a combination of these. Increased ROS leads to persistent oxidative stress, which in turn causes selective pressure for characteristics such as increased growth, invasion, and me-

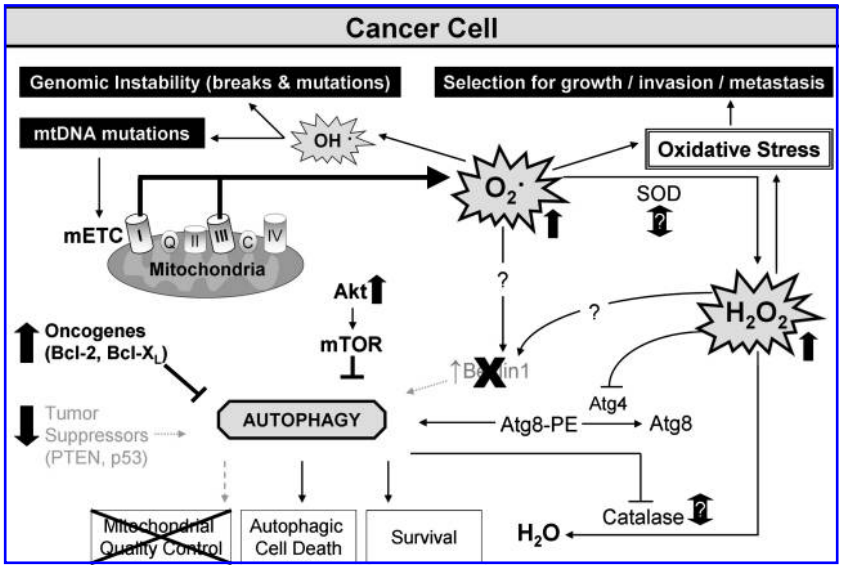

FIG. 3. Alteration of ROS and autophagy in cancer. Compared with normal cells (Fig. 6), both ROS and autophagy are altered in cancer cells. Increased Akt activation in cancer leads to inhibition of autophagy through mTOR, and monoallelic deletion of *Beclin-1* in many cancers also inhibits autophagy. Autophagy is further inhibited because of increased expression of oncogenes Bcl-2/Bcl-X_L, and decreased expression of tumor suppressors PTEN/p53. Cancer cells characteristically have high levels of ROS because of increased metabolic activity or malfunction of the mitochondrial electron-transport chain (mETC). ROS cause genomic instability and carcinogenic mutations in genomic and mitochondrial DNA (mtDNA), with mtDNA mutations contributing to mETC malfunction. Antioxidant enzymes such as superoxide dismutase (SOD) and catalase also are altered in cancers, leading to misregulation of ROS. Decreased autophagy results in loss of mitochondrial quality control, and accumulation of damaged mitochondria exacerbates ROS production.

tastasis (116). Levels of antioxidant enzymes also appear to be altered in cancer cells, although studies of this phenomenon have generated contradictory results (84). For example, some *in vitro* studies have shown that increased SOD expression leads to reduced proliferation and tumorigenicity of cancer cells (81); however, several *in vivo* studies indicate that SOD expression levels correlate with poor prognosis in patients (56). Likewise, some studies report increased catalase levels in cancers (11), whereas others report a decrease (90). Thus, ROS levels and antioxidant signaling appear to be altered in cancer cells, leading to cancer progression, although the precise nature of these changes is still under investigation.

Autophagy

Autophagy is the cellular pathway of "self digestion," a regulated lysosomal pathway for the degradation and recycling of long-lived proteins and organelles (67) (Fig. 4). During autophagy, cytoplasmic constituents are sequestered into double-membraned autophagosomes, which are delivered to lysosomes and degraded. This process generates nucleotides, amino acids, and fatty acids, which are recycled for ATP generation and macromolecular synthesis. Genetic screening in yeast has identified >30 ATG (autophagy-related) genes required for autophagy, many of which have mammalian homologues (106). Similar to ROS, autophagy is involved in both cell-survival and cell-death pathways (Fig. 5) and is altered in cancer (Fig. 3). Indeed, recent studies suggest that autophagy plays a significant role in cancer progression and could be a target for treatment (48).

As shown in Fig. 4, several upstream regulators of autophagy have been characterized (85). The class III PI3 kinase complex, including Beclin-1/Atg6, is required for generation of preautophagosome structures (106). The mammalian target of rapamycin (mTOR), a nutrient-sensing kinase complex that regulates cell growth and survival, blocks autophagy during nutrient-rich conditions by inhibiting the Atg1 complex, which is involved in the initiation stages of autophagic activity (8). Accordingly, upstream activators of mTOR (NF-κB, class I PI3 kinase, and Akt) suppress autophagy, whereas inhibitors of this pathway (PTEN) induce autophagy (7, 28). Downstream of these regulatory pathways, further Atg proteins function to build autophagosomes. The Atg5–Atg12 covalent protein complex and Atg8–phosphoethanolamine (PE) conjugates are essential components of the autophagosome membrane. Ubiquitin-like reactions involving other Atg proteins generate these conjugates (82). For example, the Atg4 cysteine proteases cleave Atg8 at the C-terminus to facilitate lipidation, generating Atg8-PE. Atg4 is also responsible for recycling Atg8 by cleaving PE at later stages of autophagy (82). The discovery of the ATG genes in yeast, and subsequently their mammalian homologues, has greatly advanced our understanding of the molecular mechanisms involved in the autophagy signaling pathway.

Autophagy in survival signaling

Conserved from yeast to humans, autophagy occurs at low basal levels to maintain cellular homeostasis through cytoplasmic and organelle turnover. For example, studies in Atg7-deficient mice determined that Atg7 was essential for autophagosome formation, amino acid supply in neonates, and starvation-induced protein and organelle degradation (62). Furthermore, Atg7 deficiency led to multiple cellular abnormalities, such as mitochondrial deformation and accumulation of ubiquitin protein aggregates. Indeed, mice lacking Atg7 specifically in the central nervous system showed neurologic defects, including abnormal clasping reflexes and decreased coordinated movement (61). These mice die within 28 weeks of birth because of massive neuronal loss in the cerebral and cerebellar cortices. Polyubiquitinated protein aggregates were found in surviving neurons as inclusion

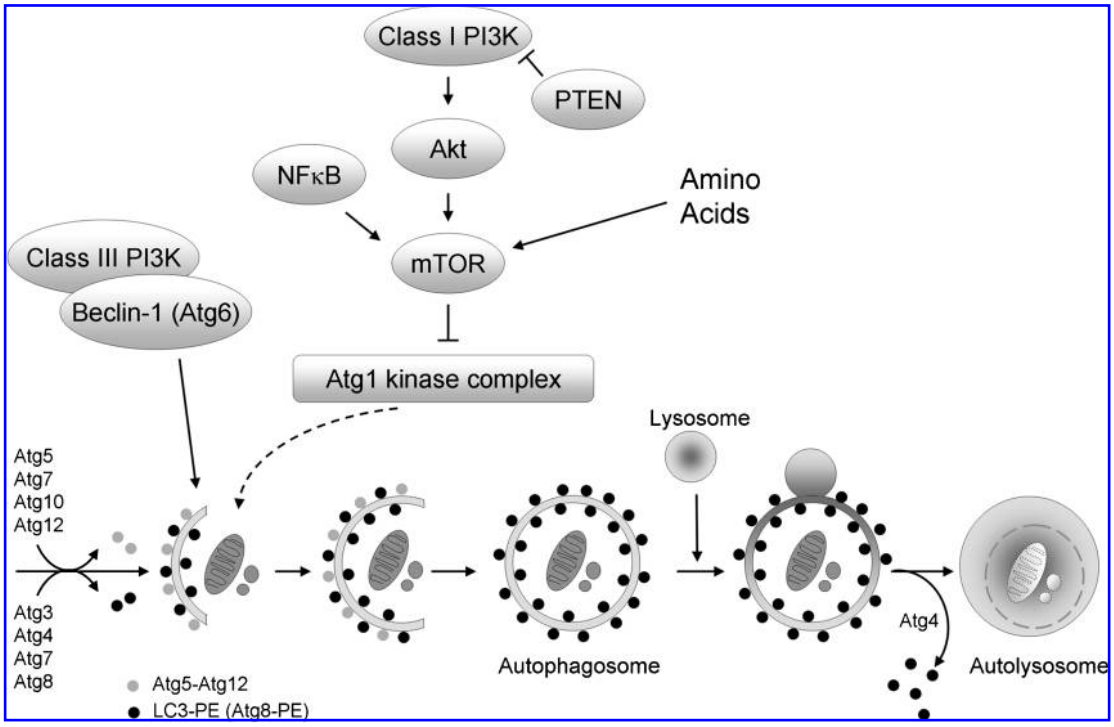

FIG. 4. Molecular mechanism of autophagy regulation and autophagosome formation. After external stimuli such as nutrient deprivation, mTOR is inhibited, leading to induction of autophagy. Accordingly, autophagy is regulated by upstream factors in the mTOR pathway: autophagy is induced by PTEN and inhibited by class I PI3 kinase, Akt, and NF-κB. The mTOR-regulated Atg1 complex is involved in autophagy initiation, and the class III PI3 kinase complex, including Beclin-1/Atg6, is required for generation of preautophagosome structures. The Atg5-Atg12 covalent protein complex and LC3/Atg8-phosphoethanolamine (PE) conjugates are essential components of the autophagosome membrane and are generated by ubiquitin-like reactions involving other Atg proteins. In particular, Atg4 mediates the initial lipidation of LC3/Atg8 as well as cleavage of LC3 from the autophagosome membrane at later stages of autophagy. As elongation of the autophagosomal membrane occurs, cytoplasmic proteins and organelles are sequestered into double-membraned autophagosomes, which are delivered to lysosomes and degraded.

bodies, which increased in size and number over time. Together these studies indicate an important role for basal autophagy in protein and organelle "quality control," and for maintaining normal neuronal function.

Apart from its homeostatic function, autophagy also is transiently induced as a survival response to various stress stimuli, such as nutrient deprivation (67) (Fig. 5). Yeast mutants defective in any autophagy gene fail to survive nutrient starvation (112), and similar results have been obtained in other plants (29). In mammalian cells, starvation induces autophagy to protect against apoptosis: if autophagy is blocked genetically by ATG-gene knockdown, or chemically by synthetic autophagy inhibitors, nutrient-deprived cells will undergo apoptosis (13). In addition, apoptosis-resistant cells that are growth-factor dependent can survive growth-factor withdrawal for several weeks through induction of autophagy (rapid cell death occurs if Atg5 or Atg7 expression is blocked). On growth-factor withdrawal, these cells lose their ability to take up extracellular nutrients but maintain ATP production from catabolism of intracellular substrates through autophagy (70).

