The Roles of Therapy-Induced Autophagy and Necrosis in Cancer Treatment

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Abstract Metabolic and therapeutic stresses activate several signal transduction pathways that regulate cell death and cell survival in cancer cells. Although decades of research unraveled the pathways that regulate apoptosis and allowed the development of novel diagnostic and therapeutic modalities in cancer treatment, only recently has the regulation and significance of tumor cell autophagy and necrosis become the focus of investigations. Necrosis is an irreversible inflammatory form of cell death. In contrast, autophagy is a reversible process that can contribute both to tumor cell death and survival. This review describes recent advances in understanding the regulation of autophagy and necrosis and their implications for cancer therapy. Currently available methods to measure autophagy and necrosis are highlighted. The effect of tumor cell autophagy and necrosis on host immunity is explored. Finally, therapeutic approaches that target autophagy and necrosis in cancer are described.

Metabolic and therapeutic stresses can simultaneously provoke a series of adaptive responses and suicide signals in cancer cells. The sum of these programmed adaptation and death signals determines the fate of the cell: cell death or cell survival. *In vitro* studies of cancer cells have allowed investigators to categorize responses to cellular stress by morphologic and biochemical criteria into at least three forms of cell death: apoptosis, autophagy, and necrosis (1). Although apoptosis and necrosis are irreversible forms of cell death, autophagy, the process by which a cell "eats" itself, can lead to cell death or paradoxically allow cells to escape cell death (2). Decades of research have unraveled the molecular underpinnings of apoptosis, paving the way for therapies that target apoptosis machinery, such as Bcl-2 family members (3), as well as apoptosis resistance mechanisms (4). As the catalogue of defects in pathways that control apoptosis in human tumors is expanding, understanding the significance of the alternative stress fates, autophagy, and necrosis has become increasingly important. Although the distinction between apoptosis, autophagy, and necrosis can be difficult to appreciate in the complex microenvironment of human tumors, there are at least three reasons that understanding the differences between these processes is important in the cancer patient: (*a*) critical regulators of these stress fates can serve as targets for novel cancer therapeutics; (*b*) measurements of cell death could serve as surrogate biomarkers for efficacy in cancer clinical trials; and (*c*) apoptosis, autophagy, and necrosis elicit different responses from the host immune system. A better understanding of the regulation, measurement, and immune consequences of autophagy and necrosis could expedite the development of therapeutic strategies that maximize irreversible tumor cell death and long-lasting antitumor immunity. Ultimately, clinical trials will have to determine the significance of targeting cell death and cell survival pathways directly in prolonging the survival of patients (5, 6).

This review will focus on the regulation of autophagy and necrosis, the currently available methods to measure autophagy and necrosis, the effect of tumor cell autophagy and necrosis on host immunity, and, finally, examples of strategies to pharmacologically manipulate autophagy and necrosis for therapeutic benefit.

Autophagy Can Lead to Cell Death or Cell Survival

Autophagy can be separated into macroautophagy, microautophagy (7), and chaperone-mediated autophagy (8). This review will focus on the macroautophagy (hereafter referred to as autophagy) as its importance in cancer is most well understood. Autophagy is a multistep process that involves the vesicular sequestration of long-lived cytoplasmic proteins and organelles such as mitochondria (2). The resulting double-membrane vesicle is called an autophagosome. Autophagosomes fuse with lysosomes, and through the action of acid-dependent enzymes, degradation of autophagosome contents ensues (Fig. 1A).

Autophagy has been observed in cancer cells faced with a variety of metabolic and therapeutic stresses. A brief and incomplete list of perturbations that can induce autophagy includes interruption of growth factor signaling pathways (9), activation of mitogen-activated protein kinase signaling (10),

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Received 6/28/07; revised 9/21/07; accepted 9/27/07.
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doi:10.1158/1078-0432.CCR-07-1595

inhibition of proteasomal degradation (11), the accumulation of intracellular calcium (12), and endoplasmic reticulum stress (13). Although the number of stress-activated pathways linked to autophagy seems to be increasing rapidly, direct biochemical associations between these stress signals and the known mammalian autophagy genes, which direct the complex structural changes associated with autophagy, have not been fully characterized (Fig. 1A). One recent report suggests that the accumulation of reactive oxygen species (ROS), which may be a common result of many of these cellular responses to stress, can directly activate autophagy. Investigators found that accumulated ROS following nutrient withdrawal lead to inactivation of the cysteine protease ATG4, leading to accumulation of the ATG8-phosphoethanolamine precursor that is required for the initiation of autophagosome formation (14). Further characterization of the interface between stress signals and the autophagy machinery may unearth new targets for drug development in cancer.

Once activated, persistent autophagy, which depletes the cell of organelles and critical proteins, can lead to a caspase-independent form of cell death (15). In cells undergoing persistent autophagy, however, hallmarks of apoptosis, such as caspase activation (16), necrotic cell death (discussed later), organellar swelling, and plasma membrane rupture, can often be observed (17). This underscores the difficulty in characterizing the predominant mode of cell death in a population of tumor cells. In addition to transforming into other irreversible modes of cell death, autophagy can be activated in a more transient fashion in cancer cells and can paradoxically contribute to cell survival. Autophagy promotes survival by serving as an intracellular mechanism by which cells dispose of damaged organelles and proteins (18, 19) and recycle macromolecules to maintain bioenergetics (9). Using cell lines deficient in apoptosis, multiple investigators have reported that autophagy activated in response to growth factor withdrawal, hypoxia, or insufficient tumor angiogenesis allowed tumor cells to survive these stresses (9, 20). Genetic depletion of essential autophagy genes in the face of these stresses enhanced cell death. In each of these contexts, autophagy was down-regulated once the stress was removed, providing evidence that, unlike the other stress fates, namely apoptosis, necrosis, or senescence described previously, autophagy is a reversible phenomenon. Current research efforts are focused on identifying critical regulators that control the down-regulation of autophagy because inhibition of these regulators may enhance autophagic cell death and convert a cell survival response into a cell death process.

In an animal model of cancer therapy, inhibition of therapy-induced autophagy either with short hairpin RNA against the autophagy gene *ATG5* or with the antimalarial drug chloroquine enhanced cell death and tumor regression of Myc-driven tumors in which either activated p53 or alkylating chemotherapy was used to drive tumor cell death (21). Although numerous other mechanisms for the antitumor activity of chloroquine have been proposed (22–24), *in vitro* studies at low micromolar doses achievable in patients have shown that chloroquine causes a dose-dependent accumulation of large autophagic vesicles and enhances alkylating therapy–induced cell death to a similar degree as knockdown of *ATG5* (21). These and other studies (25, 26) suggest that autophagy can promote resistance to DNA-damaging therapy. The regulation of damage-induced autophagy may be different than the regulation of autophagy that is activated in response to bioenergetic compromise or inhibition of growth factor signaling. Although changes in gene expression have been associated with both forms of autophagy (27, 28), the demonstration that starvation-induced ROS can directly impair ATG4-dependent inhibition of autophagy raises the possibility that autophagy secondary to bioenergetic compromise can be activated in a more rapid energy-efficient fashion using chronically expressed basal autophagy machinery (14). Because autophagy is a reversible damage response unlike apoptosis and necrosis, it is not surprising that nearly all therapeutic insults currently used in cancer therapy, including cytotoxic chemotherapy (21, 29, 30), radiation (31, 32), kinase inhibitors (33, 34) that disrupt growth factor signaling, or hormonal

