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Review

Cell death

A dynamic response concept

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Autophagy, apoptosis and necrosis have previously been described as distinct static processes that induce and execute cell death. Due to an increased use of novel techniques in mapping cellular death—techniques which allow for reporting of real-time data—the existence of "grey zones" between cell death modes and the existence of the "point of no return" within these have been revealed. This revelation demands the integration of new concepts in describing the cellular death process. Furthermore, since the contribution of autophagy in cell death or cell survival is still poorly understood, it is important to accurately describe its function within the dynamic framework of cell death.

In this review cell death is viewed as a dynamic and integrative cellular response to ensure the highest likelihood of self-preservation. Suggestions are offered for conceptualizing cell death modes and their morphological features, both individually and in relation to one another. It addresses the need for distinguishing between dying cells and dead cells so as to better locate and control the onset of cell death. Most importantly, the fundamental role of autophagy, autophagic flux, and the effects of the intracellular metabolic environment on the kinetics of the cell death modes are stressed. It also contextualizes the kinetic dimension of cell death as a process and aims to contribute towards a better understanding of autophagy as a key mechanism within this process. Understanding the dynamic nature of the cell death process and autophagy's central role can reveal new insight for therapeutic intervention in preventing cell death.

Introduction

The accurate distinction between the modes of cell death is crucial because of their association with cell loss in human pathologies.[1] The recent advances in reporting real-time data of dying cells suggest that cell death is a much more dynamic and molecularly overlapping event than previously described.[2] Currently there is a strong argument against a rigid classification of cell death mechanisms[3,4] which is supported by recent recommendations to exercise cautious use of cell death terminologies.[5,6] This motivates the current challenge of re-defining and re-describing cell death modes and may necessitate a different more dynamic contextualization of known cell death parameters.[7] Moreover, limited knowledge is available on the effects of autophagy and autophagic flux on the dynamics of apoptosis and necrosis onset within the intracellular metabolic environment, as well as the role of autophagically generated adenosine triphosphate (ATP) in this regard.

The purpose of this review is to address these challenges by highlighting the effects of autophagy and discussing contributing parameters for the onset of cell death. It therefore aims to first highlight the dynamic nature *within* each cell death mode by including the concept, meaning and significance of a "point-of-no-return" (hereafter PONR). Secondly, it aims to clarify the dynamics *between* the cell death modes by discussing their "grey zones" and molecular overlap. Finally, it aims to discuss the possibility of autophagy and its endogenous regulators of affecting the localization of the PONR and thus impacting on the onset of the cell death modes. In describing cell death as a dynamic response concept, it aims to conceptualize autophagy as a literally basal mechanism within the cellular stress response and cell death machinery. The goal is therefore to take a critical look at some important results supporting this dynamic view and to show that a metabolically stressed cell may only be salvageable, if it is identified as such, namely as dying but not dead. A conceptual model is proposed for understanding the dynamic interrelationship between autophagy, apoptosis and necrosis, by taking kinetic parameters such as autophagic capacity, its response time and the PONR into account.

It is hoped that this review contributes to a better understanding of the significance of cellular autophagic flux in context with the onset of apoptosis and necrosis. This may shed new light on the role of autophagy within the cell death-survival equation and its potential for affecting the window of opportunity for therapeutical interventions. Lastly, it aims to contribute towards facilitating communication amongst authors by addressing cell death definitions and processes and by stressing the importance of describing

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kinetic cell death parameters in context with the cell's autophagic and metabolic characteristics.

Dynamics within Modes of Cell Death

The recognition of the extensive crosstalk between different cell death pathways begins to provide insights into the complexity of the cell death decision making process. It becomes clear that cell death with autophagy, apoptosis and necrosis molecularly interdigitates on various levels and thus constructs a dynamic response. Its outcome may depend on a cell-specific net result of signals, which is governed by the type of insult, its duration, and the intracellular metabolic capacity to maintain a favorableleable cellular environment.[2,8,9] This suggests the need for consideration of stress, time and response kinetics and demands precise use of multiple detection methods[10] as well as a clear terminology when reporting measured parameters.[5,6]

Cell death morphology. Three main morphologies of cell death are described. Type I: apoptotic cell death,[11] which is characterized by cell shrinkage and chromatin condensation. Type II: cell death with autophagy,[12,13] which presents a morphology with intracellular accumulation of autophagic vacuoles. Type III: necrosis, characterized by cellular swelling and rapid loss of cellular membrane integrity.[14]

Although the morphological description of autophagy is long established,[12,13] its physiological relevance of maintaining cellular homeostasis and of influencing cell survival has only recently become clear. Three types of autophagy are known: (i) chaperone-mediated autophagy (CMA), (ii) microautophagy and (iii) macroautophagy.[15] CMA involves selective motif tagged protein translocation directly through the lysosomal membrane,[16] microautophagy involves the trapping and engulfing of cytosolic regions by lysosomes, and macroautophagy is characterized by the formation and accumulation of double membrane intermediate vesicles.[17] Most importantly, macroautophagy is the primary mechanism for cytoplasm-to-lysosome delivery and is commonly termed autophagy. Autophagy is a conserved catabolic process for long-lived proteins and organelles[18] and primarily responsible for nonspecific degradation of redundant or faulty cell components.[19] To preserve normal cellular function, a fast and responsive degradation system for irreversibly damaged proteins is essential (reviewed in refs. 20–23). This occurs as part of the cell's daily activities in response to metabolic or hypoxic stress and starvation, which also determines the flux through the autophagic pathway. It not only governs the baseline turnover of intracellular proteins and organelles,[24] but also drives the generation of amino acids under nutrient-poor conditions. The generated amino acids are recycled and provide an additional energy source for intermediary metabolism, ATP generation and biosynthetic pathways.

Apoptosis is morphologically characterized by cell shrinkage,[11] cytoplasmic condensation followed by nuclear pyknosis, and karyorhexis. At an early stage, condensed chromatin tends to marginate in semicircular structures around the nuclear envelope. The plasma membrane of the cell begins to bleb, which will lead to apoptotic bodies, if phagocytes are absent.[25] Finely tuned interplay exists between apoptosis and autophagy.[26]

Necrosis is a degenerative process where cellular integrity is completely lost, which leads to cell death[23] characterized by cytoplasmic swelling, irreversible plasma membrane damage and irreversible changes in the nucleus such as pyknosis, karyorhexis and karyolysis.[5,14] Necrosis has been described as a consequence of physical and chemical stress, which is passive and accidental. However, recent literature argues that necrosis is a result of a well-orchestrated interplay of signalling cascades,[27] with an extensive crosstalk between biochemical and molecular events.[28,29] Furthermore, some signalling molecules, which play a role in autophagy and apoptosis, also take part in this interplay.[30]

A cell may show morphological or molecular features characteristic of autophagy, apoptosis or necrosis without having *completely* lost membrane integrity, having undergone *complete* nuclear fragmentation or having been engulfed by a neighboring cell.[6] In other words, the cell is in the process of dying but still alive, as the membrane disintegration or nuclear fragmentation or engulfment is not yet complete. This bears the crucial possibility of cells being salvageable after the onset of dying. This view necessitates the recognition that cell death is induced as opposed to executed.