The prosurvival role of starvation-induced autophagy was recently confirmed in an animal model of Atg5-deficient mice (64). These mice are autophagy deficient, and although nearly normal at birth, they cannot survive the early neonatal starvation period: they have reduced circulating amino acids and decreased cardiac ATP. The Atg5-deficient neonates die prematurely because of energy depletion caused by lack of autophagy. In another study, autophagy was shown to protect cardiomyocytes after simulated ischemia/reperfusion (I/R) injury (41). I/R injury impaired both formation and downstream lysosomal degradation of autophagosomes, but overexpression of Beclin-1 increased autophagy after I/R injury and significantly reduced activation of proapoptotic Bax and induction of apoptosis, whereas knockdown of Beclin-1 increased Bax activation and apoptosis. Moreover, expression of a dominant-negative mutant of Atg5 increased cell death after I/R, indicating that autophagy provides a protective mechanism against I/R injury. Thus, both *in vivo* and *in vitro* models confirm that autophagy contributes to cell-survival functions in various physiologic contexts (Fig. 5).

Autophagy and cell death

Although autophagy can function as a cytoprotective mechanism, it also has the capacity to promote or induce cell death (Fig. 5). In some cases, autophagy may promote or ac-

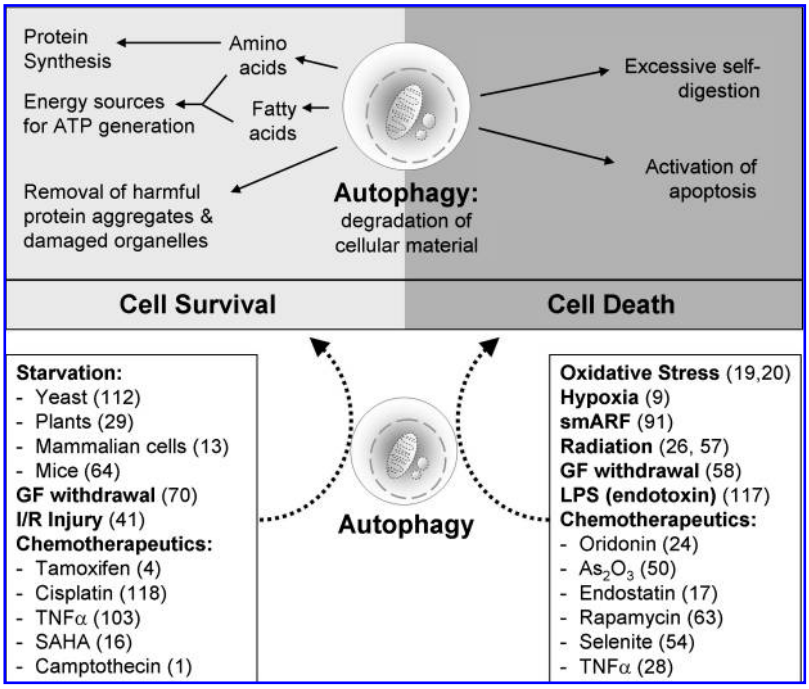

FIG. 5. Dual role of autophagy in cell survival and cell death. Autophagic degradation of cellular materials generates amino acids and fatty acids, which can be used for protein synthesis and ATP generation during stressful conditions such as starvation. Autophagy also removes protein aggregates (which can trigger apoptosis) and damaged mitochondria (source of apoptotic proteins and toxic ROS). However, prolonged autophagy can lead to cell death through excessive self-digestion or activation of apoptosis. Studies have demonstrated prosurvival autophagy in response to starvation (in yeast, plants, mammalian cells, and mouse models), growth factor (GF) withdrawal, ischemia/reperfusion (I/R) injury, and various chemotherapeutic drugs. Autophagic cell death has been observed in response to hypoxia, oxidative stress, radiation, GF withdrawal, lipopolysaccharide (LPS), overexpression of smARF, and various chemotherapeutic drugs.

tivate apoptosis (24, 38, 74). Alternatively, death can occur through apoptosis-independent "autophagic cell death." Autophagic cell death or "programmed cell death type two" (PCD II) is characterized by morphologic and molecular features that are distinct from apoptosis (PCD I) (74). In classic apoptosis, early collapse of the cytoskeleton occurs, but organelles are initially preserved. In contrast, autophagic cell death involves early degradation of some organelles with initial preservation of the cytoskeleton. Apoptosis is characterized by caspase activation and DNA cleavage, whereas autophagic cell death is caspase independent and does not involve DNA fragmentation (31, 74). In autophagic cell death, autophagosomes degrade organelles, including mitochondria, to a level at which normal cellular function is compromised and cellular death occurs.

Prolonged autophagy leading to PCD II has been demonstrated under various conditions (Fig. 5). For example, treatment of glioma cells with arsenic trioxide induced autophagy without apoptosis, and blocking autophagy resulted in decreased cell death (50). In another study, overexpression of a short isoform of tumor-suppressor p19ARF (smARF) also resulted in formation of autophagosomes and caspase-independent cell death. smARF is normally maintained at low levels through proteasomal degradation, but it increases in response to viral and cellular oncogenes. On increased expression, smARF localized to mitochondria and reduced mitochondrial membrane potential, causing increased ROS. Knockdown of *Beclin-1* and *ATG5* reduced smARF-mediated cell death, indicating PCD II (91). Finally, we reported that autophagy can function as a distinct mechanism for programmed cell death in hypoxia (9) and during oxidative stress (19, 20).

It has been suggested that PCD types I and II may converge at the mitochondrion where membrane integrity is controlled by the Bcl-2 family of proteins, crucial regulators of PCD (14). In support of this hypothesis, it was recently shown that the antiapoptotic protein Bcl-2 also has antiautophagic properties, which are mediated through direct interaction with the autophagy protein, Beclin-1 (mammalian Atg6) (86). Additionally, we and others showed that hypoxia and arsenic trioxide–induced autophagy involve increased expression of the Bcl-2 family member BNIP3 (9, 50). On overexpression, BNIP3 induces a caspase-independent cell death that requires mitochondrial localization, loss of membrane potential, and increased ROS (113). Thus, similar to apoptosis, mitochondria appear to regulate autophagic cell death.

Autophagy and cancer

The role of autophagy in cancer is both complex and controversial (44, 48) (Fig. 3). Several studies point to a cancer-promoting role for autophagy: cancer cells use autophagy as a survival pathway to sustain viability during periods of nutrient limitation, growth-factor deprivation, and metabolic stress (13, 25, 70). In stark contrast, other studies support an anticancer role for autophagy: the autophagy gene *Beclin-1* is a haploinsufficient tumor suppressor in mice (120) and is monoallelically deleted in 40–75% of sporadic human breast, ovarian, and prostate tumors (69). Furthermore, the established tumor-suppressor genes *p53* and *PTEN* are known to induce autophagy (6, 34), whereas the oncogenic proteins Bcl-2 and Bcl-X_L interact with Beclin-1 to inhibit autophagy (73, 86). In addition, activation of AKT (which is increased in many cancers) leads to increased mTOR activation and blocks autophagy (66). Rapamycin and its derivatives inhibit mTOR activity and are under clinical investigation as treatments for cancers (30) (Fig. 4). The mechanism by which autophagy inhibits tumorigenesis is unclear, but it may involve "mitochondrial quality control": prevention of oxidative damage and mutagenesis though the removal of damaged mitochondria, which are a source of toxic ROS (48).

As outlined earlier, autophagy can have entirely opposite consequences for tumor cells, depending on the circumstances: survival and tumorigenesis, or cell death and tumor

suppression. Thus, intense debate and conflicting evidence surround the role of autophagy in cancer therapy. Many anticancer agents induce autophagy, including tamoxifen, rapamycin, As_2O_3, temozolomide, histone deacetylase (HDAC) inhibitors, and ionizing radiation (63) (Fig. 4). However, it remains questionable whether the observed autophagic response is a survival attempt by tumor cells or a killing mechanism of anticancer agents. For instance, some studies have shown that autophagy counteracts the antineoplastic effects of therapies including tamoxifen (4), camptothecin (1), cisplatin (118), TNF-α (103), the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) (16), and the alkylating agent cyclophosphamide (4). In contrast, several groups have shown that induction of autophagy sensitizes cells to radiation therapy (26, 57). Moreover, autophagy has been shown to directly mediate directly the cytotoxicity of some therapies, including arsenic trioxide (50) and endostatin (17), by inducing autophagic cell death. For other antitumor agents such as the herbal compound oridonin, autophagy appears to promote or facilitate apoptosis (24). For still other antitumor agents, including imatinib (33) and two novel synthetic agents (51), autophagy induction has been observed, but its role remains unclear. Thus, autophagy is intricately involved in tumorigenesis and represents an attractive new target for cancer therapy. However, further studies are required to establish the precise role of autophagy in the various types and stages of cancer.

Regulation of Autophagy by ROS

Because ROS and autophagy are similarly involved in cell-survival and cell-death pathways, as well as cancer progression and treatment, ROS regulation of autophagy has been investigated. Studies in yeast indicate that mitochondrial oxidation events, including ROS production and oxidation of mitochondrial lipid, play a role in the induction of autophagy (59). Subsequent studies in mammalian cells have confirmed that ROS are important regulators of autophagy under various conditions (Fig. 6). For example, neurons deprived of nerve growth factor were shown to accumulate mitochondrial ROS, causing lipid peroxidation and loss of the mitochondrial inner membrane lipid cardiolipin, resulting in autophagy and cell death (58). In another study, caspase-independent cell death of lipopolysaccharide (LPS)-treated macrophages was shown to involve ROS-dependent autophagy (117). Most recently, Kim and colleagues (54) showed that superoxide mediates selenite-induced autophagic cell death in glioma cells (54). Although multiple studies have implicated ROS in autophagy regulation, few have persevered to define a mechanism. Remarkably, the mechanism for redox regulation of autophagy appears to depend on the cellular context and autophagic stimulus, because two distinct molecular mechanisms have been elucidated for starvation-induced "protective" autophagy *versus* autophagic cell death (99, 119).