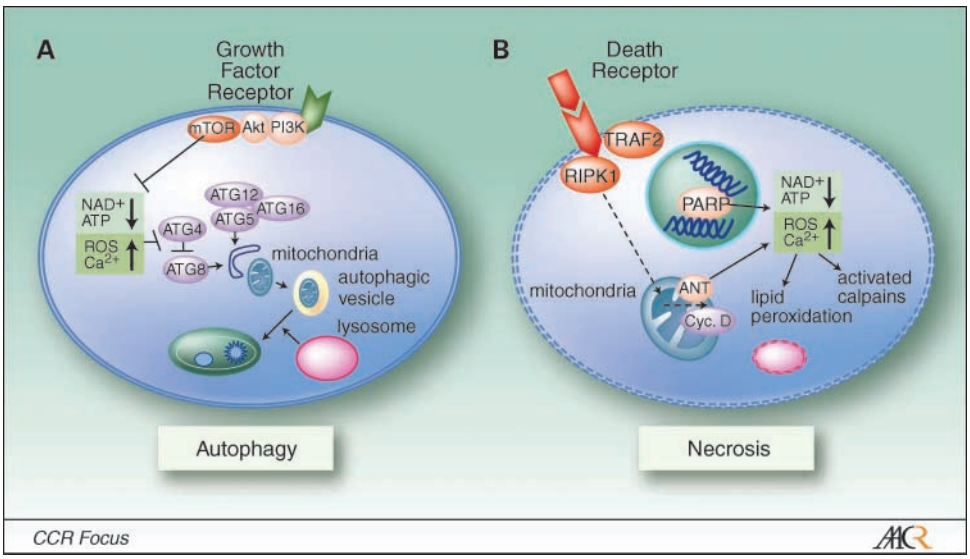

Fig. 1. Regulation of autophagy and necrosis. *A,* autophagy. Multiple stresses, including interrupted growth factor signaling, activate autophagy. Fluctuations in small molecules, such as increased ROS, may be the link between signal transduction events and the enzymatic activation of autophagy genes. Once initiated, autophagic vesicles form around damaged mitochondria and proteins. This double-membrane structure fuses with the lysosome resulting in the degradation of contents. PI3K, phosphatidylinositol 3-kinase; mTOR, mammalian target of rapamycin. *B,* necrosis. Initiators of necrosis include the NAD^+-depleting PARP and the death receptor adaptors RIPK1 and TRAF2. Activated RIPK1 translocates to the mitochondria, disrupting the association of cyclophilin D (*Cyc. D*) and adenosine nucleotide translocator (*ANT*). The subsequent depletion of NAD^+ and ATP and accumulation of calcium and ROS lead to activation of calpain proteases and lipid peroxidation followed by widespread membrane disruption.

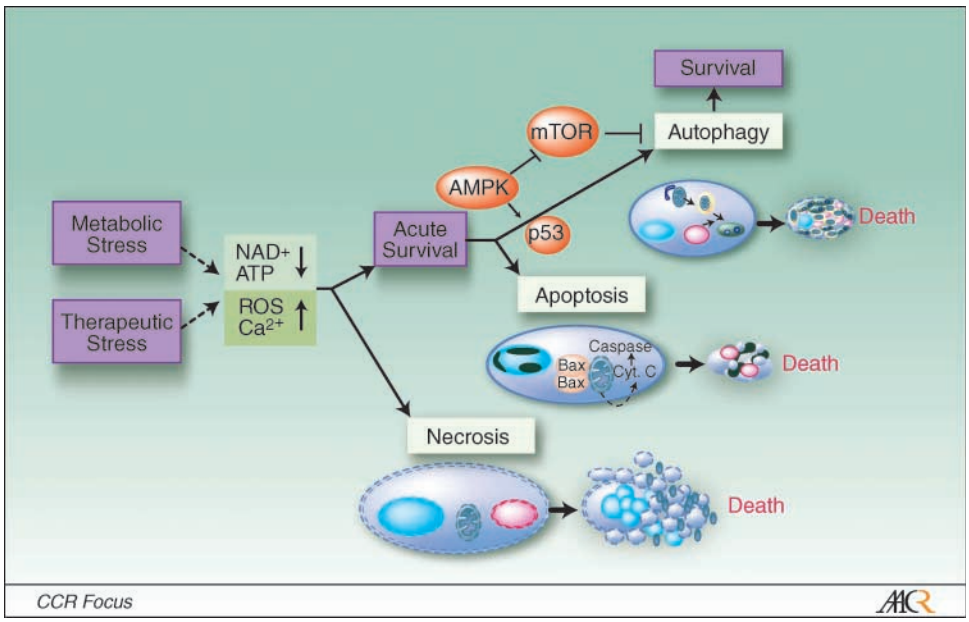

Fig. 2. The relationship between necrosis, autophagy, and apoptosis. Metabolic and therapeutic stresses lead to acute NAD^+ and ATP depletion accompanied by increased intracellular calcium and ROS. Cells that do not adapt to these changes undergo necrotic cell death. The activation of stress regulators, such as AMPK, allows cells to acutely survive these changes. AMPK-dependent phosphorylation results in the inhibition of mammalian target of rapamycin, which inhibits autophagy. AMPK-dependent phosphorylation also activates p53, which can lead to autophagy or apoptosis, through the activation of Bax and Bak, the cytoplasmic release of cytochrome *c* (*Cyt. C*), and the activation of caspases. Unlike apoptosis or necrosis, stress-induced autophagy can lead to autophagic cell death or to cell survival.

therapy (17), can activate this process. Combining autophagy-inducing therapies with autophagy inhibitors is currently being tested preclinically and clinically in several malignancies.

Necrosis Is a Form of Programmed Cell Death

When discussing necrotic cell death, it is important to distinguish tissue necrosis from molecularly defined necrotic cell death. Pathologic tissue necrosis as seen under the microscope can be the end product of apoptosis, autophagic, and/or necrotic cell death. In naturally occurring tumor tissue, necrosis occurs when the death rate exceeds the ability of cells to clear dying cells and is likely to be the summation of all three death processes. In this review, the term necrosis refers to the molecular events that lead to a form of cell death distinct from apoptosis and autophagy.

Necrotic cell death is often referred to as unscheduled cell death, implying that within a multicellular organism it is an unregulated process. The disruption of plasma membrane that is characteristic of necrotic cell death leads to the spillage of intracellular proteins that activates a damage response (see further) from the host immune system (35). This brisk inflammatory response and immune amplification of the damage signal is in sharp contrast to apoptotic cells that are silently removed by tissue macrophages. Thus, necrosis was viewed as strictly a pathologic form of cell death that is not a physiologically programmed process.

Increasing evidence suggests that, much like apoptosis, specific genes have evolved to regulate necrotic cell death (36, 37). It has been difficult to characterize the essential regulators of necrotic cell death in the absence of apoptosis. Much of the literature describing necrotic cell death consists of experiments done in cell lines subjected to death receptor ligation (38–40) or in neurons subjected to ischemia or glutamate excitotoxicity (41, 42). Because these interventions can also activate apoptosis, concomitant treatment of cells with pan-caspase inhibitors pushes cells into necrotic cell death (43). Genetic studies have identified death receptor adaptors, including receptor-interacting protein kinase 1 (RIPK1) and the tumor necrosis factor (TNF) receptor–associated factor 2 (TRAF2), as essential regulator of death receptor–induced necrotic cell death (44–46). Cells deficient in RIPK1 or TRAF2 are protected from necrotic cell death when treated with Fas ligation and caspase inhibitors or hydrogen peroxide alone (47). Activation of RIPK1 leads to its translocation to the inner mitochondrial membrane disrupting the association between the cyclophilin D and adenosine nucleotide translocator, resulting in ATP depletion and the accumulation of ROS (Fig. 1B; ref. 48).