Significance of the point-of-no-return (PONR) in autophagic, apoptotic and necrotic cell death. Defining the PONR. Although the existence of the point-of-no-return (PONR) is still controversial, unraveling the identity of a restriction point for cell death could be highly significant, as it could lead to a better understanding of the time point when a cell is still salvageable. The PONR enables the distinction of cell death induction from cell death execution; of cell death as a process from cell death as endpoint and thus the distinction between a living and an already dead cell. A better understanding of locating and characterizing the PONR may introduce novel interventions to modulate its position, so as to increase the chance of reversibility for the benefit of cell survival. Similar to the need for cautious use of nomenclature for cell death, a clear report on parameters and a combination of techniques are needed to indicate the position of the PONR. Mitochondrial membrane permeabilization has been considered as the PONR in many models of programmed cell death.[31-33] Other parameters have also been indicated such as: caspase activation[34,35] governed by Bcl-2 family proteins, a point beyond which interference with caspase cannot salvage the cell[36] and the dissipation of mitochondrial transmembrane potential $\Delta\Psi_m$[33] or cytochrome *c* interactions.[37]

However, only a dynamic perspective on the position of the PONR can reveal its significance. The PONR indicates that the cellular stress response is highly dynamic and characterized by organellar population kinetics,[38] permeability kinetics, release kinetics, diffusion and transport kinetics. These in turn will determine accumulation kinetics, thresholds, degradation capacity and turnover kinetics. This suggests that not only apoptosis, but necrosis and cell death with autophagy may also be delineated by the cell's PONR. As the cell death process is not static, but rather a net result of regulatory networks, the PONR is unlikely to be restricted in position. A common theme of these regulatory networks is a perturbation in ATP and mitochondrial function, linking stress signal transduction pathways to regulators

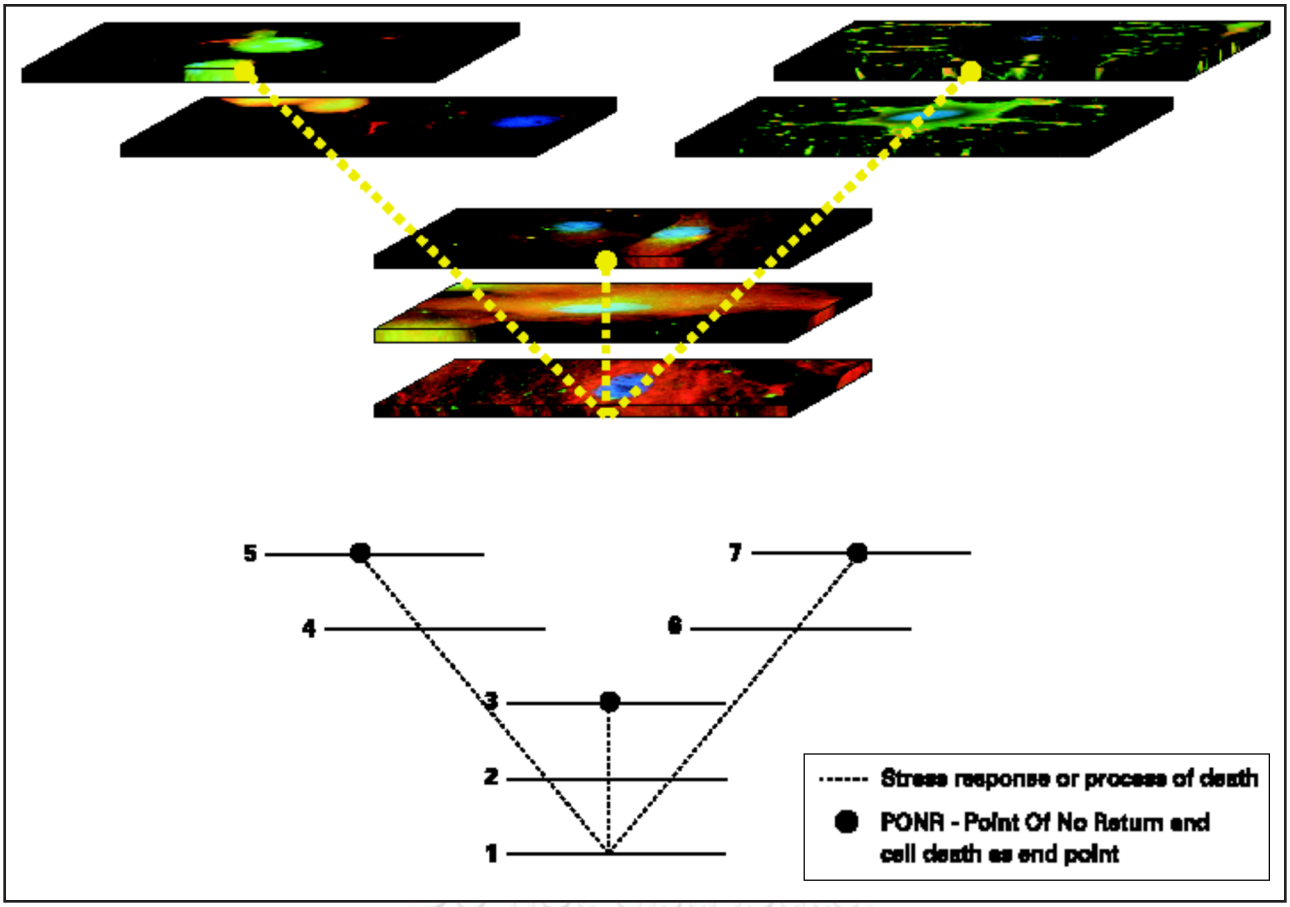

Figure 1. Hypothetical model of the cell's capacity to respond to a defined insult, indicating the typical morphological features of different types of cell death: 'autophagy baseline' 1, 'autophagy induced' 2, 'cell death with autophagy' 3, 'apoptosis induced' 4, 'apoptosis executed' 5, 'necrosis induced' 6 and 'necrosis executed' 7. Cells are counterstained for light chain 3 (LC3) (green), actin (red) and nuclei (blue). The manifestation and overlap of the above morphological features are dependent on the severity of the insult, its duration and the metabolic state of the cell.

which control characteristic[39] and often progressively developing[40] morphological changes. Control of the PONR position through the cell's metabolic efforts would ensure the highest likelihood of cellular preservation.

Conceptualizing the PONR. A conceptual model which considers kinetics, for understanding the dynamic interrelationship between autophagy, apoptosis and necrosis is therefore proposed. According to this model, characteristic morphological features for each cell death mode are layered in the z-dimension, where the z-distance between them indicates the process and endpoint of dying, separated by the PONR. The features (Fig. 1) are titled: (1) "autophagy baseline," which indicates a viable cell with basal autophagic flux, (2) "autophagy induced" indicating increased autophagic flux beyond basal levels, (3) "cell death with autophagy," indicating excessive autophagy, (4) "apoptosis induced," indicating the onset of the apoptotic cascade, (5) "apoptosis executed" indicating the endpoint of apoptotic cell death, (6) "necrosis induced," indicating the onset of a leaky membrane and (7) "necrosis executed," indicating complete loss of membrane integrity. In experimentally controlled conditions, a "pure" morphology and type of cell death may often be described. As a basal level of autophagic flux is inherent to all eukaryotic cells with a lysosomal system,[21] feature 1 is indicated in any given stress response and mode of cell death. Cell death with autophagy is thus represented by features 1, 2 and 3, apoptotic cell death by features 1, 4 and 5, and necrotic cell death by features 1, 6 and 7 (Fig. 3). However, an increase in severity of cellular insult is likely to decrease the time between cell death induction and execution,[9] and may hence shift the PONR in a position, which is reached earlier (Fig. 4). In this instance, the distance in z between 2 and 3, 4 and 5 as well as 6 and 7 decreases respectively and the endpoint of cell death is reached sooner.

Assessing the PONR. When endogenous regulators that control the intracellular metabolic environment and cell death,[1,5,10,14] are examined according to this model, their role in the time process of cell death may be indicated more clearly. Any control condition can thus be described as stage 1 with a cell- and tissue-specific basal autophagic flux, and the sum of all kinetic parameters can indicate the cell's or tissue's position (stages 2–7) within this dynamic model. Moreover, the effect of specific endogenous modulators or signaling events such as the release and translocation of key molecules may be dissected out in their relation to the PONR and the stages of this model. However, in order to assess the PONR, a combination of recommended detection methods[1,5,10,14] is suggested. Further it

Table 1 **Suggestions of kinetic parameters to be acquired for the assessment of autophagy and cell death dynamics**

Parameter	Observation- assessment	Ref.
Autophagic kinetic parameters:	Basal autophagic flux, autophagic capacity, time required to respond with increased autophagy, time required to achieve maximal attainable autophagic activity, degradative capacity, ATP levels and kinetic measure of ATP synthesis	53, 118, 119, 120
Apoptotic kinetic parameters:	Time required to: display Annexin V, start dissipating MMP, release Cyt c, cleave caspase-3 and to show onset of pyknosis/karyorhexis, p53 localization, ATP levels and kinetic measure of ATP synthesis	33, 121, 45, 46, 120
Necrotic kinetic parameters:	Time required to: display membrane leakage, show lysosomal leakage, start high mobility group box-1 (HMGB-1) release, dye uptake dynamics until complete loss of membrane integrity achieved, ATP levels and kinetic measure of ATP synthesis	122, 123, 120
Cell specific parameters:	Cell type: index for proliferation, senescence, differentiation, cell function: sub-cellular characteristics, mitochondrial load, activity of Golgi apparatus and Endoplasmic Reticulum, inherent combination of Bcl-2 family proteins	124
Intracellular metabolic parameters:	Basal ATP level, % glycolyitic flux and oxidative phosphorylation, estimated basal metabolic flux, oxygen consumption, ATP demand, substrate preference	125, 126, 127, 120
Extracellular metabolic parameters:	Microenvironment, % substrate distribution in growth media, diffusion gradients for growth factors, substrates and O_2/CO_2	128
Insult parameters:	Type of insult/stress, duration, severity, mode of action	