Regulation of Atg4 by ROS during starvation

Scherz-Shouval and colleagues (99) recently reported that ROS are essential for starvation-induced autophagy and specifically regulate the activity of Atg4 (Fig. 6). First, they determined that nutrient starvation (a well-known trigger for autophagy) stimulates production of mitochondrial ROS, specifically H_2O_2. These oxidative conditions were deemed essential for starvation-induced autophagy, because treatment with antioxidants blocked the formation of autophagosomes. The authors went on to identify the cysteine protease Atg4 as a direct target for oxidation by H_2O_2, specifying a cysteine residue located near the active site as critical for this regulation. Atg4 regulates the reversible conjugation of Atg8 (LC3 in mammals) to the autophagosomal membrane, a hallmark event in the autophagic process (82). In the model proposed by Scherz-Shouval *et al*. (99), starvation-induced oxidative inactivation of Atg4 promotes lipidation of Atg8, facilitating autophagosome formation. It remains to be seen whether ROS-mediated inhibition of Atg4 is unique to starvation-induced autophagy, or if it is a more general characteristic of autophagy signaling. Nevertheless, this study defines a novel signaling pathway in which ROS function as signaling molecules to trigger autophagy, providing one possible molecular mechanism for redox regulation of autophagy (Fig. 6).

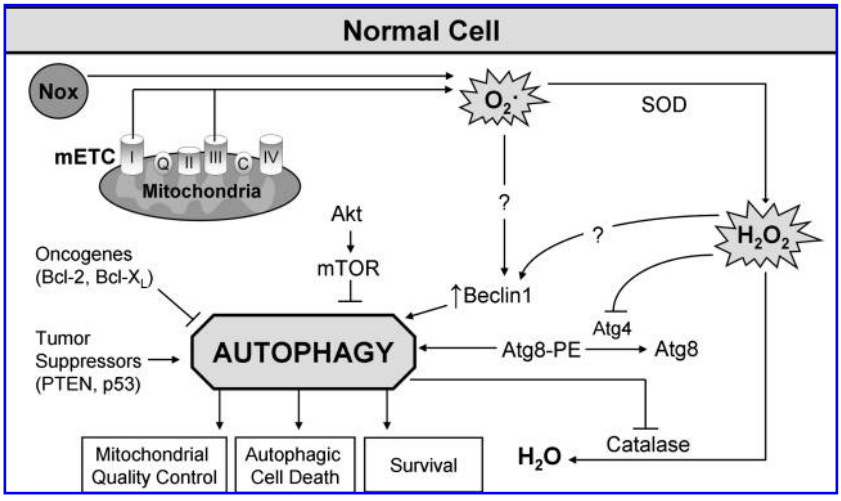

FIG. 6. Regulation of autophagy by ROS in normal cells. Intracellular ROS are generated by Nox (cytoplasmic) or by the mETC (mitochondrial) (see Fig. 1). ROS can increase expression of Beclin-1 by an unknown mechanism, leading to autophagy. H_2O_2 can directly inactivate the cysteine protease Atg4, blocking the delipidation of Atg8 to induce autophagy. Selective autophagic degradation of catalase serves as a positive-feedback loop, causing accumulation of ROS, which further enhances autophagy. Other regulators of autophagy include the Akt/mTOR pathway, oncogenes Bcl-2/Bcl-X_L, and tumor suppressors PTEN/p53.

Selective autophagic degradation of catalase during PCD II

Although cell death was not directly examined in the Scherz-Shouval study, it is well established that starvation-induced autophagy is protective (67). In contrast, Yu and colleagues (119) examined the role of ROS in autophagic cell death. The authors described a mechanism for autophagic cell death involving ROS accumulation caused by selective autophagic degradation of catalase (Fig. 6). By using siRNA and the chemical inhibitor zVAD, they demonstrated that caspase inhibition triggers autophagy, which selectively degrades the antioxidant enzyme catalase (SOD and other unrelated proteins were not degraded). Catalase degradation subsequently caused ROS accumulation and ultimately cell death. Thus, the molecular mechanisms for ROS involvement in protective versus destructive autophagy seem to be distinct. Starvation-induced (protective) autophagy depends on ROS production (99), whereas zVAD-induced (destructive) autophagy causes ROS accumulation (119). Together, these findings demonstrate that autophagy and ROS metabolism regulate each other (Fig. 6). It was recently hypothesized that catalase degradation, resulting in prolonged H_2O_2 signal, could be responsible for shifting the outcome of autophagy from survival to death (98).

Other mechanisms for redox regulation of autophagy

Other mechanisms for redox regulation of autophagy are also possible. For example, ROS can affect transcription-factor activity, leading to altered gene expression (104). Thorpe and colleagues (110) reported that autophagy genes are upregulated in response to oxidative stress in yeast, and several independent studies reported that ROS induce Beclin-1 expression in cancer cells (19, 28), although the mechanism for this upregulation remains unknown (Fig. 5). Without pinpointing specific molecular mechanisms, these studies (described later) nevertheless strongly support the involvement of ROS in autophagy regulation.

As discussed earlier, both autophagy and ROS are altered in cancer cells (Fig. 3). We recently investigated ROS regulation of autophagy by directly inducing oxidative stress in cancer cells (19). We found that two ROS-generating agents, H_2O_2 and 2-methoxyestradiol (2-ME), induced autophagic cell death independent of apoptosis in the transformed cell line HEK293 and the cancer cell lines U87 and HeLa. Both agents induced Beclin-1 expression, which was required for ROS-induced autophagy. Blocking H_2O_2– or 2-ME–induced ROS production by overexpression of SOD, or using the ROS scavenger Tiron decreased autophagy and cell death. In contrast, H_2O_2 or 2-ME preferentially induced apoptosis in normal mouse astrocytes. These findings have important implications for cancer therapy because 2-ME is a promising antitumor agent, currently in phase I and phase II clinical trials (47, 108). In a separate study, we examined the effect of the mETC inhibitors rotenone (complex I inhibitor) and TTFA (complex II inhibitor) on normal and cancer cells (20). Both inhibitors induced ROS-dependent autophagic cell death in transformed and cancer cells, but not in normal mouse astrocytes. The observed autophagic cell death was independent of apoptosis. Together, these results indicate that selective prolonged activation of autophagy (by using mETC inhibitors or other ROS-generating agents such as 2-ME) could be a viable strategy to treat cancers resistant to apoptosis (Fig. 7).

In another study of ROS-mediated autophagy in cancer cells, Djavaheri-Mergny and colleagues (28) investigated the role of the NF-κB transcription factor (28). NF-κB mediates tumor progression, based on its ability to promote cell survival and proliferation, and to inhibit apoptosis (97). In this study, the authors established that NF-κB can also mediate repression of autophagy (28). They showed that NF-κB blocks autophagy in tumor necrosis factor-alpha (TNF-α)-treated cancer cells by activating the autophagy inhibitor mTOR in a ROS-dependent manner. In contrast, TNF-α treatment in cells lacking NF-κB activation induced autophagy through ROS-dependent upregulation of Beclin-1. Notably, autophagy was not protective in this instance, as it was shown to enhance TNF-α–induced apoptosis in NF-κB–incompetent cells. While supporting a proautophagic role for ROS, these results also demonstrate that autophagy may amplify apoptosis when associated with a death-signaling pathway.

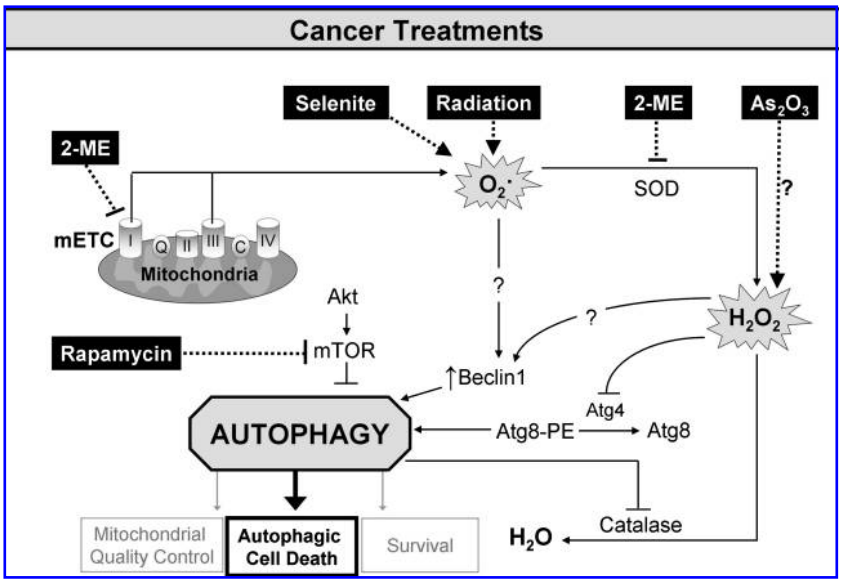

FIG. 7. Autophagy and ROS in cancer treatment. Several existing anticancer drugs function by inducing ROS and/or autophagic cell death. 2-Methoxyestradiol (2-ME) induces ROS-dependent autophagic cell death by inhibiting SOD or complex I of the mitochondrial electron-transport chain (mETC). Selenite induces superoxide-mediated autophagic cell death. Arsenic trioxide (As_2O_3) induces ROS generation and autophagic cell death, and rapamycin induces autophagy by inhibition of mTOR. The cytotoxicity of ionizing radiation is mediated by ROS generation and can lead to autophagic cell death.

Another role for ROS in autophagy was recently proposed by Scherz-Shouval and Elazar (99). They suggest that mitochondria can signal for the induction of autophagy and may supply part of the membranes required for autophagosome formation. In their proposed model, mitochondrial ROS transfer to the cytosol, creating an oxidative gradient and signaling for autophagosome formation by oxidative modification of target molecules, such as Atg4 and probably other yet unidentified factors. On oxidation, Atg4 is inactivated, allowing its substrate, Atg8/LC3, to be conjugated to autophagosomes. Because ROS are short lived, oxidation occurs only in the vicinity of mitochondria. Farther away from mitochondria, Atg4 will be active, and therefore LC3 will be cleaved from autophagosome membranes. To verify or refute this model, and to fully understand the role of ROS in autophagy signaling, it must be determined what other cellular factors, besides lipids and the Atg4 protease, are targeted by ROS in autophagy. It is also possible that the role of ROS in autophagy and other signaling pathways could depend on the species of ROS or the site of generation (mitochondrial *vs.* membrane-associated Nox) or both, but no studies to date have directly addressed these issues.

Regulation of ROS by autophagy

Although ROS play an important role in regulating autophagy, the reverse also occurs. In addition to the cellular antioxidant systems discussed earlier, autophagy is also used in cellular defense against oxidative stress (52, 78). Whereas antioxidant enzymes such as SOD and catalase (which actively scavenge ROS) can be considered the first line of defense against oxidative damage, autophagy provides a second line of defense by removing oxidatively damaged proteins and impaired organelles (12, 53). Oxidative conditions favor partial unfolding of proteins (35), and these proteins have a strong tendency to aggregate (115). If not removed, protein aggregates are toxic to cells and may initiate apoptosis (95). Because they cannot be removed by the proteasomal system (36, 102), the cell relies on autophagy to remove harmful protein aggregates. Equally important, autophagy also functions to remove selectively damaged mitochondria, the major source of ROS during oxidative stress (48). As discussed earlier, this process has been termed "mitophagy" and is protective in two ways: by removing the source of toxic ROS and by degrading damaged mitochondria before they can release apoptotic factors to induce cell death (55).