Along with experiments done in the presence of caspase inhibitors, cells deficient in the ability to undergo apoptosis have served as model systems to study necrotic cell death. When cells deficient in the proapoptotic Bcl-2 genes *Bax* and *Bak* are treated with alkylating chemotherapy, an immediate metabolic crisis develops characterized by NAD^+ depletion followed by ATP depletion. A key mediator of this process is poly(ADP-ribose) polymerase (PARP), a nuclear protein whose activation by DNA damage rapidly depletes the cell of NAD^+, the essential cofactor for aerobic glycolysis. This imposes a transient inhibition of glycolysis, and cells dependent on glycolysis for ATP production, such as cancer cells, die by necrosis. This may explain how alkylating chemotherapy is selective for cancer cells even when given at very high doses (49). PARP-dependent necrosis is also associated with TNF receptor–independent activation of RIPK1, TRAF2, and downstream effector c-Jun NH_2-terminal kinase (50). The bioenergetic crisis that occurs with acute NAD^+ and ATP depletion in glycolytic cells is associated with the accumulation of high concentrations of intracellular calcium (51) and ROS (46), which in turn cooperate to activate Ca^{2+}-dependent cytosolic proteases calpains (52). Activated calpains cleave Ca^{2+} extrusion channels (53) and permeabilize lysosomes (54), leaking

executioner cathepsins (55). High intracellular calcium concentrations and ROS also activate phospholipase A2 (56, 57). Proteolysis and lipid peroxidation ensues, leading to widespread membrane permeabilization and irreversible necrotic cell death.

The Relationship between Necrosis, Autophagy, and Apoptosis

Unlike apoptosis, the necrosis that ensues after a metabolic or therapeutic stress results from a bioenergetic compromise that leads to rapid cell death (Fig. 2). Tumor cells can evade this earliest form of therapy-induced cell death by adapting to declining ATP levels through the action of the energy sensor LKB1/AMPK complex (58). The LBK1/AMPK complex has been proposed as the master regulator of autophagy through its ability to inhibit mammalian target of rapamycin signaling (59) and activate the tumor suppressors p27 (60) and p53 (61). One of the target genes that is induced after p53 activation is the *damage-related autophagy modulator*, a lysosomal membrane protein essential for p53-induced autophagy (27). In addition to direct transcription of autophagy-associated proteins, p53 may indirectly activate autophagy through feedback inhibition of mammalian target of rapamycin signaling (62). Often, apoptosis and autophagy after p53 activation can occur in different cells within the same tumor (see Fig. 3A). It is still unclear what factors dictate the predominant stress fate following p53 activation. In one Akt-driven tumor model, nutrient limitation and hypoxia preferentially activated apoptosis (20). In tumor cells genetically deficient in the ability to undergo apoptosis, autophagy was activated and protected the cell from metabolic compromise and death. Autophagy can often be observed in cells undergoing necrosis (Fig. 3B). These findings suggest that, within the same tumor necrosis, autophagy and apoptosis may coexist, and the relative contributions of these three processes can dictate the trajectory of tumor growth or regression and the host response.

Measuring Autophagy and Necrosis

Although markers of apoptosis, such as specific caspase-3 cleavage products, can be measured in the blood of patients receiving cytotoxic chemotherapy and can predict survival in these patients (63), currently there are very few specific methods to detect autophagy or necrotic cell death *in vivo* (Fig. 3C). Electron microscopy provides the most information about tumor cell morphology and has served as a "gold standard" for distinguishing apoptosis from autophagy and necrosis. Although apoptotic cells are characterized by pyknotic nuclei with condensed chromatin, and nuclear and cytoplasmic blebbing, autophagy is characterized by vacuolization of the cytoplasm with intact nuclear and cytoplasmic membranes (Fig. 3A). The number of double-membrane autophagic vesicles per cell and the percentage of cells with an increased number of

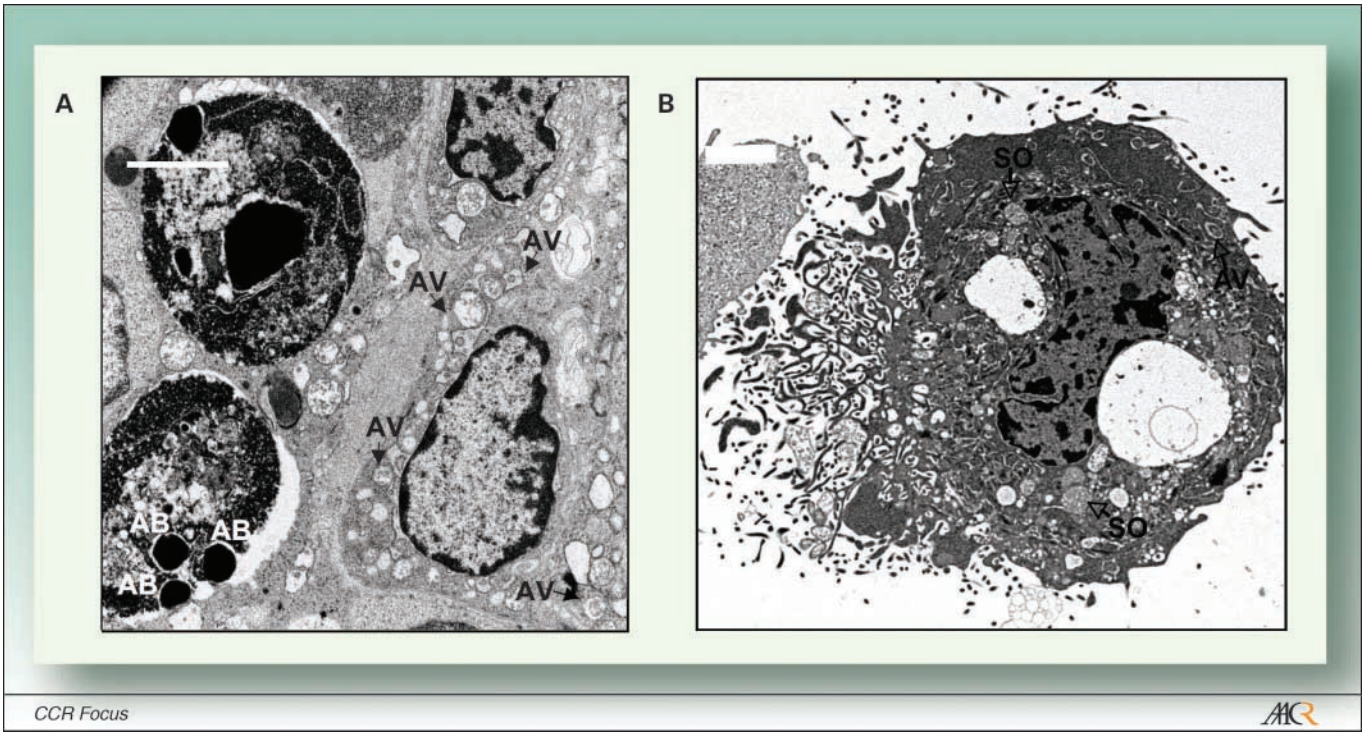

Fig. 3. Distinguishing apoptosis, autophagy, and necrosis. *A,* electron micrograph of lymphoma tissue from a mouse treated with hydroxychloroquine. Left, morphologic changes characteristic of apoptosis, including chromatin condensation and the formation of apoptotic bodies (*AB*), are present in the cells. A neighboring cell (*right*) has an accumulation of autophagic vesicles (*AV*) with intact nuclear and cytoplasmic membranes. *B,* electron micrograph of a steroid-withdrawn MCF-7 breast cancer cell undergoing necrotic cell death. Cytoplasmic membrane rupture is accompanied by partially condensed chromatin and swollen organelles (*SO*). Autophagic vesicles are also present. Bar, 2 μm.

