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

Ravi K. Amaravadi and Craig B. Thompson

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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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), organelar 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...
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 regulators 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 NH2-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 Ca2+-dependent cytosolic proteases calpains (52). Activated calpains cleave Ca2+-extrusion channels (53) and permeabilize lysosomes (54), leaking calcium and ROS into the cytoplasm.
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 LKB1/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...
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, lysosomal weakly basic 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 autophagosome 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 organelar 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,
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).

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

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

**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 liquefication 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 signficant 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+ (93). 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 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


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