Suggestions of kinetic parameters to be acquired to complement detection methods[1,5,10,14] for the assessment of autophagy and cell death dynamics. It is advised to report acquired data as measured (e.g., FRET efficiency, intensity profile in arbitrary units), avoiding terminology like '%autophagy', '%apoptosis', '%necrosis', '%viability', and to contextualize the listed parameters in relation to one another.

is suggested that kinetic parameters for autophagy, apoptosis and necrosis as well as cell specific parameters, intra- and extracellular metabolic parameters and insult parameters be carefully considered (Table 1). For example, a combination of data on basal autophagic flux,[10,23,41] the time needed to reach its maximal attainable activity, as well as vital dye uptake dynamics[42-44] and onset of dissipation of mitochondrial transmembrane potential $\Delta\Psi_m$[33] should be placed into context with the cell's metabolic substrate preference and ATP household over time[39] in order to describe the PONR. It is crucial that multiple parameters be acquired at multiple points in time, and examined in context with one another, to capture the dynamic character of temporal signalling during cellular response. This approach may be aided by: (i) increased utilization of live cell imaging techniques which report the dynamics of dye exclusion between the onset of a leaky membrane up to the loss of its integrity during necrosis or (ii) assessment of the time scale of caspase activation through fluorescence resonance energy transfer (FRET) microscopy.[45,46] To allow increased understanding of such dynamic events on a cell population level, this approach may be strengthened by data generated from living cells with fluorescence-activated cell sorting, particularly when tandem dyes are available.[47]

Autophagy and the Dynamics between Modes of Cell Death

In addition to the dynamics within a mode of cell death described above, a growing body of knowledge disputes the view that the cell death process is merely one static "suicide" pathway a cell commits to, but rather argues that it is the net result of a dynamic integrative signalling network, which permits molecular overlap and grey zones. Recognition of the extensive crosstalk between different cell death pathways begins to provide insight into the complexity of the cell death decision-making process. Although in many conditions a distinct morphology of one of the above types of cell death may be encountered, multiple pathological conditions present themselves with morphological overlap.[2,48] As the position of the PONR is likely to be dynamic, so is the onset of cell death modes. This contributes to the shared morphological features. Furthermore, the order of the appearance of morphological characteristics for cell death with autophagy, apoptosis and necrosis over time may not necessarily suggest a preferred cellular way of dying, but may rather result from the metabolic condition that governs the cell's stress response at that point in time. Importantly, autophagy appears to be a central and upstream role player when cells respond to metabolic perturbations.[49] Autophagy helps cells avoid a metabolic crisis and thus affects the downstream cell death pathways. The cell protects itself from dying, therefore

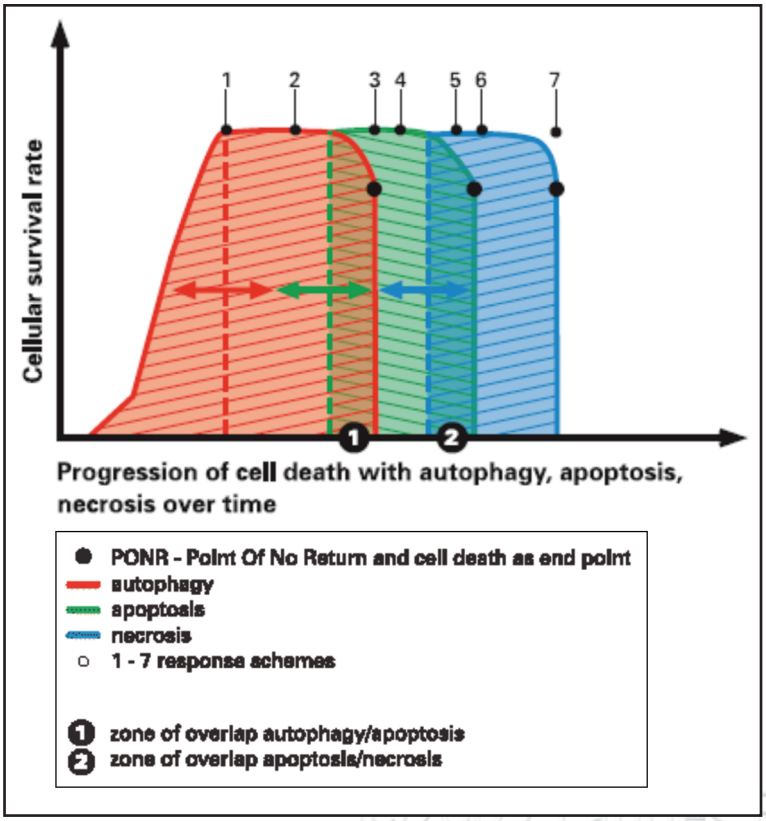

Figure 2. The overlap of autophagy, apoptosis and necrosis pathways, their onset and the PONR position translates time-dependently into the observed morphology and cellular survival rate. The cell's response to an insult is highly dynamic and morphological features between the death modes can overlap. The control of the PONR position through the cells metabolic efforts would maintain a high survival rate, ensuring highest likelihood of cellular preservation.

autophagic activity has an impact on the onset of other death modes through intricating the cell's metabolic balance sheet. Cell death may therefore be viewed from the perspective of a dynamic response that provides cellular adaptability to a given stressful condition which can, context specific, extend into the process and endpoint of death.[5] If there is a genetically encoded cellular intention, then it is likely to foster a will for survival as opposed to death. The overlap of autophagy, apoptosis and necrosis pathways, their onset and the PONR position translates time dependently into the observed morphology and cellular survival rate (Fig. 2). It is therefore important to understand these dynamics, because only then can the opportunities presented, by delaying an onset of cell death, as opposed to inhibiting a pathway, be utilized. This will be addressed in the following chapter.

Role of autophagy in delaying onset of dying and in delaying the PONR. Literature suggests that autophagy plays a fundamental role in the cell's survival mechanism.[2,48] Although it may be activated in cell death, evidence suggests that in most cases it is not a contributing factor.[50] When cell death is viewed as a dynamic response, interchanging the terminology for apoptosis or necrosis "inhibition" with "onset delay" is needed. This view suggests that autophagy promotes cell survival primarily by delaying the onset of apoptosis and necrosis. It also proposes that autophagic response time, capacity and net flux determine the time of death onset by creating a metabolically favorable intracellular environment. Using the available literature, this chapter discusses the molecular mechanism and endogenous regulators supporting this hypothesis. Based on our model (Fig. 4), it may be more accurate to indicate an additional level of autophagy above baseline levels, which occurs prior to the induction of apoptosis or necrosis. This additional level indicates autophagic flux (z-distance between the stages 1 and 2 in Fig. 5).