autophagic vesicles can be scored to quantify autophagy by electron microscopy. When this analysis is done by an investigator who has specific experience in the electron microscopic analysis of autophagy, it is a reliable and specific method of characterizing changes in autophagy. This methodology, however, is expensive and laborious. Moreover, the special fixation and processing and small specimen size required for electron microscopic analysis present hurdles to the use of this methodology for clinical samples, such as surgically resected tumors and blind tumor biopsies. Other methods to detect autophagy in cells include the use of weakly basic dyes, such as monodansylcadaverine (64) or acridine orange (65), which accumulate in the acidic autophagosome-lysosome compartment. Quantification of fluorescence can provide an objective measurement of these cellular compartments. Besides difficulty with establishing signal-to-noise ratios and reproducible staining, like electron microscopy, lysosomotropic dyes are not specific for autophagosomes. Currently, the "gold standard" for molecularly defined detection of autophagy is antibody-based detection of the autophagy gene *LC3*. LC3 is constitutively expressed at low levels in most cells (66). LC3 is located diffusely within the cytoplasm. LC3 is cleaved to form LC3-I and LC3-II. LC3-II is localized to the autophagosome membrane and therefore serves as a specific marker of autophagy. Although immunoblotting against the LC3 protein has been used to draw conclusions about autophagy in cells, the dynamic nature of autophagic flux and its effects on LC3 processing can result in misleading conclusions from experimental results. A recent review on the interpretation of LC3 immunoblotting suggests that conclusions about whether an intervention induces or inhibits autophagy can best be drawn by including sample conditions in which the intervention is combined with inhibitors of lysosomal function (67).

In conjunction with LC3 immunoblotting, stable overexpression of the GFP-LC3 fusion protein has facilitated the study of autophagy in cancer cells (68). The GFP-LC3 construct produces diffuse fluorescence in the absence of autophagy and punctuate fluorescence when autophagy is activated. Cleavage of LC3-I and relocalization of LC3-II to the autophagosomal membranes is the most robust test of autophagy available. Although other molecular markers of autophagy are being explored *in vitro*, an assay for autophagy that can be widely applied to clinical samples is needed to move the field forward.

Characterizing necrotic cell death also has many limitations. Electron microscopic morphologic criteria for necrotic cell death are the presence of organellar swelling and a loss of integrity of the cytoplasmic membrane (Fig. 3B; ref. 1). The lack of activation of caspase-3 distinguishes necrotic cell death and autophagy from apoptosis. The leakage of certain measurable intracellular proteins has been a promising approach to define necrotic cell death. Extracellular lactate dehydrogenase has often been used as a marker of necrotic cell death; however, numerous studies have measured lactate dehydrogenase in the medium of apoptotic cells (69). The nonhistone DNA-binding protein high-mobility group B1 (HMGB1) is currently the best candidate for a specific marker of necrotic cell death. HMGB1 is loosely bound to chromatin and binds more tightly to the chromatin of apoptotic cells as it condenses. In contrast, in cells undergoing necrotic cell death, PARP activation leads to the release of HMGB1 from chromatin and export out of the nucleus (70) and eventually to the extracellular space where it acts as an inflammatory cytokine (69). Once plasma membrane integrity is lost, HMGB1 can be measured in the extracellular space. HMGB1 binds to receptors, such as RAGE, on endothelial cells (71) and Toll-like receptors on macrophages (72). Activated macrophages amplify the damage signal by releasing HMGB1 (73) to recruit additional inflammatory cells to the site of necrosis. HMGB1 is detectable in the serum of patients. ELISA assays against HMGB1 in patients with severe sepsis have shown that in these inflammatory conditions increased levels of HMGB1 are prognostic of poorer survival (74). Levels of HMGB1 are higher in ambulatory cancer patients than in patients critically ill with sepsis, but the significance of this finding has not been fully explored. Additional preclinical studies are needed to determine how validated assays of markers for necrotic cell death should best be incorporated into cancer clinical trials.

Host Immune Reaction to Apoptosis, Autophagy, and Necrosis

Whereas the presence of tumor-infiltrating lymphocytes is indicative of improved survival in several malignancies (75, 76), the significance of tumor-associated macrophages is not as well understood. Although macrophages secrete growth factors that promote angiogenesis, tumor cell growth, and proliferation (77), tissue macrophages as professional antigen-presenting cells are a link between innate immunity and adaptive immunity and can contribute directly to tumor cell death through the generation of nitric oxide and ROS.

Apoptotic cells are cleared by both professional (tissue macrophages) and nonprofessional phagocytes (neighboring tumor cells). Numerous molecular signals present on the apoptotic cells that are recognized by phagocytes have been described. The exposure of the membrane phospholipid phosphatidylserine and calreticulin (78), a protein normally localized to the endoplasmic reticulum, on the surface of apoptotic cells has been well characterized. Whereas recognition of phosphatidylserine by phagocytes suppresses the expression of proinflammatory cytokines (79), the recognition of calreticulin may lead to the production of proinflammatory cytokines (80). When measured in bulk populations, the end result of engulfment of apoptotic cells is inhibition of proinflammatory mediators. In most circumstances, apoptosis is therefore an immune-silent form of cell death. In contrast, tamoxifen-treated breast cancer cells that had activated autophagy were still efficiently phagocytosed by professional and nonprofessional macrophages (81). Although both phosphatidylserine and calreticulin were exposed on autophagic cells, engulfment by macrophages occurred in a calreticulin-dependent manner that led to the expression of proinflammatory cytokines. Further work is needed to determine the *in vivo* significance of this finding.

Necrosis is always an inflammatory cell death, in contrast to apoptosis and autophagy, in which the balance of anti-inflammatory and proinflammatory signals dictates the immune outcome. Extracellular HMGB1 can bind to RAGE receptors (82) and Toll-like receptors (83) on macrophages,

Table 1. Characteristics of apoptosis, autophagy, and necrotic cell death

	Apoptosis	Autophagy	Necrotic cell death
Morphology	Chromatin condensation, nuclear and cytoplasmic blebbing, apoptotic bodies	Cytoplasmic vesicles, intact nuclear and cytoplasmic membranes	Swollen organelles, cytoplasmic membrane rupture
Key regulators	Caspases, cytochrome *c*, Bcl-2 family members	Autophagy genes: *beclin, LC3, ATG1, ATG5, ATG7*	RIPK1, TRAF2, PARP, calpains
Measurements	Activated caspase-3, caspase-3 cleavage products, TUNEL, Annexin V	LC3 relocalization, number of autophagic vesicles (EM)	Extracellular HMGB1, S100 family members
Immune reaction	Suppression of inflammation	Inflammatory	Inflammatory

Abbreviations: TUNEL, terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling; EM, electron microscopy.

stimulating the secretion of proinflammatory cytokines (69). The binding of HMGB1 to the RAGE receptor is not sufficient to activate macrophages but instead amplifies the production of proinflammatory cytokines in macrophages activated by other environmental factors, such as DNA-protein complexes (84). The necrosis-induced recruitment of macrophages and the subsequent macrophage-associated production of angiogenic and growth factors may be the explanation of how cancers often arise at the site of chronic inflammation (77), and investigators have suggested that inflammation promoted by tumor necrosis can accelerate tumorigenesis (20). In the context of anticancer therapy aimed at established tumors, however, necrosis-associated damage signals, such as HMGB1, may also be capable of initiating antitumor immunity. HMGB1 is an effective immune adjuvant that promotes the migration and maturation of dendritic cells, clonal selection, and the expansion and survival of naive T cells. *In vivo* studies suggest that immunization with HMGB1 can enhance antitumor immunity against otherwise poorly immunogenic apoptotic tumors (85). The link between necrosis and adaptive immunity may be an explanation for how alkylating chemotherapy can induce long-term remissions and cures in indolent and aggressive lymphomas with well-characterized apoptotic defects.