ROS and Autophagy in Cancer Therapy

Because both ROS and autophagy are significantly involved in cancer initiation and progression (Fig. 3), it is not surprising that many existing treatment strategies involve the manipulation of one or the other or both of these factors (3, 63) (Fig. 7). Theoretically, two ROS-related cancer therapy strategies are possible: inhibition of ROS "survival signaling" or stimulation of ROS production at high enough levels to trigger death pathways. Similarly, two autophagy-related cancer therapies are possible: to inhibit autophagy and block its prosurvival effects, or to induce high levels of autophagy and activate autophagic cell death. As we discussed, ROS and autophagy signaling are entwined in ways that are not yet fully understood. Therefore, both must be considered in the development of cancer therapeutics.

Existing cancer therapies exploiting ROS and autophagy

Ionizing radiation—one of the most commonly used interventions in cancer therapy—causes cellular damage through the generation of ROS (OH^- and H_2O_2). Radiation also induces autophagy, and it is known that some radiosensitizing agents function by promoting autophagy (26). An alternative to radiation-induced ROS production is drug-induced ROS. Many conventional anticancer drugs have been found to induce formation of ROS (3). Among these are several agents known to also induce autophagic cell death, such as 2-methyoxyestradiol (2-ME), selenite, and arsenic trioxide (As_2O_3) (Fig. 7). Selenite was recently shown to induce autophagic cell death in gliomas cells, mediated by superoxide (54). As_2O_3 can induce apoptotic or autophagic cell death, or both, in various types of cancer cells (50), and although not all studies have addressed the role of oxidative stress, several have demonstrated that ROS formation is essential for As_2O_3 cytotoxicity (39). 2-ME, a promising antitumor agent currently in phase I and II clinical trials, enhances ROS formation by inhibiting complex I of the mETC (40) and mitochondrial SOD (45). We recently showed that 2-ME induces ROS-dependent autophagic cell death in cancer cells (19). Although both As_2O_3 and 2-ME are capable of inducing apoptosis, the fact that they can also cause autophagic cell death suggests that they could be effective in treating apoptosis-resistant cancers.

Developing novel cancer therapies to target ROS and autophagy

In developing novel therapies for cancer, careful consideration should be given to manipulation of cellular ROS and autophagy. Because many cancers are intrinsically resistant to apoptosis, or develop resistance during treatment, causing cell death through autophagy is an attractive strategy (66). ROS generation is an established anticancer mechanism for many existing drugs (3), and because ROS can induce autophagy (98), combining ROS- and autophagy-inducing agents could generate synergistic tumor-killing activity. Conversely, autophagy can promote tumor cell survival (25) and has been shown to attenuate the cytotoxicity of several anticancer drugs (1, 4). Thus, it could be effective to combine autophagy inhibitors with ROS-inducing agents to promote apoptosis. The most effective drug combinations will be achieved through determining the mechanism of cytotoxicity for each agent: autophagy should be enhanced if it mediates cytotoxicity, or inhibited if it antagonizes drug activity. Ideally, treatment strategies should be selected based on tumor characteristics such as basal autophagic activity, ROS levels, and apoptosis resistance or sensitivity, all of which are affected by common genetic alterations in cancer. Consequently, the most effective treatments will be designed based on individual genetic signatures of cancers and may be targeted against ROS, autophagy or apoptosis, or any combination of these three pathways.

Conclusions

Both ROS and autophagy have been studied for decades. Initially, ROS were known to cause cellular damage and mutagenesis, and autophagy was known to protect against nutrient deprivation and other stress stimuli. In recent years, it

was discovered that besides causing oxidative damage, ROS are important signaling molecules; and besides protecting cells from starvation, autophagy can cause cell death. It is also now widely accepted that ROS participate in the regulation of autophagy, and *vice versa*. Additionally, both ROS and autophagy participate in cancer initiation and progression. Even though the association between ROS and autophagy is established, many unanswered questions remain. For example, do ROS control the "switch" from prosurvival to prodeath autophagy, and if so, how? Besides Atg4, what are the targets of ROS in the autophagy pathway? Do the species and/or the site of generation of ROS influence their role in regulating autophagy? Future studies will address these questions, and full understanding of the interplay between ROS and autophagy signaling should lead to the development of effective new therapies for cancer.

Acknowledgments

M. B. Azad is supported by the National Sciences and Engineering Research Council of Canada and the Manitoba Graduate Scholarship Program. S. B. Gibson is a Manitoba Research Chair, supported by the Manitoba Health Research Council.

Abbreviations

2-ME, 2-methoxyestradiol; ANT, adenine nucleotide translocase; As_2O_3, arsenic trioxide; Atg, ATG gene product; ATG, autophagy-related gene; BNIP3, BCL2/adenovirus E1B 19-kDa interacting protein 3; EGF, epidermal growth factor; GPx, glutathione peroxidase; H_2O_2, hydrogen peroxide; HDAC, histone deacetylase; HIF-1α, hypoxia-inducible factor α; HSFs, heat-shock factors; HSPs, heat-shock proteins; I/R, ischemia/reperfusion; IAP, inhibitor of apoptosis protein; IGF-2, insulin-like growth factor 2; IKK, inhibitor of kappa B kinase; IκB, inhibitor of kappa B; LC3, microtubule-associated light chain 3; mETC, mitochondrial electrón-transport chaín; mtDNA, mitochondrial DNA; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa B; NIK, NF-κB–inducing kinase; NO, nitric oxide; NO_2, nitrogen dioxide radical; Nox, NADPH oxidase; O_2^-, superoxide; OH^-, hydroxyl radical; $ONOO^-$, peroxynitrite; PCD, programmed cell death; PDGF, platelet-derived growth factor; PE, phosphoethanolamine; PHD, prolyl 4-hydroxylase; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol (4, 5)-bisphosphate; PIP3, phosphatidylinositol (3, 4, 5)-triphosphate; PKB, protein kinase B; PrxIII, peroxiredoxin III; PT pore, permeability transition pore; PTEN, phosphatase and tensin homologue; ROS, reactive oxygen species; SOD, superxide dismutase; TNF-α, tumor necrosis factor alpha; VDAC, voltage-dependent ion channel; VEGF, vascular endothelial growth factor.

References

1. Abedin MJ, Wang D, McDonnell MA, Lehmann U, and Kelekar A. Autophagy delays apoptotic death in breast cancer cells following DNA damage. *Cell Death Differ* 14: 500–510, 2007.
2. Adam-Vizi V and Chinopoulos C. Bioenergetics and the formation of mitochondrial reactive oxygen species. *Trends Pharmacol Sci* 27: 639–645, 2006.
3. Agostinelli E and Seiler N. Non-irradiation-derived reactive oxygen species (ROS) and cancer: therapeutic implications. *Amino Acids* 31: 341–355, 2006.
4. Amaravadi RK, Yu D, Lum JJ, Bui T, Christophorou MA, Evan GI, Thomas-Tikhonenko A, and Thompson CB. Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. *J Clin Invest* 117: 326–336, 2007.
5. Ames BN, Shigenaga MK, and Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci U S A* 90: 7915–7922, 1993.
6. Arico S, Petiot A, Bauvy C, Dubbelhuis PF, Meijer AJ, Codogno P, and Ogier-Denis E. The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. *J Biol Chem* 276: 35243–35246, 2001.
7. Arico S, Petiot A, Bauvy C, Dubbelhuis PF, Meijer AJ, Codogno P, and Ogier-Denis E. The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. *J Biol Chem* 276: 35243–35246, 2001.
8. Arsham AM and Neufeld TP. Thinking globally and acting locally with TOR. *Curr Opin Cell Biol* 18: 589–597, 2006.
9. Azad MB, Chen Y, Henson ES, Cizeau J, McMillan-Ward E, Israels SJ, and Gibson SB. Hypoxia induces autophagic cell death in apoptosis-competent cells through a mechanism involving BNIP3. *Autophagy* 4: 195–204, 2008.
10. Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB, and Rhee SG. Epidermal growth factor (EGF)-induced generation of hydrogen peroxide: role in EGF receptor-mediated tyrosine phosphorylation. *J Biol Chem* 272: 217–221, 1997.
11. Beno I, Staruchova M, Volkovova K, and Batovsky M. Increased antioxidant enzyme activities in the colorectal adenoma and carcinoma. *Neoplasma* 42: 265–269, 1995.
12. Bergamini E, Cavallini G, Donati A, and Gori Z. The anti-ageing effects of caloric restriction may involve stimulation of macroautophagy and lysosomal degradation, and can be intensified pharmacologically. *Biomed Pharmacother* 57: 203–208, 2003.
13. Boya P, Gonzalez-Polo RA, Casares N, Perfettini JL, Dessen P, Larochette N, Metivier D, Meley D, Souquere S, Yoshimori T, Pierron G, Codogno P, and Kroemer G. Inhibition of macroautophagy triggers apoptosis. *Mol Cell Biol* 25: 1025–1040, 2005.
14. Bras M, Queenan B, and Susin SA. Programmed cell death via mitochondria: different modes of dying. *Biochemistry (Mosc)* 70: 231–239, 2005.
15. Carew JS and Huang P. Mitochondrial defects in cancer. *Mol Cancer* 1: 9, 2002.
16. Carew JS, Nawrocki ST, Kahue CN, Zhang H, Yang C, Chung L, Houghton JA, Huang P, Giles FJ, and Cleveland JL. Targeting autophagy augments the anticancer activity of the histone deacetylase inhibitor SAHA to overcome Bcr-Abl-mediated drug resistance. *Blood* 110: 313–322, 2007.
17. Chau YP, Lin SY, Chen JH, and Tai MH. Endostatin induces autophagic cell death in EAhy926 human endothelial cells. *Histol Histopathol* 18: 715–726, 2003.
18. Chen Y and Gibson SB. Is mitochondrial generation of reactive oxygen species a trigger for autophagy? *Autophagy* 4: 246–248, 2008.
19. Chen Y, McMillan-Ward E, Kong J, Israels SJ, and Gibson SB. Oxidative stress induces autophagic cell death inde-