Autophagic responsiveness. The cell, as a highly dynamic structure, has a sophisticated control mechanism for ensuring self-preservation. Self-preservation can only be accomplished when energy is preserved to match the cellular energetic demand. Under stressful conditions, a major threat to the cell's self-preservation is its incapability to react within the needed time frame with sufficient energetic capacity. To maintain this capacity and ATP homeostasis, several mechanisms can be utilized by the cell to modulate ATP supply and demand in order to minimize cell damage. To assess an autophagic response, it may firstly be necessary to consider the cell's own energetic profile and energy demand under control conditions, to define its response to starvation (Table 1). The cell's metabolic turnover may be a crucial factor in determining the context-dependent behavior of autophagy[18,24] and may explain tissue-specific autophagic response kinetics. Autophagy is active at a basal level in most of the cells in the organism, although individual autophagic capacity may vary.[21] An indirect indication of a causal link between the cell's metabolism and basal autophagy may be given through metabolically different, yet histologically identical, tissue types which rely differentially on glycolysis and oxidative phosphorylation.[51] A transgenic mouse model expressing green fluorescent protein-light chain 3 (GFP-LC3) revealed differential autophagic response kinetics, possibly indicating tissue specific metabolic demands. Organs with a high degree of energy requirement, such as the myocardium, often show the highest rates of autophagy, which possibly reflects a continuous ATP demand.[51] Mice lacking Atg5, which is part of the conjugation system, die during the neonatal period. Atg5$^{-/-}$ mice also show myocardial damage, have decreased amino acid levels and decreased cardiac ATP production.[20] As ATP production indicates the energy contribution of basal autophagy turnover, this is of importance when describing autophagy kinetically. The baseline level of autophagy (stage 1, Fig. 5), the threshold which triggers additional autophagy (stage 2, Fig. 5), the time needed in doing so (z-distance between stages 1 and 2, Fig. 5), as well as the flux through the autophagic pathway are likely to determine the degree of resistance to a given stress stimulus.[52,53]

An increase in autophagic responsiveness and flux may provide a selective advantage for tissue against injury, by maintaining intracellular ATP levels and nutrients and shifting the PONR for cell death with autophagy, apoptosis and necrosis to a later point (z-distance between stages 2 and 3, 2 and 5 as well as 2 and 7

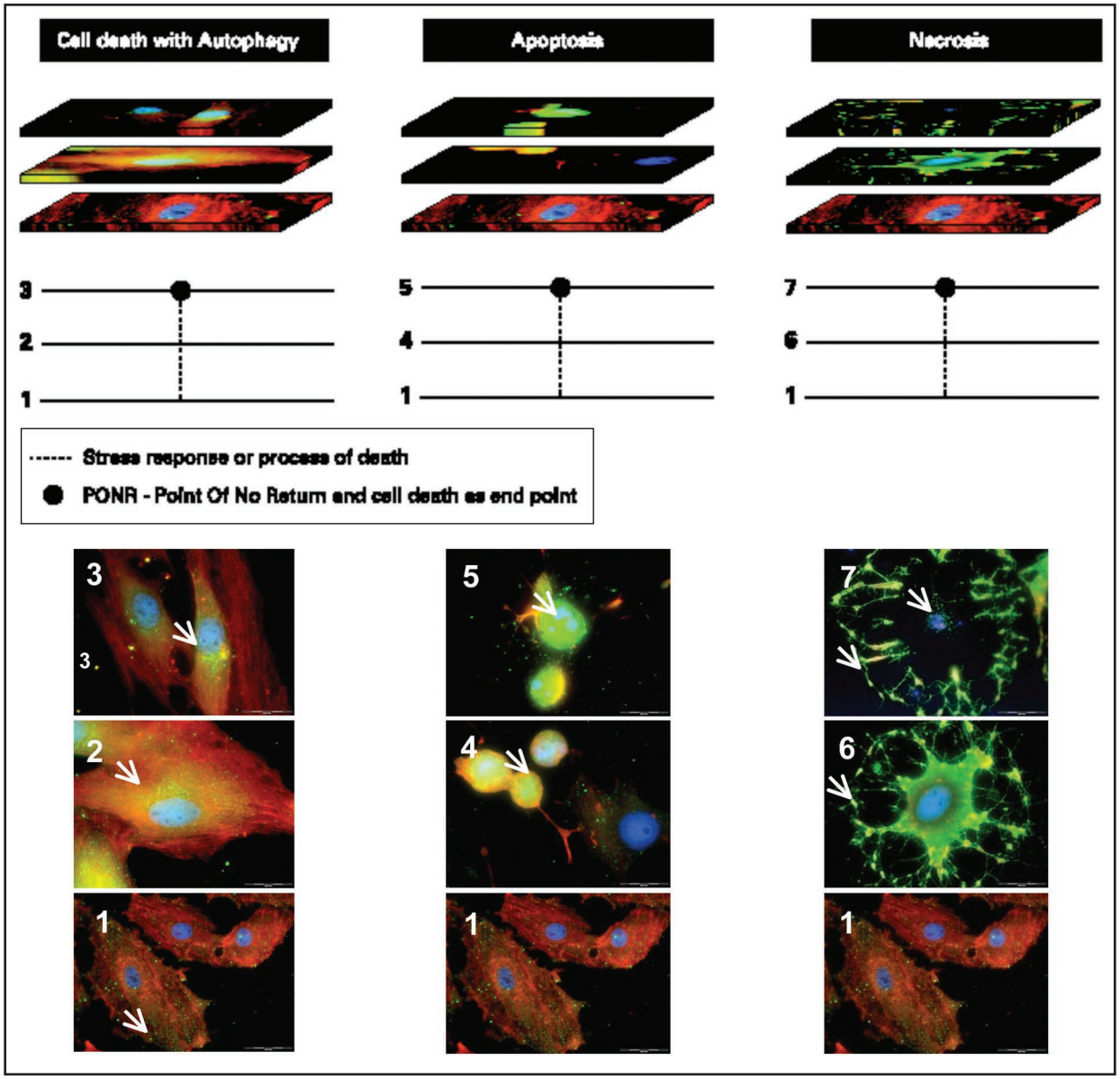

Figure 3. Model of typical appearance of different types of cell death. Cells are counterstained for LC3 (green), actin (red) and nuclei (blue). Fluorescence micrographs indicate morphological features for cell death with autophagy (micrograph 1, 2 and 3), apoptosis (micrograph 1, 4 and 5) and necrosis (micrograph 1, 6 and 7). Cell death with autophagy is indicated with increasing accumulation of autophagic vacuoles (micrograph 2 and 3), apoptosis shows cell shrinkage, nuclear pyknosis and karyorhexis (micrograph 4 and 5) and necrosis shows cell swelling, membrane impairment and karyolysis (micrograph 6 and 7). The z-distance between the micrographs may indicate the process and endpoint of dying, separated by the point-of-no-return, PONR.

increases, Fig. 5). What is crucial for inducing additional autophagy is a fast response to a decreasing intracellular ATP concentration. The 5'-AMP-activated protein kinase (AMPK) which is required for autophagy and is activated in response to changes in the inracellular ATP/AMP ratio,[54] switches off ATP-dependent processes[55] and therefore contributes to a favorable metabolic environment. In many cell death cases, such as during hypoxic stress, it is known that AMPK is activated and that autophagic flux is increased.[54] mTOR (mammalian target of rapamycin), which receives negative input from AMPK and which acts as a nutrient sensor kinase and negatively regulates autophagy, is ideally situated to sense changes in the ATP/AMP ratio as it localizes with the outer mitochondrial membrane.[32] The autophagic response controlled by AMPK and mTOR is crucial for maintaining ionic homeostasis through catabolic energy production,[56] thus delaying the onset of apoptosis and necrosis, as opposed to inhibiting these processes of cell death (Fig. 6). Dynamically seen, an onset delay may be equal to an inhibition, given a sufficiently large time window of delay.

Autophagy and oxidative stress. An important parameter for maintaining a metabolically favorable environment is the role of autophagy in degenerating damaged and oxidized proteins, thus indicating its role in the homeostasis of cellular oxidative stress.