Manipulating Autophagy and Necrosis for Therapeutic Benefit

Table 1 summarizes the morphologic characteristics, key regulators, measurement techniques, and immune response to apoptosis, autophagy, and necrosis. Because autophagy and necrosis are complex, multicomponent dynamic processes, they present multiple potential targets for therapeutic intervention. The goals of therapy that modulates autophagy or necrosis may be different in the setting of chemoprevention versus the treatment of established tumors. Given that *beclin1* is a haploinsufficient tumor suppressor gene (86), and autophagy limits genomic instability in early tumor formation (87),

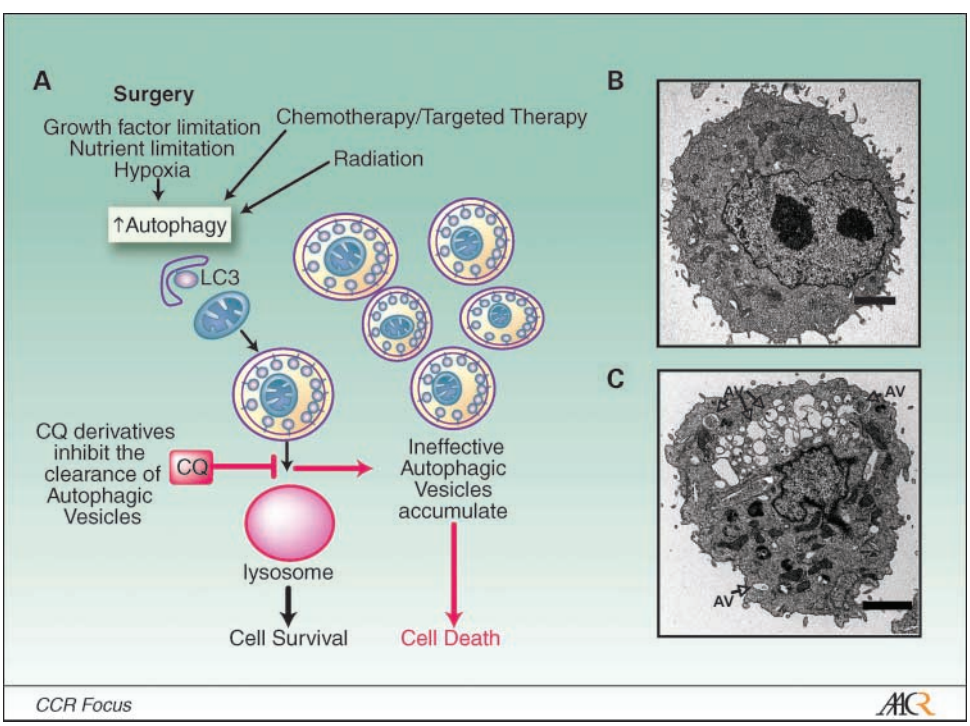

Fig. 4. Inhibiting autophagy for cancer therapy. *A,* surgery, chemotherapy, targeted therapies, and radiation can all activate autophagy. Treatment of cancer cells with chloroquine (*CQ*) derivatives leads to deacidification of lysosomes followed by an accumulation of ineffective autophagic vesicles. In cells dependent on autophagy for survival, autophagy inhibition with chloroquine leads to cell death. *B* and *C,* electron micrographs of PC3 prostate cancer cells either untreated (*B*) or treated with chloroquine (*C*). Bar, 2 μm.

therapies that induce autophagy may limit tumor formation in patients at high risk for cancer. In established tumors faced with metabolic or therapeutic stress, autophagy can promote tumor survival, and therefore, combining apoptosis-inducing therapies with autophagy inhibitors may lead to improved tumor regression and prolonged survival.

As mentioned above, pharmacologic induction of autophagy can be achieved with several drugs commonly used in oncology. In addition, the paradoxically low response rates and prolonged progression-free survival, along with the central liquification of tumors by computed tomography scan that is observed with more novel angiogenesis inhibitors alone and in combination with chemotherapy (83), suggest that autophagy and/or necrosis may be activated in these clinical scenarios as well.

Early clinical trials testing the strategy of eliminating tumor cells using autophagy to survive are already being developed. Currently, few inhibitors of autophagy are well characterized, and most do not have favorable pharmacologic properties. The exception is the antimalarial chloroquine and chloroquine derivatives such as hydroxychloroquine. For more than 60 years since their initial synthesis, these 4-aminoquinolines have been used worldwide for the treatment of malaria and autoimmune diseases such as rheumatoid arthritis. As weak bases, their chemical structures predispose these drugs to accumulation in acidic compartments, such as lysosomes (88). Studies of tissue lysosomes indicate that intralysosomal pH rises and lysosomes swell in animals treated with chloroquine (89). Treatment of a wide variety of cell types with chloroquine derivatives results in a rapid and massive accumulation of autophagic vesicles, presumably because the last step of autophagy is blocked (Fig. 4). Evidence of dysfunctional autophagy in chloroquine-treated cells is the accumulation of larger autophagosomes with increased oxidized waste material lipofuscin in epithelial cancer cells (Fig. 4B) and the cytosolic accumulation of intralysosomal enzymes such as cathepsin D (90).

In *Myc*-driven lymphomas in which tumor regression was initiated with either p53 activation or alkylating chemotherapy, either chloroquine treatment or knockdown of the essential autophagy gene *ATG5* resulted in a similar enhancement of therapy-induced apoptosis. Chloroquine treatment of cells deficient in autophagy did not further enhance cell death, suggesting that, at low micromolar concentrations, the anticancer effects of chloroquine were due to its action as an autophagy inhibitor. In addition to acting as an autophagy inhibitor, chloroquine at different intracellular concentrations and in different cellular contexts may have other antineoplastic effects, including acting as a noncatalytic topoisomerase inhibitor (22), an inhibitor of the drug-detoxifying enzyme NAD(P)H dehydrogenase, quinine 2 (23), and an activator of ATM (24). Whether these are direct effects of chloroquine or phenomenon associated with the increased ROS secondary to the inhibition of autophagy remains to be determined. Based on some of these other reported mechanisms of action, Sotelo et al. (91) conducted a single-institution, double-blind, placebo-controlled randomized phase III trial of carmustine and radiation therapy with or without daily chloroquine for patients with newly diagnosed glioblastoma multiforme. Although this small study did not detect a statistically significant difference in survival, the median survival in patients treated with chloroquine was 24 months compared with 11 months in patients treated with placebo. This trend toward improved overall survival in patients was accompanied by no significant increase in toxicity.