pendent of apoptosis in transformed and cancer cells. *Cell Death Differ* 15: 171–182, 2008.
20. Chen Y, McMillan-Ward E, Kong J, Israels SJ, and Gibson SB. Mitochondrial electron-transport-chain inhibitors of complexes I and II induce autophagic cell death mediated by reactive oxygen species. *J Cell Sci* 120: 4155–4166, 2007.
21. Chiarugi P. PTPs versus PTKs: the redox side of the coin. *Free Radic Res* 39: 353–364, 2005.
22. Cocheme HM and Murphy MP. Complex I is the major site of mitochondrial superoxide production by paraquat. *J Biol Chem* 283: 1786–1798, 2008.
23. Costantini P, Belzacq AS, Vieira HL, Larochette N, de Pablo MA, Zamzami N, Susin SA, Brenner C, and Kroemer G. Oxidation of a critical thiol residue of the adenine nucleotide translocator enforces Bcl-2-independent permeability transition pore opening and apoptosis. *Oncogene* 19: 307–314, 2000.
24. Cui Q, Tashiro S, Onodera S, Minami M, and Ikejima T. Autophagy preceded apoptosis in oridonin-treated human breast cancer MCF-7 cells. *Biol Pharm Bull* 30: 859–864, 2007.
25. Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, Mukherjee C, Shi Y, Gelinas C, Fan Y, Nelson DA, Jin S, and White E. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell* 10: 51–64, 2006.
26. Demasters G, Di X, Newsham I, Shiu R, and Gewirtz DA. Potentiation of radiation sensitivity in breast tumor cells by the vitamin D3 analogue, EB 1089, through promotion of autophagy and interference with proliferative recovery. *Mol Cancer Ther* 5: 2786–2797, 2006.
27. Deshpande SS, Angkeow P, Huang J, Ozaki M, and Irani K. Rac1 inhibits TNF-alpha-induced endothelial cell apoptosis: dual regulation by reactive oxygen species. *FASEB J* 14: 1705–1714, 2000.
28. Djavaheri-Mergny M, Amelotti M, Mathieu J, Besancon F, Bauvy C, Souquere S, Pierron G, and Codogno P. NF-kappaB activation represses tumor necrosis factor-alpha-induced autophagy. *J Biol Chem* 281: 30373–30382, 2006.
29. Doelling JH, Walker JM, Friedman EM, Thompson AR, and Vierstra RD. The APG8/12-activating enzyme APG7 is required for proper nutrient recycling and senescence in *Arabidopsis thaliana*. *J Biol Chem* 277: 33105–33114, 2002.
30. Easton JB and Houghton PJ. mTOR and cancer therapy. *Oncogene* 25: 6436–6446, 2006.
31. Edinger AL and Thompson CB. Death by design: apoptosis, necrosis and autophagy. *Curr Opin Cell Biol* 16: 663–669, 2004.
32. Engel RH and Evens AM. Oxidative stress and apoptosis: a new treatment paradigm in cancer. *Front Biosci* 11: 300–312, 2006.
33. Ertmer A, Huber V, Gilch S, Yoshimori T, Erfle V, Duyster J, Elsasser HP, and Schatzl HM. The anticancer drug imatinib induces cellular autophagy. *Leukemia* 21: 936–942, 2007.
34. Feng Z, Zhang H, Levine AJ, and Jin S. The coordinate regulation of the p53 and mTOR pathways in cells. *Proc Natl Acad Sci U S A* 102: 8204–8209, 2005.
35. Fink AL. Protein aggregation: folding aggregates, inclusion bodies and amyloid. *Fold Des* 3: R9–R23, 1998.
36. Friguet B and Szweda LI. Inhibition of the multicatalytic proteinase (proteasome) by 4-hydroxy-2-nonenal cross-linked protein. *FEBS Lett* 405: 21–25, 1997.
37. Fruehauf JP and Meyskens FL Jr. Reactive oxygen species: a breath of life or death? *Clin Cancer Res* 13: 789–794, 2007.
38. Gonzalez-Polo RA, Boya P, Pauleau AL, Jalil A, Larochette N, Souquere S, Eskelinen EL, Pierron G, Saftig P, and Kroemer G. The apoptosis/autophagy paradox: autophagic vacuolization before apoptotic death. *J Cell Sci* 118: 3091–3102, 2005.
39. Gupta S, Yel L, Kim D, Kim C, Chiplunkar S, and Gollapudi S. Arsenic trioxide induces apoptosis in peripheral blood T lymphocyte subsets by inducing oxidative stress: a role of Bcl-2. *Mol Cancer Ther* 2: 711–719, 2003.
40. Hagen T, D'Amico G, Quintero M, Palacios-Callender M, Hollis V, Lam F, and Moncada S. Inhibition of mitochondrial respiration by the anticancer agent 2-methoxyestradiol. *Biochem Biophys Res Commun* 322: 923–929, 2004.
41. Hamacher-Brady A, Brady NR, and Gottlieb RA. Enhancing macroautophagy protects against ischemia/reperfusion injury in cardiac myocytes. *J Biol Chem* 281: 29776–29787, 2006.
42. Hampton MB and Orrenius S. Dual regulation of caspase activity by hydrogen peroxide: implications for apoptosis. *FEBS Lett* 414: 552–556, 1997.
43. Hanukoglu I. Antioxidant protective mechanisms against reactive oxygen species (ROS) generated by mitochondrial P450 systems in steroidogenic cells. *Drug Metab Rev* 38: 171–196, 2006.
44. Hippert MM, O'Toole PS, and Thorburn A. Autophagy in cancer: good, bad, or both? *Cancer Res* 66: 9349–9351, 2006.
45. Huang P, Feng L, Oldham EA, Keating MJ, and Plunkett W. Superoxide dismutase as a target for the selective killing of cancer cells. *Nature* 407: 390–395, 2000.
46. Ishii N. Role of oxidative stress from mitochondria on aging and cancer. *Cornea* 26(9 suppl)1: S3–S9, 2007.
47. James J, Murry DJ, Treston AM, Storniolo AM, Sledge GW, Sidor C, and Miller KD. Phase I safety, pharmacokinetic and pharmacodynamic studies of 2-methoxyestradiol alone or in combination with docetaxel in patients with locally recurrent or metastatic breast cancer. *Invest New Drugs* 25: 41–48, 2007.
48. Jin S and White E. Role of autophagy in cancer: management of metabolic stress. *Autophagy* 3: 28–31, 2007.
49. Kaltschmidt B, Sparna T, and Kaltschmidt C. Activation of NF-kappa B by reactive oxygen intermediates in the nervous system. *Antioxid Redox Signal* 1: 129–144, 1999.
50. Kanzawa T, Zhang L, Xiao L, Germano IM, Kondo Y, and Kondo S. Arsenic trioxide induces autophagic cell death in malignant glioma cells by upregulation of mitochondrial cell death protein BNIP3. *Oncogene* 24: 980–991, 2005.
51. Kessel D, Reiners JJ Jr, Hazeldine ST, Polin L, and Horwitz JP. The role of autophagy in the death of L1210 leukemia cells initiated by the new antitumor agents, XK469 and SH80. *Mol .Cancer Ther* 6: 370–379, 2007.
52. Kiffin R, Bandyopadhyay U, and Cuervo AM. Oxidative stress and autophagy. *Antioxid Redox Signal* 8: 152–162, 2006.
53. Kiffin R, Christian C, Knecht E, and Cuervo AM. Activation of chaperone-mediated autophagy during oxidative stress. *Mol Biol Cell* 15: 4829–4840, 2004.
54. Kim EH, Sohn S, Kwon HJ, Kim SU, Kim MJ, Lee SJ, and Choi KS. Sodium selenite induces superoxide-mediated mitochondrial damage and subsequent autophagic cell death in malignant glioma cells. *Cancer Res* 67: 6314–6324, 2007.
55. Kim I, Rodriguez-Enriquez S, and Lemasters JJ. Selective degradation of mitochondria by mitophagy. *Arch Biochem Biophys* 462: 245–253, 2007.
56. Kim JJ, Chae SW, Hur GC, Cho SJ, Kim MK, Choi J, Nam SY, Kim WH, Yang HK, and Lee BL. Manganese superoxide