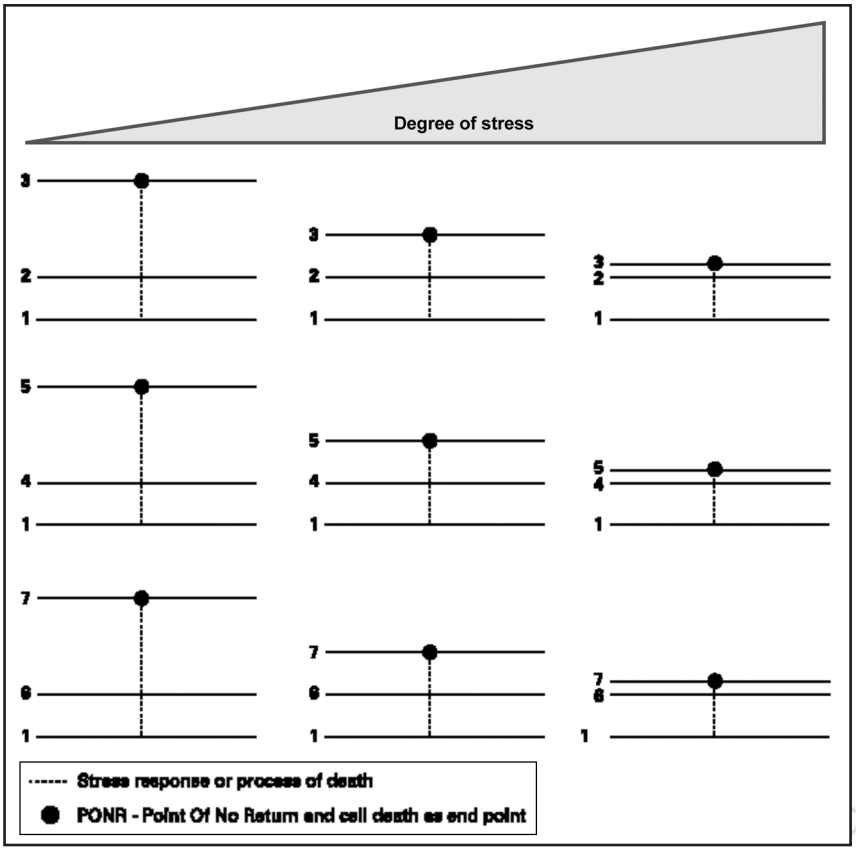

Figure 4. Relationship between the severity of the insult and the induction, execution of cell death: With increasing degree of a stressful insult, but a steady autophagic flux, cell death is reached earlier, the z-distance between 2 and 3, 4 and 5 as well as 6 and 7 decreases. 'autophagy baseline' 1, 'autophagy induced' 2, 'cell death with autophagy' 3, 'apoptosis induced' 4, 'apoptosis executed' 5, 'necrosis induced' 6 and 'necrosis executed' 7. The z-distance between the micrographs may indicate the process (induction) and endpoint (execution) of dying, separated by the point-of-no-return, PONR. The degree of stress may determine the position of the PONR.

Reactive oxygen species (ROS) is a side product of mitochondrial respiration which initiates cell damaging peroxidation chain reactions. Amino acid starvation-induced H_2O_2 generation in mitochondria has been shown to lead to Atg4 oxidization which enables autophagosome formation to proceed.[57] Sensing ROS and reacting to it through increased degradation of oxidatively damaged proteins[52,58] would decrease the load of oxidatively damaged structures, thus preserving mitochondrial function for longer. It has been proposed that autophagy can be more selective than previously thought.[59,60] For example, caspase inhibition, which leads to cell death with autophagy, directly induces catalase degradation and subsequent ROS accumulation. This ROS accumulation is then blocked through the inhibition of autophagy by the knockdown of Atg7, Atg8 and the serine/threonine kinase RIP,[61] indicating another level of autophagy which can regulate oxidative stress. Atg4, caspase-3, cathepsins and calpains all undergo redox regulation through modulation of their active sites,[57] which may highlight the upstream role of increased autophagy in modulating apoptosis and necrosis by reacting to oxidative stress. Rapamycin renders cells more susceptible to the cytotoxic effect of tumor necrosis factorα (TNFα),[62] indicating autophagy as a primary response scheme to decrease ROS. Furthermore, both TNFα and ROS cause an increase in Beclin 1 expression.[62] As damaged or depolarized mitochondria are selectively eliminated through autophagy,[19] a decrease in mitochondrial load is rapidly achieved, creating a role for autophagy in sequestering death promoting molecules, protein aggregates and proapoptotic signals. This is consistent with the observation that the protective effect by rapamycin is lost when inhibiting cytochrome *c* release.[63] This mechanism of delaying apoptosis onset would provide a protective time-window in which the primary inducing stimulus could cease and the cell could more likely recover.

This model would also explain the presence of autophagy in dying cells. The mitochondria might thereby represent a nexus of interaction between autophagy and apoptosis. Rapamycin seems to protect particularly against proapoptotic insults which is mediated through the intrinsic pathway, supporting the role of mitochondria as nexus.[63] Rapamycin-induced autophagy in a cell culture model expressing the aggregate-prone, disease-causing mutant protein, Huntington, has been shown to clear the protein aggregate.[64] Raised glucose concentration in the growth medium also reduced Huntington aggregation and cell death by increasing autophagy. This effect was mediated on the level of glucose-6-phosphate as well as through the reduction of Akt and mTOR phosphorylation.[65]

Autophagy and apoptosis onset. The dynamic relationship between autophagy and apoptosis is most clear before the activation of caspases is triggered.[66] Similar stimuli can either induce autophagy, apoptosis or both because several signals are shared between them. Autophagy may end with apoptosis and apoptosis may begin with autophagy. Caspases and autophagy can be simultaneously activated within a dying cell.[67] Cells with substantial amounts of autophagic vacuoles mostly do not die.[48] Cells with high levels of autophagy induced by Atg1 overexpression present themselves with a hybrid cell death-showing cell death with autophagy and apoptotic characteristics.[68] When using caspase inhibitors, their survival is enhanced.[68]

There is an indication that autophagy occurs or is de-inhibited, when apoptosis is not functional. In cells that are unable to undergo Bax/Bak mediated apoptosis (Bax/Bak$^{-/-}$), irradiation massively upregulates autophagy, which suggests that autophagy may be the first line of defence against a cellular insult. Fibroblasts from double knockout Bax$^{-/-}$ Bak$^{-/-}$ respond with substantial autophagy followed by delayed cell death, when treated with DNA-damaging agents.[3] In addition, autophagy not only limits metabolic stress but also minimizes genomic instability.[49] These findings support the dynamic response concept of autophagy mediating a direct onset

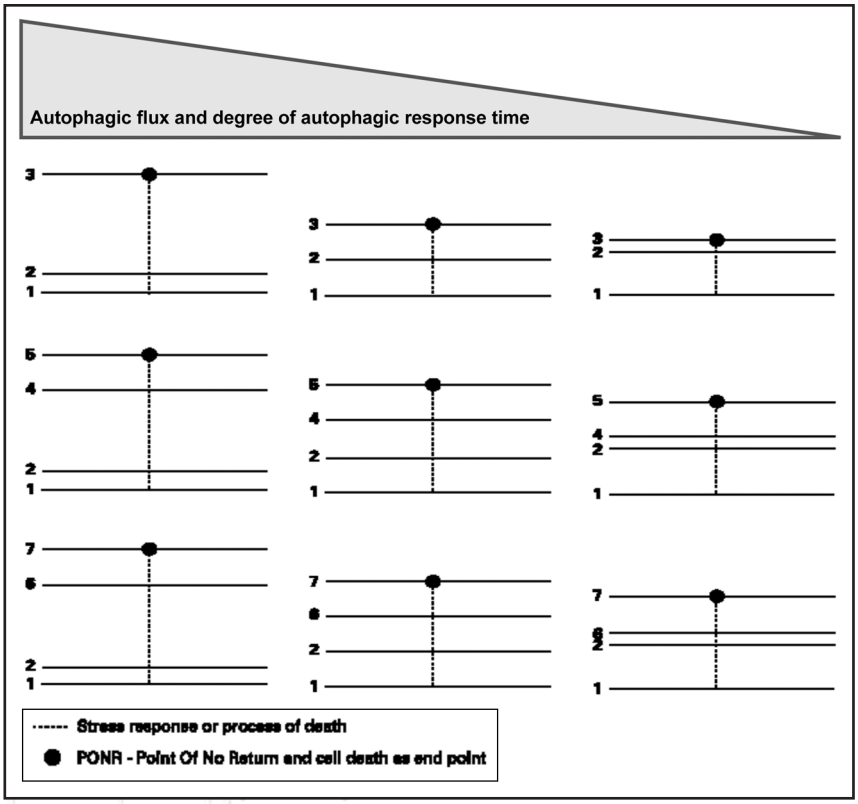

Figure 5. Relationship between autophagic flux, autophagic response time (time required to induce additional autophagy) and the induction/execution of cell death: With decreasing autophagic flux and more time needed to induce additional autophagy, cell death is reached earlier, the z-distance between 2 and 3, 2 and 5 as well as 2 and 7 decreases. 'autophagy baseline' 1, 'autophagy induced' 2, 'cell death with autophagy' 3, 'apoptosis induced' 4, 'apoptosis executed' 5, 'necrosis induced' 6 and 'necrosis executed' 7. The z-distance between the micrographs may indicate the process (induction) and endpoint (execution) of dying, separated by the point-of-no-return, PONR. The degree of stress and autophagic flux may determine the position of the PONR.