Currently, a phase I/II trial of hydroxychloroquine with radiation therapy and concomitant and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme is being conducted through the New Approaches to Brain Tumor Therapy consortium. Hydroxychloroquine was chosen to pair with cytotoxic therapy because it can be more safely dose escalated than chloroquine. Other phase I/II clinical trials of hydroxychloroquine in combination with an autophagy inducer are currently being developed in several malignancies. Correlative studies measuring pharmacokinetic variables of hydroxychloroquine, changes in autophagy, and the genotyping of genes that regulate autophagy will hopefully elucidate further the connection between chloroquine derivatives and therapy-induced autophagy in patients and will guide the development of further clinical trials of autophagy inhibitors.

Therapeutic enhancement of necrotic cell death to augment anticancer immunity has not been a focus of drug development. In fact, many drugs are entering clinical trials that will predictably inhibit necrotic cell death. PARP inhibitors, such as AG014699, have been developed as chemosensitizers. Because PARP promotes DNA repair, inhibition of PARP to potentiate the effects of DNA-damaging chemotherapy has sound rationale and extensive preclinical evidence of efficacy in xenograft tumors to warrant clinical testing (92). PARP activation by chemotherapy can induce necrotic cell death through the depletion of NAD^+. In contrast, PARP inhibitors in combination with chemotherapy could inhibit necrotic cell death and, simultaneously, through the inhibition of PARP-dependent DNA repair, enhance apoptotic cell death. At least five PARP inhibitors are currently in phase I and II cancer clinical trials. Although tolerable as single agents, PARP inhibitors seem to enhance chemotherapy-induced myelosuppression as expected. Data from a phase II clinical trial of temozolomide with AG014699 found a 10% response rate, similar to previously reported responses for temozolomide alone (93). Additional phase II trial results are awaited to determine if this strategy of enhancing apoptosis potentiates antitumor activity in patients with metastatic cancer. Because PARP inhibitors are well tolerated, necrosis inhibition as a chemopreventative strategy could be tested with PARP inhibitors in models of cancer arising out of chronic inflammation. Because the relationship between acute or chronic inflammation and cancer remains controversial, while necrosis inhibition with PARP inhibitors is being evaluated in preclinical and clinical studies, drugs designed to enhance necrotic cell death should also be developed and evaluated in similar contexts.

Multiple early-phase clinical trials of molecules that act as death receptor ligands have been launched in hopes of inducing caspase-8–dependent apoptosis in tumor cells. Early clinical trials of death receptor ligands were stalled due to hepatotoxicity and lymphocyte depletion (94). Early-phase clinical trials of the death receptor ligand TNF-related apoptosis-inducing ligand have found this generation of death receptor ligands to be better tolerated (95). Phase II trials for

several TNF-related apoptosis-inducing ligand molecules are being conducted. Although it will be important to enroll only patients whose tumors express TNF-related apoptosis-inducing ligand receptor to guide further development of this class of drugs, it may be equally important to measure markers of apoptosis and necrosis in patients to determine the predominant form of cell death that occurs in patients that respond. Finally, the development of pan-caspase inhibitors (96) may open up the possibility of testing the hypothesis that pharmacologic enhancement of necrotic cell death for established tumors can improve clinical outcomes. Preclinical allograft experiments combining pharmacologic caspase inhibitors with chemotherapy or death receptor ligands, with measurements of immune responses, and antitumor activity could be a first step toward testing the strategy of necrosis enhancement for therapeutic benefit.

Conclusion

The fundamental goal of cancer research should continue to be the identification of therapies that induce selective cancer cell death. Understanding the processes that contribute to cell death and survival is critical in reducing morbidity and mortality from cancer. The extensive characterization of the regulation of apoptosis has allowed the development of targeted therapies designed to induce apoptotic cell death and biomarkers that can measure therapy-induced apoptosis in patients. Given the wide variety of genetic and epigenetic defects that can suppress apoptosis in most cancers, understanding the regulation and significance of nonapoptotic consequences of cancer therapy is important. Autophagy and necrosis are two nonapoptotic consequences of cancer therapy that may have overlapping but separable regulatory networks. A common theme in both autophagy and necrosis is that perturbations in small molecules, such as ATP, NAD^+, ROS, and calcium link stress-activated signal transduction pathways, to specific regulators that give rise to the morphologic changes that are characteristic for each phenotype. Although apoptosis is largely immune silent or immunosuppressive, therapy-induced necrotic cell death initiates an immune response to tumor cells. Whether the inflammation associated with necrosis is desirable or leads to further tumor growth is still under debate. Therapy-induced autophagy, unlike apoptosis or necrosis, can lead to cell survival and therefore has been identified as a target for drug inhibition. Clinical trials of chloroquine derivatives in combination with therapies that induce autophagy and the preclinical identification of more specific autophagy inhibitors are under way. Therapeutics that target regulators of necrotic cell death are already in early-phase clinical trials. Validated markers of autophagy and necrosis are not yet in clinical use, limiting the rational design of clinical trials testing agents that might be targeted to these processes.