dismutase expression correlates with a poor prognosis in gastric cancer. *Pathobiology* 70: 353–360, 2002.
57. Kim KW, Mutter RW, Cao C, Albert JM, Freeman M, Hallahan DE, and Lu B. Autophagy for cancer therapy through inhibition of pro-apoptotic proteins and mammalian target of rapamycin signaling. *J Biol Chem* 281: 36883–36890, 2006.
58. Kirkland RA, Adibhatla RM, Hatcher JF, and Franklin JL. Loss of cardiolipin and mitochondria during programmed neuronal death: evidence of a role for lipid peroxidation and autophagy. *Neuroscience* 115: 587–602, 2002.
59. Kissova I, Deffieu M, Samokhvalov V, Velours G, Bessoule JJ, Manon S, and Camougrand N. Lipid oxidation and autophagy in yeast. *Free Radic Biol Med* 41: 1655–1661, 2006.
60. Klimova T and Chandel NS. Mitochondrial complex III regulates hypoxic activation of HIF. *Cell Death Differ* 15: 660–666, 2008.
61. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E, and Tanaka K. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* 441: 880–884, 2006.
62. Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, Ezaki J, Mizushima N, Ohsumi Y, Uchiyama Y, Kominami E, Tanaka K, and Chiba T. Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. *J Cell Biol* 169: 425–434, 2005.
63. Kondo Y and Kondo S. Autophagy and cancer therapy. *Autophagy* 2: 85–90, 2006.
64. Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, and Mizushima N. The role of autophagy during the early neonatal starvation period. *Nature* 432: 1032–1036, 2004.
65. Lambeth JD. Nox enzymes, ROS, and chronic disease: an example of antagonistic pleiotropy. *Free Radic Biol Med* 43: 332–347, 2007.
66. Lefranc F, Facchini V, and Kiss R. Proautophagic drugs: a novel means to combat apoptosis-resistant cancers, with a special emphasis on glioblastomas. *Oncologist* 12: 1395–1403, 2007.
67. Levine B. Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. *Cell* 120: 159–162, 2005.
68. Li Q and Engelhardt JF. Interleukin-1beta induction of NFkappaB is partially regulated by H2O2-mediated activation of NFkappaB-inducing kinase. *J Biol Chem* 281: 1495–1505, 2006.
69. Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, and Levine B. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* 402: 672–676, 1999.
70. Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T, and Thompson CB. Growth factor regulation of autophagy and cell survival in the absence of apoptosis. *Cell* 120: 237–248, 2005.
71. Lushchak VI. Free radical oxidation of proteins and its relationship with functional state of organisms. *Biochemistry (Mosc)* 72: 809–827, 2007.
72. Madesh M and Hajnoczky G. VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release. *J Cell Biol* 155: 1003–1015, 2001.
73. Maiuri MC, Le Toumelin G, Criollo A, Rain JC, Gautier F, Juin P, Tasdemir E, Pierron G, Troulinaki K, Tavernarakis N, Hickman JA, Geneste O, and Kroemer G. Functional and physical interaction between Bcl-X(L) and a BH3-like domain in Beclin-1. *EMBO J* 26: 2527–2539, 2007.
74. Maiuri MC, Zalckvar E, Kimchi A, and Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol* 8: 741–752, 2007.
75. Mocanu MM and Yellon DM. PTEN, the Achilles' heel of myocardial ischaemia/reperfusion injury? *Br J Pharmacol* 150: 833–838, 2007.
76. Mohr S, Zech B, Lapetina EG, and Brune B. Inhibition of caspase-3 by S-nitrosation and oxidation caused by nitric oxide. *Biochem Biophys Res Commun* 238: 387–391, 1997.
77. Mollen KP, McCloskey CA, Tanaka H, Prince JM, Levy RM, Zuckerbraun BS, and Billiar TR. Hypoxia activates c-Jun N-terminal kinase via Rac1-dependent reactive oxygen species production in hepatocytes. *Shock* 28: 270–277, 2007.
78. Moore MN, Allen JI, and Somerfield PJ. Autophagy: role in surviving environmental stress. *Mar Environ Res* 62(suppl): S420–S425, 2006.
79. Nian M, Lee P, Khaper N, and Liu P. Inflammatory cytokines and postmyocardial infarction remodeling. *Circ Res* 94: 1543–1553, 2004.
80. Nicotera P and Melino G. Regulation of the apoptosis-necrosis switch. *Oncogene* 23: 2757–2765, 2004.
81. Oberley LW. Mechanism of the tumor suppressive effect of MnSOD overexpression. *Biomed Pharmacother* 59: 143–148, 2005.
82. Ohsumi Y. Molecular dissection of autophagy: two ubiquitin-like systems. *Nat Rev Mol Cell Biol* 2: 211–216, 2001.
83. Ozaki M, Deshpande SS, Angkeow P, Suzuki S, and Irani K. Rac1 regulates stress-induced, redox-dependent heat shock factor activation. *J Biol Chem* 275: 35377–35383, 2000.
84. Ozben T. Oxidative stress and apoptosis: impact on cancer therapy. *J Pharm Sci* 96: 2181–2196, 2007.
85. Pattingre S, Espert L, Biard-Piechaczyk M, and Codogno P. Regulation of macroautophagy by mTOR and Beclin 1 complexes. *Biochimie* 90: 313–323, 2008.
86. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, and Levine B. Bcl-2 anti-apoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* 122: 927–939, 2005.
87. Pelicano H, Carney D, and Huang P. ROS stress in cancer cells and therapeutic implications. *Drug Resist Update* 7: 97–110, 2004.
88. Poli G, Leonarduzzi G, Biasi F, and Chiarpotto E. Oxidative stress and cell signalling. *Curr Med Chem* 11: 1163–1182, 2004.
89. Pouyssegur J, Dayan F, and Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature* 441: 437–443, 2006.
90. Punnonen K, Ahotupa M, Asaishi K, Hyoty M, Kudo R, and Punnonen R. Antioxidant enzyme activities and oxidative stress in human breast cancer. *J Cancer Res Clin Oncol* 120: 374–377, 1994.
91. Reef S, Zalckvar E, Shifman O, Bialik S, Sabanay H, Oren M, and Kimchi A. A short mitochondrial form of p19ARF induces autophagy and caspase-independent cell death. *Mol Cell* 22: 463–475, 2006.
92. Riley PA. Free radicals in biology: oxidative stress and the effects of ionizing radiation. *Int J Radiat Biol* 65: 27–33, 1994.
93. Rudolph J. Redox regulation of the Cdc25 phosphatases. *Antioxid Redox Signal* 7: 761–767, 2005.
94. Rygiel TP, Mertens AE, Strumane K, van der Kammen R, and Collard JG. The Rac activator Tiam1 prevents

keratinocyte apoptosis by controlling ROS-mediated ERK phosphorylation. *J Cell Sci* 121: 1183–1192, 2008.
95. Sanchez I, Xu CJ, Juo P, Kakizaka A, Blenis J, and Yuan J. Caspase-8 is required for cell death induced by expanded polyglutamine repeats. *Neuron* 22: 623–633, 1999.
96. Sanlioglu S, Williams CM, Samavati L, Butler NS, Wang G, McCray PB Jr, Ritchie TC, Hunninghake GW, Zandi E, and Engelhardt JF. Lipopolysaccharide induces Rac1-dependent reactive oxygen species formation and coordinates tumor necrosis factor-alpha secretion through IKK regulation of NF-kappa B. *J Biol Chem* 276: 30188–30198, 2001.
97. Sarkar FH and Li Y. NF-kappaB: a potential target for cancer chemoprevention and therapy. *Front Biosci* 13: 2950–2959, 2008.
98. Scherz-Shouval R, and Elazar Z. ROS, mitochondria and the regulation of autophagy. *Trends Cell Biol* 17: 422–427, 2007.
99. Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L, and Elazar Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. *EMBO J* 26: 1749–1760, 2007.
100. Schrader M and Fahimi HD. Peroxisomes and oxidative stress. *Biochim Biophys Acta* 1763: 1755–1766, 2006.
101. Simon HU, Haj-Yehia A, and Levi-Schaffer F. Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis* 5: 415–418, 2000.
102. Sitte N, Huber M, Grune T, Ladhoff A, Doecke WD, Von Zglinicki T, and Davies KJ. Proteasome inhibition by lipofuscin/ceroid during postmitotic aging of fibroblasts. *FASEB J* 14: 11: 1490–1498, 2000.
103. Sivaprasad U and Basu A. Inhibition of ERK attenuates autophagy and potentiates tumor necrosis factor-alpha-induced cell death in MCF-7 cells. *J Cell Mol Med* 12: 1265–1271, 2008.
104. Storz G and Polla BS. Transcriptional regulators of oxidative stress-inducible genes in prokaryotes and eukaryotes. *EXS* 77: 239–254, 1996.
105. Sundaresan M, Yu ZX, Ferrans VJ, Irani K, and Finkel T. Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. *Science* 270: 296–299, 1995.
106. Suzuki K and Ohsumi Y. Molecular machinery of autophagosome formation in yeast, *Saccharomyces cerevisiae*. *FEBS Lett* 581: 2156–2161, 2007.
107. Svobodova A, Walterova D, and Vostalova J. Ultraviolet light induced alteration to the skin. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 150: 25–38, 2006.
108. Sweeney C, Liu G, Yiannoutsos C, Kolesar J, Horvath D, Staab MJ, Fife K, Armstrong V, Treston A, Sidor C, and Wilding G. A phase II multicenter, randomized, double-blind, safety trial assessing the pharmacokinetics, pharmacodynamics, and efficacy of oral 2-methoxyestradiol capsules in hormone-refractory prostate cancer. *Clin Cancer Res* 11: 6625–6633, 2005.
109. Takada Y, Mukhopadhyay A, Kundu GC, Mahabeleshwar GH, Singh S, and Aggarwal BB. Hydrogen peroxide activates NF-kappa B through tyrosine phosphorylation of I kappa B alpha and serine phosphorylation of p65: evidence for the involvement of I kappa B alpha kinase and Syk protein-tyrosine kinase. *J Biol Chem* 278: 24233–24241, 2003.
110. Thorpe GW, Fong CS, Alic N, Higgins VJ, and Dawes IW. Cells have distinct mechanisms to maintain protection against different reactive oxygen species: oxidative-stress-response genes. *Proc Natl Acad Sci U S A* 101: 6564–6569, 2004.
111. Traber MG, van der Vliet A, Reznick AZ, and Cross CE. Tobacco-related diseases: is there a role for antioxidant micronutrient supplementation? *Clin Chest Med* 21: 173–187, 2000.
112. Tsukada M and Ohsumi Y. Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. *FEBS Lett* 333: 169–174, 1993.
113. Vande Velde C, Cizeau J, Dubik D, Alimonti J, Brown T, Israels S, Hakem R, and Greenberg AH. BNIP3 and genetic control of necrosis-like cell death through the mitochondrial permeability transition pore. *Mol Cell Biol* 20: 5454–5468, 2000.
114. Wochna A, Niemczyk E, Kurono C, Masaoka M, Kedzior J, Slominska E, Lipinski M, and Wakabayashi T. A possible role of oxidative stress in the switch mechanism of the cell death mode from apoptosis to necrosis: studies on rho0 cells. *Mitochondrion* 7: 119–124, 2007.
115. Wolozin B and Behl C. Mechanisms of neurodegenerative disorders: Pt. 1: protein aggregates. *Arch Neurol* 57: 793–796, 2000.
116. Wu WS. The signaling mechanism of ROS in tumor progression. *Cancer Metastasis Rev* 25: 695–705, 2006.
117. Xu Y, Kim SO, Li Y, and Han J. Autophagy contributes to caspase-independent macrophage cell death. *J Biol Chem* 281: 19179–19187, 2006.
118. Yang C, Kaushal V, Shah SV, and Kaushal GP. Autophagy is associated with apoptosis in cisplatin injury to renal tubular epithelial cells. *Am J Physiol Renal Physiol* 294: F777–F787, 2008.
119. Yu L, Wan F, Dutta S, Welsh S, Liu Z, Freundt E, Baehrecke EH, and Lenardo M. Autophagic programmed cell death by selective catalase degradation. *Proc Natl Acad Sci U S A* 103: 4952–4957, 2006.
120. Yue Z, Jin S, Yang C, Levine AJ, and Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci U S A* 100: 15077–15082, 2003.

Address reprint requests to:
Manitoba Institute of Cell Biology
675 McDermot Ave.
Winnipeg
Manitoba R3E 0V9
Canada

E-mail: gibsonsb@cc.umanitoba.ca

Date of first submission to ARS Central, September 2, 2008; date of acceptance, September 29, 2008.