delay of apoptosis (Fig. 6). Apoptosis-deficient cells with functional autophagy machinery induce additional autophagy under stress which increases the likelihood of recovery. In support of the concept of cell death being dynamic is the recent investigation of caspase regulatory network dynamics, where the use of live cell reporters revealed a partial state of cell death, which can persist for hours.[69]

The inverse relationship between autophagy and the onset of apoptosis is likely to manifest when decreasing or inhibiting autophagy. If the autophagic energy control mechanism is inhibited, apoptosis competent cells become sensitized and undergo apoptosis earlier. The use of inhibitors of autophagy leads to cell death through apoptosis which can be reduced with caspase inhibitors.[70] Cells deprived of the cytoprotective functions of autophagy might be more vulnerable and sensitized to insult. This view is supported by the notion that RNAi against Beclin 1 and atg5 enhances starvation-induced apoptotic cell death.[20] A decrease in autophagic responsiveness and flux may induce tissue injury earlier, due to increased accumulation of damaged protein aggregates and large dysfunctional mitochondria, leading to increased ROS, defective ATP generation, less efficient metabolic pathways and a decrease in lysosomal stability (Fig. 7). Loss of lysosomal integrity leads to a release of lysosomal hydrolases into the cytosol,[71] which brings about an inhibition in autophagic maturation, contributing to the accumulation of dysfunctional mitochondria. This process sensitizes cells to proapoptotic signals.[72] The inactivation of lysosome-associated membrane protein-1 (LAMP1), through RNA interference, inhibits the fusion of autophagosomes and lysosomes in HeLa cells, which adopt characteristics of both autophagy and apoptosis.[73] According to our model, this vicious cycle may indicate a "premature" onset of apoptosis and necrosis (Fig. 7). Therefore, by reducing effective ATP generation and decreasing degradative capacity, the PONR for cell death with autophagy, apoptosis and necrosis may shift to an earlier point (z-distance between stages 2 and 3, 2 and 5 as well as 2 and 7, decreases) (Fig. 5).

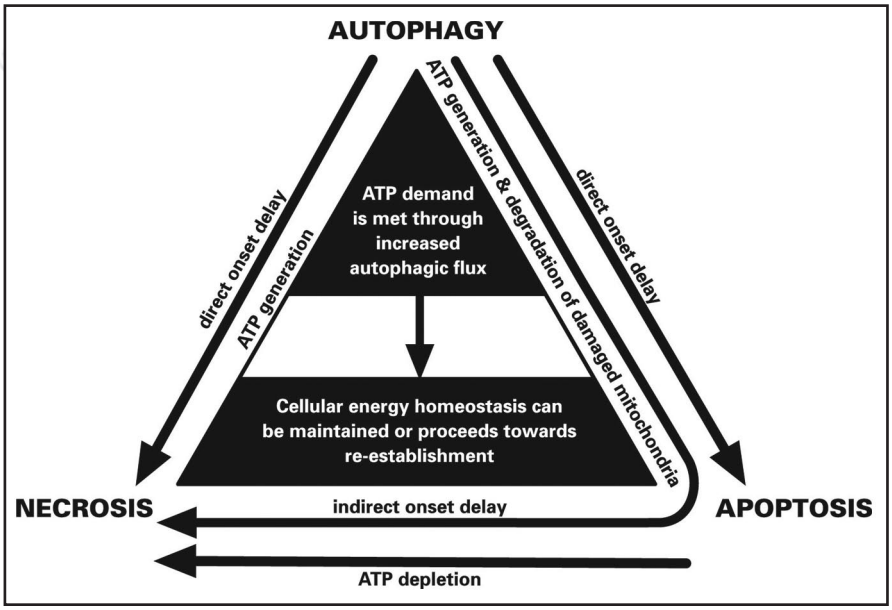

Figure 6. ATP-dependent relationship between autophagy, apoptosis and necrosis. Autophagy induces direct and indirect onset delay of apoptosis and necrosis through ATP generation and removal of damaged mitochondria and apoptotic stimuli. Energy homeostasis is more likely to be maintained.

Controlling additional autophagy beyond baseline. Most interesting is the finding that Beclin 1 binds to Bcl-2, an important

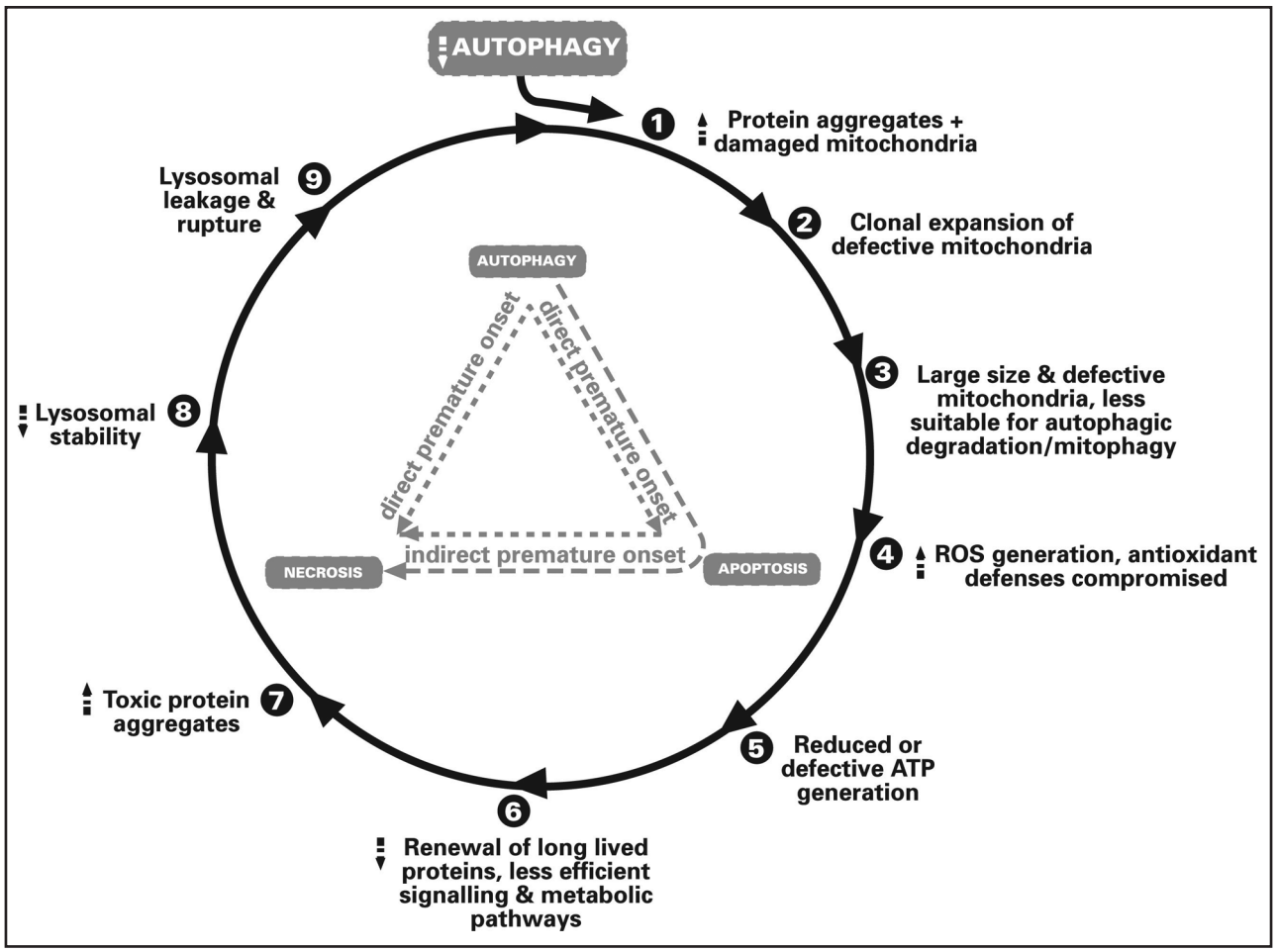

Figure 7. Decreased autophagy may induce a vicious cycle leading to a direct and indirect premature onset of apoptosis and necrosis. Defective mitochondria, increased ROS generation and defective ATP generation may lead to lysosomal leakage and the acceleration of cell death induction.