References

1. Kroemer G, El-Deiry WS, Golstein P, et al. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. Cell Death Differ 2005; 12 Suppl 2:1463–7.
2. Lum JJ, DeBerardinis RJ, Thompson CB. Autophagy in metazoans: cell survival in the land of plenty. Nat Rev Mol Cell Biol 2005;6:439–48.
3. Danial NN. BCL-2 family proteins: critical checkpoints of apoptotic cell death. Clin Cancer Res 2007; 13:7254–63.
4. Verdine GL, Walensky LD. The challenge of drugging undruggable targets in cancer: lessons learned from targeting BCL-2 family members. Clin Cancer Res 2007;13:7264–70.
5. Benz EJ, Jr., Nathan DG, Amaravadi RK, Danial NN. Targeting the cell death-survival equation. Clin Cancer Res 2007;13:7250–3.
6. Rixe O, Fojo T. Is cell death a critical endpoint for anticancer therapies or is cytostasis sufficient? Clin Cancer Res 2007;13:7280–8.
7. Uttenweiler A, Schwarz H, Neumann H, Mayer A. The vacuolar transporter chaperone (VTC) complex is required for microautophagy. Mol Biol Cell 2007;18: 166–75.
8. Massey AC, Zhang C, Cuervo AM. Chaperone-mediated autophagy in aging and disease. Curr Top Dev Biol 2006;73:205–35.
9. Lum JJ, Bauer DE, Kong M, et al. Growth factor regulation of autophagy and cell survival in the absence of apoptosis. Cell 2005;120:237–48.
10. Corcelle E, Nebout M, Bekri S, et al. Disruption of autophagy at the maturation step by the carcinogen lindane is associated with the sustained mitogen-activated protein kinase/extracellular signal-regulated kinase activity. Cancer Res 2006;66:6861–70.
11. Pandey UB, Nie Z, Batlevi Y, et al. HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. Nature 2007;447: 860–4.
12. Hoyer-Hansen M, Bastholm L, Szyniarowski P, et al. Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-β, and Bcl-2. Mol Cell 2007;25:193–205.
13. Ogata M, Hino S, Saito A, et al. Autophagy is activated for cell survival after endoplasmic reticulum stress. Mol Cell Biol 2006;26:9220–31.
14. Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L, Elazar Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. EMBO J 2007;26:1749–60.
15. Yu L, Alva A, Su H, et al. Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. Science 2004;304:1500–2.
16. Gonzalez-Polo RA, Boya P, Pauleau AL, et al. The apoptosis/autophagy paradox: autophagic vacuolization before apoptotic death. J Cell Sci 2005;118: 3091–102.
17. Bursch W, Ellinger A, Kienzl H, et al. Active cell death induced by the anti-estrogens tamoxifen and ICI 164 384 in human mammary carcinoma cells (MCF-7) in culture: the role of autophagy. Carcinogenesis 1996; 17:1595–607.
18. Gu Y, Wang C, Cohen A. Effect of IGF-1 on the balance between autophagy of dysfunctional mitochondria and apoptosis. FEBS Lett 2004;577:357–60.
19. Iwata JI, Ezaki J, Komatsu M, et al. Excess peroxisomes are degraded by autophagic machinery in mammals. J Biol Chem 2006;281:4035–41.
20. Degenhardt K, Mathew R, Beaudoin B, et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 2006;10:51–64.
21. Amaravadi RK, Yu D, Lum JJ, et al. Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. J Clin Invest 2007;117: 326–36.
22. Langer SW, Schmidt G, Sorensen M, Sehested M, Jensen PB. Inhibitors of topoisomerase II as pH-dependent modulators of etoposide-mediated cytotoxicity. Clin Cancer Res 1999;5:2899–907.
23. Kwiek JJ, Haystead TA, Rudolph J. Kinetic mechanism of quinone oxidoreductase 2 and its inhibition by the antimalarial quinolines. Biochemistry 2004;43: 4538–47.
24. Schneider JG, Finck BN, Ren J, et al. ATM-dependent suppression of stress signaling reduces vascular disease in metabolic syndrome. Cell Metabol 2006;4: 377–89.
25. Katayama M, Kawaguchi T, Berger MS, Pieper RO. DNA damaging agent-induced autophagy produces a cytoprotective adenosine triphosphate surge in malignant glioma cells. Cell Death Differ 2007;14:548–58.
26. Abedin MJ, Wang D, McDonnell MA, Lehmann U, Kelekar A. Autophagy delays apoptotic death in breast cancer cells following DNA damage. Cell Death Differ 2007;14:500–10.
27. Crighton D, Wilkinson S, O'Prey J, et al. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. Cell 2006;126:121–34.
28. Rose TL, Bonneau L, Der C, Marty-Mazars D, Marty F. Starvation-induced expression of autophagy-related genes in Arabidopsis. Biol Cell 2006;98:53–67.
29. Kanzawa T, Germano IM, Komata T, Ito H, Kondo Y, Kondo S. Role of autophagy in temozolomide-induced cytotoxicity for malignant glioma cells. Cell Death Differ 2004;11:448–57.
30. Rez G, Csak J, Fellinger E, et al. Time course of vinblastine-induced autophagocytosis and changes in the endoplasmic reticulum in murine pancreatic acinar cells: a morphometric and biochemical study. Eur J Cell Biol 1996;71:341–50.
31. Paglin S, Hollister T, Delohery T, et al. A novel response of cancer cells to radiation involves autophagy and formation of acidic vesicles. Cancer Res 2001;61: 439–44.
32. Yao KC, Komata T, Kondo Y, Kanzawa T, Kondo S, Germano IM. Molecular response of human glioblastoma multiforme cells to ionizing radiation: cell cycle arrest, modulation of the expression of cyclin-dependent kinase inhibitors, and autophagy. J Neurosurg 2003;98:378–84.

33. Ertmer A, Huber V, Gilch S, et al. The anticancer drug imatinib induces cellular autophagy. Leukemia 2007; 21:936–42.

34. Takeuchi H, Kanzawa T, Kondo Y, Kondo S. Inhibition of platelet-derived growth factor signalling induces autophagy in malignant glioma cells. Br J Cancer 2004;90:1069–75.

35. Zeh HJ III, Lotze MT. Addicted to death: invasive cancer and the immune response to unscheduled cell death. J Immunother (1997) 2005;28:1–9.

36. Golstein P, Kroemer G. Cell death by necrosis: towards a molecular definition. Trends Biochem Sci 2007;32:37–43.

37. Festjens N, Vanden Berghe T, Vandenabeele P. Necrosis, a well-orchestrated form of cell demise: signalling cascades, important mediators and concomitant immune response. Biochim Biophys Acta 2006;1757: 1371–87.

38. Eguchi Y, Shimizu S, Tsujimoto Y. Intracellular ATP levels determine cell death fate by apoptosis or necrosis. Cancer Res 1997;57:1835–40.

39. Goossens V, Grooten J, De Vos K, Fiers W. Direct evidence for tumor necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. Proc Natl Acad Sci U S A 1995; 92:8115–9.

40. Boone E, Vanden Berghe T, Van Loo G, et al. Structure/function analysis of p55 tumor necrosis factor receptor and fas-associated death domain. Effect on necrosis in L929sA cells. J Biol Chem 2000;275: 37596–603.

41. Ankarcrona M, Dypbukt JM, Bonfoco E, et al. Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. Neuron 1995;15:961–73.

42. Zhu C, Wang X, Xu F, et al. The influence of age on apoptotic and other mechanisms of cell death after cerebral hypoxia-ischemia. Cell Death Differ 2005;12: 162–76.

43. Vercammen D, Beyaert R, Denecker G, et al. Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by tumor necrosis factor. J Exp Med 1998;187:1477–85.

44. Holler N, Zaru R, Micheau O, et al. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol 2000;1:489–95.

45. Chan FK, Shisler J, Bixby JG, et al. A role for tumor necrosis factor receptor-2 and receptor-interacting protein in programmed necrosis and antiviral responses. J Biol Chem 2003;278:51613–21.

46. Lin Y, Choksi S, Shen HM, et al. Tumor necrosis factor-induced nonapoptotic cell death requires receptor-interacting protein-mediated cellular reactive oxygen species accumulation. J Biol Chem 2004;279: 10822–8.

47. Shen HM, Lin Y, Choksi S, et al. Essential roles of receptor-interacting protein and TRAF2 in oxidative stress-induced cell death. Mol Cell Biol 2004;24: 5914–22.

48. Temkin V, Huang Q, Liu H, Osada H, Pope RM. Inhibition of ADP/ATP exchange in receptor-interacting protein-mediated necrosis. Mol Cell Biol 2006;26: 2215–25.

49. Zong WX, Ditsworth D, Bauer DE, Wang ZQ, Thompson CB. Alkylating DNA damage stimulates a regulated form of necrotic cell death. Genes Dev 2004;18:1272–82.

50. Xu Y, Huang S, Liu ZG, Han J. Poly(ADP-ribose) polymerase-1 signaling to mitochondria in necrotic cell death requires RIP1/TRAF2-mediated JNK1 activation. J Biol Chem 2006;281:8788–95.

51. Xu K, Tavernarakis N, Driscoll M. Necrotic cell death in *C. elegans* requires the function of calreticulin and regulators of Ca(2+) release from the endoplasmic reticulum. Neuron 2001;31:957–71.

52. Syntichaki P, Xu K, Driscoll M, Tavernarakis N. Specific aspartyl and calpain proteases are required for neurodegeneration in *C. elegans*. Nature 2002;419: 939–44.

53. Bano D, Young KW, Guerin CJ, et al. Cleavage of the plasma membrane Na^+/Ca^{2+} exchanger in excitotoxicity. Cell 2005;120:275–85.