This article has been cited by:

1. Xian-ling Guo, Ding Li, Kai Sun, Jin Wang, Yan Liu, Jian-rui Song, Qiu-dong Zhao, Shan-shan Zhang, Wei-jie Deng, Xue Zhao, Meng-chao Wu, Li-xin Wei. 2012. Inhibition of autophagy enhances anticancer effects of bevacizumab in hepatocarcinoma. *Journal of Molecular Medicine* . [CrossRef]
2. Lucia Laura Policastro , Irene Laura Ibañez , Cintia Notcovich , Hebe Alicia Duran , Osvaldo Luis Podhajcer . The Tumor Microenvironment: Characterization, Redox Considerations, and Novel Approaches for Reactive Oxygen Species-Targeted Gene Therapy. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
3. Lin Li, Ganchimeg Ishdorj, Spencer B. Gibson. 2012. Reactive oxygen species regulation of autophagy in cancer: Implications for cancer treatment. *Free Radical Biology and Medicine* **53**:7, 1399-1410. [CrossRef]
4. Ana Coto-Montes, Jose Antonio Boga, Sergio Rosales-Corral, Lorena Fuentes-Broto, Dun-Xian Tan, Russel J. Reiter. 2012. Role of melatonin in the regulation of autophagy and mitophagy: A review. *Molecular and Cellular Endocrinology* **361**:1-2, 12-23. [CrossRef]
5. Irena Szumiel. 2012. Radiation hormesis: Autophagy and other cellular mechanisms. *International Journal of Radiation Biology* **88**:9, 619-628. [CrossRef]
6. Xiao-Li He, Peng Zhang, Xian-Zhe Dong, Mei-Hua Yang, Shi-Lin Chen, Ming-Gang Bi. 2012. JR6, a new compound isolated from Justicia procumbens, induces apoptosis in human bladder cancer EJ cells through caspase-dependent pathway. *Journal of Ethnopharmacology* . [CrossRef]
7. David Kessel, John Reiners. 2012. Light-Activated Pharmaceuticals: Mechanisms and Detection. *Israel Journal of Chemistry* **52**:8-9, 674-680. [CrossRef]
8. Lin Li, Yongqiang Chen, Spencer B. Gibson. 2012. Starvation-induced autophagy is regulated by mitochondrial reactive oxygen species leading to AMPK activation. *Cellular Signalling* . [CrossRef]
9. Vanessa J. Lavallard, Alfred J. Meijer, Patrice Codogno, Philippe Gual. 2012. Autophagy, signaling and obesity. *Pharmacological Research* . [CrossRef]
10. Xin-Xin Tong, Dan Wu, Xue Wang, Hua-Li Chen, Jia-Xiang Chen, Xiao-Xiao Wang, Xu-Lei Wang, Lu Gan, Zhi-Yun Guo, Gui-Xiu Shi, Yi-Zheng Zhang, Wei Jiang. 2012. Ghrelin protects against cobalt chloride-induced hypoxic injury in cardiac H9c2 cells by inhibiting oxidative stress and inducing autophagy. *Peptides* . [CrossRef]
11. Subash C. Gupta , David Hevia , Sridevi Patchva , Byoungduck Park , Wonil Koh , Bharat B. Aggarwal . 2012. Upsides and Downsides of Reactive Oxygen Species for Cancer: The Roles of Reactive Oxygen Species in Tumorigenesis, Prevention, and Therapy. *Antioxidants & Redox Signaling* **16**:11, 1295-1322. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
12. Fred Lozy, Vassiliki Karantza. 2012. Autophagy and cancer cell metabolism. *Seminars in Cell & Developmental Biology* **23**:4, 395-401. [CrossRef]
13. Gregory L. Bellot, Dan Liu, Shazib Pervaiz. 2012. ROS, Autophagy, Mitochondria and Cancer: Ras, The Hidden Master?. *Mitochondrion* . [CrossRef]
14. Jwa-Jin Kim, Hye-Mi Lee, Dong-Min Shin, Wonho Kim, Jae-Min Yuk, Hyo Sun Jin, Sang-Hee Lee, Guang-Ho Cha, Jin-Man Kim, Zee-Won Lee, Sung Jae Shin, Heekyung Yoo, Young Kil Park, Jin Bong Park, JongKyeong Chung, Tamotsu Yoshimori, Eun-Kyeong Jo. 2012. Host Cell Autophagy Activated by Antibiotics Is Required for Their Effective Antimycobacterial Drug Action. *Cell Host & Microbe* **11**:5, 457-468. [CrossRef]
15. Jian-Xiang Liu, Guang-Biao Zhou, Sai-Juan Chen, Zhu Chen. 2012. Arsenic compounds: revived ancient remedies in the fight against human malignancies. *Current Opinion in Chemical Biology* **16**:1-2, 92-98. [CrossRef]
16. Saverio Marchi, Carlotta Giorgi, Jan M. Suski, Chiara Agnoletto, Angela Bononi, Massimo Bonora, Elena De Marchi, Sonia Missiroli, Simone Patergnani, Federica Poletti, Alessandro Rimessi, Jerzy Duszynski, Mariusz R. Wieckowski, Paolo Pinton. 2012. Mitochondria-Ros Crosstalk in the Control of Cell Death and Aging. *Journal of Signal Transduction* **2012**, 1-17. [CrossRef]
17. Aijun Liao, Rong Hu, Qihui Zhao, Jia Li, Yingchun Li, Kun Yao, Rong Zhang, Huihan Wang, Wei Yang, Zhuogang Liu. 2012. Autophagy induced by FTY720 promotes apoptosis in U266 cells. *European Journal of Pharmaceutical Sciences* . [CrossRef]
18. Jae-Min Yuk, Tamotsu Yoshimori, Eun-Kyeong Jo. 2012. Autophagy and bacterial infectious diseases. *Experimental & Molecular Medicine* **44**:2, 99. [CrossRef]

19. Giuseppina Di Giacomo, Salvatore Rizza, Costanza Montagna, Giuseppe Filomeni. 2012. Established Principles and Emerging Concepts on the Interplay between Mitochondrial Physiology and S-(De)nitrosylation: Implications in Cancer and Neurodegeneration. *International Journal of Cell Biology* **2012**, 1-20. [CrossRef]
20. Yuxing Zhang , Yanzhi Du , Weidong Le , Kankan Wang , Nelly Kieffer , Ji Zhang . 2011. Redox Control of the Survival of Healthy and Diseased Cells. *Antioxidants & Redox Signaling* **15**:11, 2867-2908. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
21. Filipe V. Duarte, João S. Teodoro, Anabela P. Rolo, Carlos M. Palmeira. 2011. Exposure to dibenzofuran triggers autophagy in lung cells. *Toxicology Letters* . [CrossRef]
22. Ling-Juan Zheng, Thomas Peer, Volker Seybold, Ursula Lütz-Meindl. 2011. Pb-induced ultrastructural alterations and subcellular localization of Pb in two species of Lespedeza by TEM-coupled electron energy loss spectroscopy. *Environmental and Experimental Botany* . [CrossRef]
23. Vittorio Calabrese, Carolin Cornelius, Albena T. Dinkova-Kostova, Ivo Iavicoli, Rosanna Di Paola, Aleardo Koverech, Salvatore Cuzzocrea, Enrico Rizzarelli, Edward J. Calabrese. 2011. Cellular stress responses, hormetic phytochemicals and vitagenes in aging and longevity. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* . [CrossRef]
24. Mohd Imran Khan, Akbar Mohammad, Govil Patil, S.A.H. Naqvi, L.K.S. Chauhan, Iqbal Ahmad. 2011. Induction of ROS, mitochondrial damage and autophagy in lung epithelial cancer cells by iron oxide nanoparticles. *Biomaterials* . [CrossRef]
25. Chien-Ju Lin, Chin-Cheng Lee, Yung-Luen Shih, Tsung-Yao Lin, Sheng-Hao Wang, Yuh-Feng Lin, Chwen-Ming Shih. 2011. Resveratrol enhances the therapeutic effect of temozolomide against malignant glioma in vitro and in vivo by inhibiting autophagy. *Free Radical Biology and Medicine* . [CrossRef]
26. Regina Brigelius-Flohé , Leopold Flohé . 2011. Basic Principles and Emerging Concepts in the Redox Control of Transcription Factors. *Antioxidants & Redox Signaling* **15**:8, 2335-2381. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
27. Daolin Tang , Rui Kang , Kristen M. Livesey , Herbert J. Zeh III , Michael T. Lotze . 2011. High Mobility Group Box 1 (HMGB1) Activates an Autophagic Response to Oxidative Stress. *Antioxidants & Redox Signaling* **15**:8, 2185-2195. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
28. Xiaowei Ma, Yanyang Wu, Shubin Jin, Yuan Tian, Xiaoning Zhang, Yuliang Zhao, Li Yu, Xing-Jie Liang. 2011. Gold Nanoparticles Induce Autophagosome Accumulation through Size-Dependent Nanoparticle Uptake and Lysosome Impairment. *ACS Nano* 111011170117002. [CrossRef]
29. Zi-yue Li, Yu Yang, Miao Ming, Bo Liu. 2011. Mitochondrial ROS generation for regulation of autophagic pathways in cancer. *Biochemical and Biophysical Research Communications* . [CrossRef]
30. Ching-Chuan Kuo, Tsang-Wu Liu, Li-Tzong Chen, Her-Shyong Shiah, Ching-Ming Wu, Yen-Ting Cheng, Wen-Yu Pan, Jin-Fen Liu, Kuo-Li Chen, Yun-Ning Yang, Shan-Na Chen, Jang-Yang Chang. 2011. Combination of arsenic trioxide and BCNU synergistically triggers redox-mediated autophagic cell death in human solid tumors. *Free Radical Biology and Medicine* . [CrossRef]
31. Yu Jeong Byun, Seong-Beom Lee, Hwa Ok Lee, Min Jeong Son, Ho-Shik Kim, Oh-Joo Kwon, Seong-Whan Jeong. 2011. Vacuolar H+-ATPase c protects glial cell death induced by sodium nitroprusside under glutathione-depleted condition. *Journal of Cellular Biochemistry* **112**:8, 1985-1996. [CrossRef]
32. R Coriat, W Marut, M Leconte, L B Ba, A Vienne, C Chéreau, J Alexandre, B Weill, M Doering, C Jacob, C Nicco, F Batteux. 2011. The organotelluride catalyst LAB027 prevents colon cancer growth in the mice. *Cell Death and Disease* **2**:8, e191. [CrossRef]
33. Virginia Motilva, Sofía García-Mauriño, Elena Talero, Matilde Illanes. 2011. New paradigms in chronic intestinal inflammation and colon cancer: role of melatonin. *Journal of Pineal Research* **51**:1, 44-60. [CrossRef]
34. Juan-Cheng Yang, Mei-Chin Lu, Chia-Lin Lee, Guan-Yu Chen, Yan-Yu Lin, Fang-Rong Chang, Yang-Chang Wu. 2011. Selective targeting of breast cancer cells through ROS-mediated mechanisms potentiates the lethality of paclitaxel by a novel diterpene, gelomulide K. *Free Radical Biology and Medicine* **51**:3, 641-657. [CrossRef]
35. Siegfried Hekimi, Jérôme Lapointe, Yang Wen. 2011. Taking a “good” look at free radicals in the aging process. *Trends in Cell Biology* . [CrossRef]
36. Weijie Guo, Yingjun Zhao, Zhenfeng Zhang, Ning Tan, Fangyu Zhao, Chao Ge, Linhui Liang, Deshui Jia, Taoyang Chen, Ming Yao, Jinjun Li, Xianghuo He. 2011. Disruption of xCT inhibits cell growth via the ROS/autophagy pathway in hepatocellular carcinoma. *Cancer Letters* . [CrossRef]