negative regulator of apoptosis, and that its binding kinetics have been suggested to function as a homeostat, which maintains autophagy at levels needed for cellular survival.[20] Beclin 1 has recently been identified as a novel BH-3 only protein, carrying a Bcl-2-homology-3 (BH3) domain. It interacts with Bcl-2 and its homologue Bcl-x_L,[74,75] binds to the Class III Phosphatidylinositol 3 (PI3) kinase Vps34 and interacts with a number of co-activators forming a complex regulatory interactome.[76] BH3 domains of other proteins, such as Bad, competitively disrupt this Bcl-2/Beclin 1 interaction, which causes autophagy of mitochondria (mitophagy). It is therefore likely that additional induction of autophagy and the time required (z-distance between stages 1 and 2, Fig. 5) are driven by kinetic, concentration and affinity-dependent characteristics, which result in a defined displacement probability of the Bcl-2/Beclin 1 complex, disrupting its interaction. Bcl-2 functions as an anti-apoptotic and anti-autophagic protein.[26] A cytoprotective role for Bcl-2 in this context has been described, where ATP depleted and Bcl-2 transfected cells were treated with the apoptosis inducer, staurosporine.[77] It inhibits autophagy through direct interaction with Beclin 1, while Bcl-2 downregulation increases autophagy. The cell type specific combination of Bcl-2 family proteins[76] may therefore contribute to define cell type specific autophagic capacity and response kinetics. Moreover, phosphorylation of Bcl-2 has been shown to disrupt the Bcl-2/Beclin 1 complex and to induce autophagy.[78] The engagement of Beclin 1 with the lipid kinase Vps 34 is essential for initiating autophagosome formation.[79] Vps 34 is recruited to autophagosomes when induced by starvation.[80] This indicates its role in sensing and responding to metabolic perturbations[81] thus integrating a cellular response. This process may also influence autophagic response time. An increase in disruptions of the interactome may lead to increased Beclin-Vps34 binding, which increases autophagy[85] and thus contributes to a metabolically favorable microenvironment.

Cancer cells seem particularly advanced in evading cell death in a nonfavorable metabolic environment, indicating existence of a high degree of flexibility in delaying the onset of apoptosis and necrosis. Although autophagy displays context-dependent behavior, sometimes favoring and sometimes preventing ongoing proliferation,[18] it has been indicated to play a role during survival, rather than during death.[35] Adequate ATP availability plays a central role in mediating this behavior. One survival strategy may be the minimization of intracellular ATP demand achieved

through undergoing a hibernation-like state by decreasing the cellular metabolism to baseline levels[82] or increasing the ATP supply by increasing ATP generation.

The maternally imprinted Ras-related tumor suppressor gene, aplasia Ras homolog member I (ARHI), induces autophagy at physiological levels,[83] inhibits mTOR and upregulates AMPK, indicating its close relationship to the cell's energy and nutrient sensing mechanisms.[84] The role of ARHI seems also time-dependent, as chronic expression leads to cell death. In the tumor environment, ARHI-induced autophagy allows hibernating cells to survive and to recur as tumors at a later point, when ARHI is downregulated again.[82] Additionally, however, the complex metabolic scenarios in cancerous tissue, which create diffusion gradients for growth factors,[85] which in turn may differentially influence the autophagic response, need to be considered.

Furthermore, the recently discovered Beclin 1 associated autophagy-related key regulator (barkor) shows direct interaction with Beclin 1, and induces autophagy when upregulated.[86] It competes with the regulatory protein UVRAG (UV radiation resistance associated gene product) for Beclin 1 binding,[86] and may therefore modulate the onset of additional autophagy. The absence of autophagy sensitizes cells to death following radiotherapy,[87] thus supporting the view of autophagy's role in delaying death onset.

Another regulator for the level of autophagy (and hence z-distance between stages 1 and 2, Fig. 5), is the tumor suppressor protein p53, which is often inactivated in cancer cells. Its deletion, depletion or inhibition has been shown to induce enhanced autophagy in metabolically stressed cancer cells to maintain ATP levels even in the absence of glucose.[88] However, its localization within the cell determines its role during the autophagic response, which may be governed by the metabolic characteristics of the cell and its microenvironment.[89] The cytoplasmic p53 localization inhibits autophagy, whereas its nuclear localization induces autophagy.[90] Most interesting is the fact that ATP hydrolysis is a requirement for nucleolar localization of p53, which translocates to the nucleoplasm when ATP decreases.[91] This may indicate yet another causal link of ATP availability and the onset of autophagy. Moreover, p53 mediates a checkpoint response under metabolically challenging conditions through its AMPK-dependent phosphorylation.[92] p53-induced autophagy is also dependent on the lysosomal protein damage-regulated-autophagy-regulator (DRAM), which in turn is induced by cellular stress.[93] Additionally, the autophagic response may also depend on the cells' degradative capacity, as degradation of the cytoplasmic p53 pool, which is stimulated by nutrient depletion, is required for autophagy onset.[90] Furthermore, the growth and tumor suppressor, ARF, induces a p53-dependent apoptosis programme, acts as a guardian against oncogenic insults and is often inactivated during tumor progression.[94] Autophagy may also be induced by ARF in a p53-dependent[94] and -independent[95] manner. Interestingly, ARF decreases the Beclin 1/Bcl-x_L complex, thereby controlling the onset of autophagy. Bcl-x_L is a mitochondrial ARF binding protein, and may therefore impact the Beclin-1/Bcl-x_L complex.[96] On the other hand, independent of p53 and the Bcl-2 family, the expression of the short mitochondrial form of ARF (smARF) dissipates mitochondrial membrane potential,[95] indicating the close relationship between autophagy and an often described PONR.[33] Smarf-induced mitochondrial dysfunction may then contribute to an increased level of autophagy. Taken together, it becomes apparent that cancer cells possess a finely controlled sensing mechanism for inducing an additional autophagic response beyond basal levels, and that the intracellular metabolic environment is central in regulating cellular fate and in controlling the onset of cell death.

Autophagy and necrosis onset. Recent studies also show a molecular link between autophagy and necrosis. It is suggested that necrosis manifests as a result of an unsuccessful autophagic stress response,[29] which supports our view that autophagy is induced as a stress response to increase likelihood of survival. When autophagy and apoptosis are inhibited in mouse-derived photo-receptor cells, necrotic cell death is induced.[97] It was also observed that a fetal mouse heart kept for 1 hr in organ culture undergoes autophagy followed by progressive necrosis.[98,99] This confirms that autophagy acts as the first line of defense during a stress response.

The heart's capacity for sensing ROS and reacting to it through increased degradation of oxidatively damaged proteins to maintain intracellular ATP may lead to a direct onset delay of necrosis (Fig. 6). This scenario may be the case during death receptor-induced necrosis, where ROS is generated from either mitochondrial or nonmitochondrial sources.[100] On the one hand, the autophagic removal of oxidatively damaged mitochondria may preserve the cell for a specific time which may depend on the intensity and duration of the initial insult.[9] On the other hand, mutation studies demonstrated that autophagosomes and lysosomes synergize as proteolytic systems to facilitate necrosis.[101] This suggests that autophagy may be using proteases that also mediate necrosis or that parts of the machinery for apoptosis and necrosis are recruited to complete execution initiated by autophagy. Despite the close relationship of proteolytic systems in autophagy and necrosis, another level of pathway interaction becomes clear, as autophagically generated amino acids such as glutamine may act through the integrin-p38 mitogen-activated protein kinase (MAPK)-pathway, causing cell swelling and inhibition of autophagy.[65] This interaction suggests that necrosis can be induced when autophagic capacity is insufficient to maintain a favorable environment. In *C. elegans*, autophagy is induced early during necrotic cell death.[101]

Despite a possible direct onset delay of necrotic cell death we, however, propose that the profound effect of autophagy on necrosis is brought about indirectly. The indirect onset delay of necrosis is due to autophagy's role in causing onset delay of apoptotic cell death (Fig. 6). This necessitates a closer look at the cell's metabolic performance in the light of the dynamic relationship between apoptotic and necrotic cell death. Common denominators for both apoptotic and necrotic cell death are triggers such as hypoxia, heat shock, viruses or oxidative stress, and second messengers such as Ca^{2+}, capase-8 or stress-dependent transcription factors.[4] It is suggested that necrosis serves as the cell's default pathway, as it can be induced by the inhibition of apoptosis and autophagy.[1] It is important to note that ATP availability plays a crucial role in determining the onset of necrosis. When ATP depletion is achieved in the cell through culture in glucose-free

medium with pyruvate, allowing only mitochondrial ATP production, apoptosis is induced. However, when cells are treated with a blocker of mitochondrial ATP synthesis, necrosis is induced. ATP depletion of >50% is needed to change the mode of cell death from apoptotic to necrotic.[102] Also, restoration of glycolytic ATP generation is sufficient to allow apoptosis to take place. We propose that autophagically generated amino acids and substrate intermediates may be involved in maintaining ATP at sufficient levels for longer, thus delaying the switch from apoptosis to necrosis. Conversely, when autophagy is dysfunctional or an additional autophagic response is not occurring timely, one may speak of an indirect premature onset of necrosis (Fig. 6).