54. Yamashima T, Tonchev AB, Tsukada T, et al. Sustained calpain activation associated with lysosomal rupture executes necrosis of the postischemic CA1 neurons in primates. Hippocampus 2003;13:791–800.

55. Yamashima T, Kohda Y, Tsuchiya K, et al. Inhibition of ischaemic hippocampal neuronal death in primates with cathepsin B inhibitor CA-074: a novel strategy for neuroprotection based on 'calpain-cathepsin hypothesis'. Eur J Neurosci 1998;10:1723–33.

56. El Mahdani NE, Ameyar M, Cai Z, Colard O, Masliah J, Chouaib S. Resistance to TNF-induced cytotoxicity correlates with an abnormal cleavage of cytosolic phospholipase A2. J Immunol 2000;165:6756–61.

57. Hayakawa M, Ishida N, Takeuchi K, et al. Arachidonic acid-selective cytosolic phospholipase A2 is crucial in the cytotoxic action of tumor necrosis factor. J Biol Chem 1993;268:11290–5.

58. Buzzai M, Bauer DE, Jones RG, et al. The glucose dependence of Akt-transformed cells can be reversed by pharmacologic activation of fatty acid β-oxidation. Oncogene 2005;24:4165–73.

59. Hoyer-Hansen M, Jaattela M. AMP-activated protein kinase: a universal regulator of autophagy? Autophagy 2007;3:381–3.

60. Liang J, Shao SH, Xu ZX, et al. The energy sensing LKB1-AMPK pathway regulates p27(kip1) phosphorylation mediating the decision to enter autophagy or apoptosis. Nat Cell Biol 2007;9:218–24.

61. Jones RG, Plas DR, Kubek S, et al. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Mol Cell 2005;18:283–93.

62. Feng Z, Zhang H, Levine AJ, Jin S. The coordinate regulation of the p53 and mTOR pathways in cells. Proc Natl Acad Sci U S A 2005;102:8204–9.

63. Olofsson MH, Ueno T, Pan Y, et al. Cytokeratin-18 is a useful serum biomarker for early determination of response of breast carcinomas to chemotherapy. Clin Cancer Res 2007;13:3198–206.

64. Munafo DB, Colombo MI. A novel assay to study autophagy: regulation of autophagosome vacuole size by amino acid deprivation. J Cell Sci 2001;114:3619–29.

65. Ollinger K, Roberg K. Nutrient deprivation of cultured rat hepatocytes increases the desferrioxamine-available iron pool and augments the sensitivity to hydrogen peroxide. J Biol Chem 1997;272:23707–11.

66. Tanida I, Ueno T, Kominami E. LC3 conjugation system in mammalian autophagy. Int J Biochem Cell Biol 2004;36:2503–18.

67. Mizushima N, Yoshimori T. How to interpret LC3 immunoblotting. Autophagy 2007;3:e1–4.

68. Mizushima N. Methods for monitoring autophagy. Int J Biochem Cell Biol 2004;36:2491–502.

69. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 2002;418:191–5.

70. Ditsworth D, Zong WX, Thompson CB. Activation of poly(ADP)-ribose polymerase (PARP-1) induces release of the pro-inflammatory mediator HMGB1 from the nucleus. J Biol Chem 2007;282:17845–54.

71. Fiuza C, Bustin M, Talwar S, et al. Inflammation-promoting activity of HMGB1 on human microvascular endothelial cells. Blood 2003;101:2652–60.

72. Yu M, Wang H, Ding A, et al. HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. Shock 2006;26: 174–9.

73. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999; 285:248–51.

74. Sunden-Cullberg J, Norrby-Teglund A, Rouhiainen A, et al. Persistent elevation of high mobility group box-1 protein (HMGB1) in patients with severe sepsis and septic shock. Crit Care Med 2005;33:564–73.

75. Sharma P, Shen Y, Wen S, et al. CD8 tumor-infiltrating lymphocytes are predictive of survival in muscle-invasive urothelial carcinoma. Proc Natl Acad Sci U S A 2007;104:3967–72.

76. Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 2003;348:203–13.

77. Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. Nat Rev Immunol 2004;4:641–8.

78. Gardai SJ, McPhillips KA, Frasch SC, et al. Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. Cell 2005;123:321–34.

79. Savill J, Dransfield I, Gregory C, Haslett C. A blast from the past: clearance of apoptotic cells regulates immune responses. Nat Rev Immunol 2002;2:965–75.

80. Henson PM, Hume DA. Apoptotic cell removal in development and tissue homeostasis. Trends Immunol 2006;27:244–50.

81. Petrovski G, Zahuczky G, Katona K, et al. Clearance of dying autophagic cells of different origin by professional and non-professional phagocytes. Cell Death Differ 2007;14:1117–28.

82. Dumitriu IE, Baruah P, Valentinis B, et al. Release of high mobility group box 1 by dendritic cells controls T cell activation via the receptor for advanced glycation end products. J Immunol 2005;174:7506–15.

83. Park JS, Svetkauskaite D, He Q, et al. Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. J Biol Chem 2004; 279:7370–7.

84. Tian J, Avalos AM, Mao SY, et al. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. Nat Immunol 2007;8:487–96.

85. Rovere-Querini P, Capobianco A, Scaffidi P, et al. HMGB1 is an endogenous immune adjuvant released by necrotic cells. EMBO Rep 2004;5:825–30.

86. Qu X, Yu J, Bhagat G, et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest 2003;112:1809–20.

87. Mathew R, Kongara S, Beaudoin B, et al. Autophagy suppresses tumor progression by limiting chromosomal instability. Genes Dev 2007;21:1367–81.

88. O'Neill PM, Bray PG, Hawley SR, Ward SA, Park BK. 4-Aminoquinolines—past, present, and future: a chemical perspective. Pharmacol Ther 1998;77:29–58.

89. Poole B, Ohkuma S. Effect of weak bases on the intralysosomal pH in mouse peritoneal macrophages. J Cell Biol 1981;90:665–9.

90. Carew JS, Nawrocki ST, Kahue CN, et al. Targeting autophagy augments the anticancer activity of the histone deacetylase inhibitor SAHA to overcome Bcr-Abl-mediated drug resistance. Blood 2007;110:313–22.

91. Sotelo J, Briceno E, Lopez-Gonzalez MA. Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial. Ann Intern Med 2006;144:337–43.

92. Ratnam K, Low JA. Current development of clinical inhibitors of poly(ADP-ribose) polymerase in oncology. Clin Cancer Res 2007;13:1383–8.

93. Plummer R, Lorigan P, Evans J, et al. First and final report of a phase II study of the poly(ADP-ribose) polymerase (PARP) inhibitor, AG014699, in combination with temozolomide (TMZ) in patients with metastatic malignant melanoma (MM) [abstract 8013]. Proc Am Soc Clin Oncol 2006;24:18S.

94. Gajewski TF. On the TRAIL toward death receptor-based cancer therapeutics. J Clin Oncol 2007;25: 1305–7.

95. Tolcher AW, Mita M, Meropol NJ, et al. Phase I pharmacokinetic and biologic correlative study of mapatumumab, a fully human monoclonal antibody with agonist activity to tumor necrosis factor-related apoptosis-inducing ligand receptor-1. J Clin Oncol 2007; 25:1390–5.

96. Baskin-Bey ES, Washburn K, Feng S, et al. Clinical trial of the pan-caspase inhibitor, IDN-6556, in human liver preservation injury. Am J Transplant 2007;7:218–25.