37. Pathrapol Lithanatudom, Tirawat Wannatung, Amporn Leecharoenkiat, Saovaros Svasti, Suthat Fucharoen, Duncan R. Smith. 2011. Enhanced activation of autophagy in #-thalassemia/Hb E erythroblasts during erythropoiesis. *Annals of Hematology* **90**:7, 747-758. [CrossRef]

38. Ya-Na Wu, Li-Xing Yang, Xuan-Yu Shi, I-Chen Li, Joanna M. Biazik, Kyle R. Ratinac, Dong-Hwang Chen, Pall Thordarson, Dar-Bin Shieh, Filip Braet. 2011. The selective growth inhibition of oral cancer by iron core-gold shell nanoparticles through mitochondria-mediated autophagy. *Biomaterials* **32**:20, 4565-4573. [CrossRef]

39. Yi Luo, Ping Zou, Jing Zou, Jie Wang, Daohong Zhou, Lingbo Liu. 2011. Autophagy regulates ROS-induced cellular senescence via p21 in a p38 MAPK# dependent manner. *Experimental Gerontology* . [CrossRef]

40. Ignacio Vega-Naredo, Beatriz Caballero, Verónica Sierra, Marina García-Macia, David de Gonzalo-Calvo, Paulo J. Oliveira, María Josefa Rodríguez-Colunga, Ana Coto-Montes. 2011. Melatonin modulates autophagy through a redox-mediated action in female Syrian hamster Harderian gland controlling cell types and gland activity. *Journal of Pineal Research* no-no. [CrossRef]

41. Ju Huang , Grace Y. Lam , John H. Brumell . 2011. Autophagy Signaling Through Reactive Oxygen Species. *Antioxidants & Redox Signaling* **14**:11, 2215-2231. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]

42. Michael Dewaele, Wim Martinet, Noemí Rubio, Tom Verfaillie, Peter A. de Witte, Jacques Piette, Patrizia Agostinis. 2011. Autophagy pathways activated in response to PDT contribute to cell resistance against ROS damage. *Journal of Cellular and Molecular Medicine* **15**:6, 1402-1414. [CrossRef]

43. Tao Cui, Chunxiang Fan, Li Gu, Hua Gao, Qi Liu, Tao Zhang, Zhifeng Qi, Chunli Zhao, Huanying Zhao, Qing Cai, Hui Yang. 2011. Silencing of PINK1 induces mitophagy via mitochondrial permeability transition in dopaminergic MN9D cells. *Brain Research* **1394**, 1-13. [CrossRef]

44. Kai Bartkowiak, Sabine Riethdorf, Klaus Pantel. 2011. The Interrelating Dynamics of Hypoxic Tumor Microenvironments and Cancer Cell Phenotypes in Cancer Metastasis. *Cancer Microenvironment* . [CrossRef]

45. Si Jin , Fan Zhou , Foad Katirai , Pin-Lan Li . Lipid Raft Redox Signaling: Molecular Mechanisms in Health and Disease. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]

46. Zhihui Feng, Liyuan Bai, Jiong Yan, Yuan Li, Weili Shen, Ying Wang, Karin Wertz, Peter Weber, Yong Zhang, Yan Chen, Jiankang Liu. 2011. Mitochondrial dynamic remodeling in strenuous exercise-induced muscle and mitochondrial dysfunction: Regulatory effects of hydroxytyrosol. *Free Radical Biology and Medicine* **50**:10, 1437-1446. [CrossRef]

47. L-U Ling, K-B Tan, H Lin, G N C Chiu. 2011. The role of reactive oxygen species and autophagy in safingol-induced cell death. *Cell Death and Disease* **2**:3, e129. [CrossRef]

48. Danielle G Smith, Tapiwanashe Magwere, Susan A Burchill. 2011. Oxidative stress and therapeutic opportunities: focus on the Ewing's sarcoma family of tumors. *Expert Review of Anticancer Therapy* **11**:2, 229-249. [CrossRef]

49. Ruth Scherz-Shouval, Zvulun Elazar. 2011. Regulation of autophagy by ROS: physiology and pathology. *Trends in Biochemical Sciences* **36**:1, 30-38. [CrossRef]

50. K. J. Thomas, M. K. McCoy, J. Blackinton, A. Beilina, M. van der Brug, A. Sandebring, D. Miller, D. Maric, A. Cedazo-Minguez, M. R. Cookson. 2011. DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. *Human Molecular Genetics* **20**:1, 40-50. [CrossRef]

51. Hwa Ok Lee, Yu Jeong Byun, Kyung-Ok Cho, Seong Yun Kim, Seong-Beom Lee, Ho-Shik Kim, Oh-Joo Kwon, Seong-Whan Jeong. 2011. GS28 Protects Neuronal Cell Death Induced by Hydrogen Peroxide under Glutathione-Depleted Condition. *The Korean Journal of Physiology and Pharmacology* **15**:3, 149. [CrossRef]

52. Ida Perrotta, Valentina Carito, Emilio Russo, Sandro Tripepi, Saveria Aquila, Giuseppe Donato. 2011. Macrophage Autophagy and Oxidative Stress: An Ultrastructural and Immunoelectron Microscopical Study. *Oxidative Medicine and Cellular Longevity* **2011**, 1-8. [CrossRef]

53. Deep Agnani, Olga Camacho-Vanegas, Catalina Camacho, Shashi Lele, Kunle Odunsi, Samantha Cohen, Peter Dottino, John A Martignetti. 2011. Decreased levels of serum glutathione peroxidase 3 are associated with papillary serous ovarian cancer and disease progression. *Journal of Ovarian Research* **4**:1, 18. [CrossRef]

54. Charles E. Wenner. 2011. Targeting mitochondria as a therapeutic target in cancer. *Journal of Cellular Physiology* n/a-n/a. [CrossRef]

55. Vittorio Calabrese, Carolin Cornelius, Anna Maria Giuffrida Stella, Edward J. Calabrese. 2010. Cellular Stress Responses, Mitostress and Carnitine Insufficiencies as Critical Determinants in Aging and Neurodegenerative Disorders: Role of Hormesis and Vitagenes. *Neurochemical Research* **35**:12, 1880-1915. [CrossRef]

56. Simone Reuter, Subash C. Gupta, Madan M. Chaturvedi, Bharat B. Aggarwal. 2010. Oxidative stress, inflammation, and cancer: How are they linked?. *Free Radical Biology and Medicine* **49**:11, 1603-1616. [CrossRef]
57. Maria C. Messner, Myles C. Cabot. 2010. Cytotoxic responses to N-(4-hydroxyphenyl)retinamide in human pancreatic cancer cells. *Cancer Chemotherapy and Pharmacology* . [CrossRef]
58. Susana Martin, Eugenio Fernandez-Alanis, Veronica Delfosse, Pablo Evelson, Juan S. Yakisich, Paulo H. Saldiva, Deborah R Tasat. 2010. Low doses of urban air particles from Buenos Aires promote oxidative stress and apoptosis in mice lungs. *Inhalation Toxicology* **22**:13, 1064-1071. [CrossRef]
59. Meghan B. Azad, Spencer B. Gibson. 2010. Role of BNIP3 in proliferation and hypoxia-induced autophagy: implications for personalized cancer therapies. *Annals of the New York Academy of Sciences* **1210**:1, 8-16. [CrossRef]
60. D Tang, R Kang, C-W Cheh, K M Livesey, X Liang, N E Schapiro, R Benschop, L J Sparvero, A A Amoscato, K J Tracey, H J Zeh, M T Lotze. 2010. HMGB1 release and redox regulates autophagy and apoptosis in cancer cells. *Oncogene* **29**:38, 5299-5310. [CrossRef]
61. Mariarita Perri, Attilio Pingitore, Erika Cione, Emma Vilardi, Valentina Perrone, Giuseppe Genchi. 2010. Proliferative and anti-proliferative effects of retinoic acid at doses similar to endogenous levels in Leydig MLTC-1/R2C/TM-3 cells. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1800**:9, 993-1001. [CrossRef]
62. R. Castino, N. Bellio, C. Follo, D. Murphy, C. Isidoro. 2010. Inhibition of PI3k Class III-Dependent Autophagy Prevents Apoptosis and Necrosis by Oxidative Stress in Dopaminergic Neuroblastoma Cells. *Toxicological Sciences* **117**:1, 152-162. [CrossRef]
63. Cheol-Hee Choi, Yong-Keun Jung, Seon-Hee Oh. 2010. Selective induction of catalase-mediated autophagy by dihydrocapsaicin in lung cell lines. *Free Radical Biology and Medicine* **49**:2, 245-257. [CrossRef]
64. Daniela Belloni, Lorenzo Veschini, Chiara Foglieni, Giacomo Dell'Antonio, Federico Caligaris-Cappio, Marina Ferrarini, Elisabetta Ferrero. 2010. Bortezomib induces autophagic death in proliferating human endothelial cells. *Experimental Cell Research* **316**:6, 1010-1018. [CrossRef]
65. Georg T. Wondrak . 2009. Redox-Directed Cancer Therapeutics: Molecular Mechanisms and Opportunities. *Antioxidants & Redox Signaling* **11**:12, 3013-3069. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
66. Karim Bensaad, Eric C Cheung, Karen H Vousden. 2009. Modulation of intracellular ROS levels by TIGAR controls autophagy. *The EMBO Journal* **28**:19, 3015-3026. [CrossRef]
67. Y Chen, M B Azad, S B Gibson. 2009. Superoxide is the major reactive oxygen species regulating autophagy. *Cell Death and Differentiation* **16**:7, 1040-1052. [CrossRef]
68. Claire M. Payne, Cheray Crowley-Skillicorn, Hana Holubec, Katerina Dvorak, Carol Bernstein, Mary Pat Moyer, Harinder Garewal, Harris Bernstein. 2009. Deoxycholate, an Endogenous Cytotoxin/Genotoxin, Induces the Autophagic Stress-Survival Pathway: Implications for Colon Carcinogenesis. *Journal of Toxicology* **2009**, 1-14. [CrossRef]