Investigation of the effect of three different homocysteine concentrations on cardiomyocyte viability shows an ATP-dependent effect on apoptosis and necrosis.[103] Firstly, low homocysteine concentrations result in mitochondrial hyperpolarization and an increase in ATP, described as first signs of mitochondrial disturbance. Secondly, an intermediate homocysteine concentration induces a slight decrease in ATP and a flip-flop of the cell membrane phospholipids. Finally, a high concentration of homocysteine decreases ATP further and shows annexin-V and propidium iodide (PI) positive cells.[103] This finding supports the view that a dynamic relationship exists between the modes of cell death, because the cells first undergo ATP-dependent metabolic disturbances, then apoptosis, followed by necrosis. Furthermore, nitric oxide (NO) also drives the decision-making process between apoptosis and necrosis through NO-dependent failure of mitochondrial ATP synthesis. In this model, cells undergoing staurosporine induced apoptosis converted to necrosis, where the cell's ability to activate caspases and undergoing apoptosis could be recovered when replenished with glucose supplementation.[104]

In the ischaemic myocardium it is demonstrated that ATP levels at reperfusion are replenished faster and necrosis is reduced when using an ATP-hydrolase inhibitor.[105] In glucose-deprived rat neonatal cardiac myocytes exposed to hypoxia, a positive correlation between myocyte ATP concentration and the percentage of apoptotic cells is described. By increasing the glucose concentration, apoptosis progressively replaces necrotic cells,[106] underlining the importance of substrate availability in the cell death decision-making process. ATP generation, through either glycolysis or mitochondrial oxidative phosphorylation, is needed to execute nuclear pyknosis, DNA fragmentation and phosphatidylserine exposure.[25] It may be speculated that the duration and severity of the insult determines whether autophagy can be induced timely, in order to have an effect on the cell's ATP household. It also becomes clear that the cell's inherent ATP supply and demand behavior influences the prevalence of either apoptosis or necrosis.[107]

Most interesting is the finding that during poly (ADP-ribose) polymerase-(PARP) induced cytoplasmic NAD depletion, glycolysis is inhibited and an ATP-dependent necrotic cell death is induced.[30] This would indicate that PARP activation runs a metabolic test on the cell, which results in a decision between necrosis or cell survival and DNA repair. Inactivation of PARP through caspase-mediated cleavage would preserve the intracellular ATP pool and allow apoptosis to proceed and be completed.[25] How much PARP-induced DNA repair a cell can metabolically afford before entering necrosis, may depend on the cell's autophagic capacity.

The above data strongly indicate that the cell's metabolic profile needs to be considered when characterizing autophagy and cell death and that cell death with autophagy, apoptosis and necrosis needs to be discussed in relation to each other (Table 1).

Cell Death with Autophagy—A Rare Exception?

The exact relationship of cell death with autophagy and its role in pathophysiological conditions remains to be elucidated. Only a few conditions are known where cell death with autophagy functions as a physiological mechanism. In cells of salivary glands of Drosophila larvae, autophagy is not a failed survival attempt, but rather a physiologically controlled cell death mechanism.[18] Moreover, cell death with autophagy also seems to play a role in cardiovascular diseases such as degenerative aortic valve disease.[108] In these rare cases, autophagy may be a "complete" cell suicide mode.[109] The other point of view is that excessive levels of autophagy can induce cell death with autophagy without the activation of caspases, leading to a degradation of organelles with a preservation of the cytoskeleton until late stages.[20] Cells that lack critical apoptosis regulators appear to die with massive amounts of autophagic vacuoles,[66] possibly compensating for the defects in apoptosis. Thus it can be argued that there is some indication that cell death with autophagy, when excessively induced, is due to bioenergetic failure.[110] Such cases deserve more study in highlighting the central role of the cell's metabolic capacity (Table 1).

Conclusion and Future Outlook

This review emphasizes the importance of understanding cell death as a dynamic response concept by discussing the dynamics within and between cell death modes. By proposing a conceptual model for understanding the dynamic interrelationship between autophagy, apoptosis and necrosis it is hoped that this can contribute towards clarifying cell death definitions and processes in order to accelerate communication. It is also hoped that it can shed light onto the centrality of autophagy within the cell death machinery in context of cellular energy homeostasis and its effect on the onset of apoptosis and necrosis. It becomes apparent that kinetic parameters such as autophagic capacity, its response time and the cell's metabolic capacity may affect the PONR and offer opportunities for cell survival. The importance of locating and characterizing the PONR has been stressed, as this may introduce novel interventions to modulate the PONR position—interventions which may be applied to increase the chance of reversibility for the benefit of cell survival.

The high degree of plasticity in the induction and execution of cell death will require the use of more sophisticated and inclusive assays. The use of advanced real-time observation techniques in combination with simulated network models[111] will offer clues to unravel the dynamic behavior of cellular commitment to survival and may offer the platform for analyzing the position of each PONR between the individual death modes. We therefore suggest the careful consideration of parameters which

focus on cell death dynamics (Table 1) to complement current detection methods.[1,5,10,14] Moreover, it is hoped to have shown that autophagic response parameters provide additional time to the process of dying, and thus ultimately increase the window of opportunity for novel therapeutic interventions.

This review highlights new treatment strategies that may focus on delaying the onset of each death mode, rather than inhibiting it. Such strategies may be based on a combined therapy, targeting autophagic as well as apoptotic pathways.[112] This may have particular implications for the treatment of terminal differentiated cells during brain[113] or myocardial injury,[114,115] which ranks among the primary causes of morbidity and mortality.[98] Going forward, such treatment strategies may also make use of energy transfer systems[116] to stabilize cellular metabolic capacity and to allow the stressed or dying cell to fully utilize Gibbs free energy during ATP hydrolysis.[117] Most crucially, the role of autophagy in delaying the onset of cell death by the modification of cellular metabolic performance deserves more study.

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References

1. Golstein P and Kroemer G. Cell death by necrosis: towards a molecular definition. TiBS 2006; 32:37-42.
2. Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, et al. Cemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. Nat Chem Biol 2005; 1:112-9.
3. Maiuri MC, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. Mol Cell Biol 2007; 8:741-52.
4. Leist M, Nicotera P. The shape of cell death. Biochemical and biophysical research communications 1997; 236:1-9.
5. Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Baehrecke EH, Blagosklonny MV, et al. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ 2009; 16:3-11.
6. Kroemer G, El-Deiry WS, Golstein P, Peter ME, Vaux D, Vandenabeele P, et al. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. Cell Death Differ 2005; 12:1463-7.
7. Lemasters JJ. Mechanism of Hepatic Toxicity V. Necrapoptosis and the mitochondrial permeability transition: shared pathways to necrosis and apoptosis. Am J Physiol 1999; 276:1-6.
8. Izyumov DS, Avetisyan AV, Pletjushkina OY, Sakharov DV, Wirtz KW, Chernyak BV, et al. "Wages of Fear": transient threefold decrease in intracellular ATP level imposes apoptosis. Biochim Biophys Acta 2004; 1658:141-7.
9. Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA. Apoptosis and necrosis: Two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. Neurobiology 1995; 92:7162-6.
10. Klionsky DJ, Abeliovich H, Agostinis P, Agrawal DK, Aliev G, Askew DS, et al. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. Autophagy 2008; 2:151-75.
11. Kerr JFR. Shrinking necrosis: a distinct mode of cell death. J Pathol 1971; 105:13-20.
12. De Duve C, Wattiaux R. Functions Of Lysosomes. Ann Rev Physiol 1966; 28:435-92.
13. Schweichel JU, Merker HJ. The morphology of various types of cell death in prenatal tissues. Teratology 1973; 3:253-66.
14. Festiens N, Vanden Berghe T and Vandenabeele P. Necrosis, a well-orchestrated form of cell demise: Signalling cascades, important mediators and concominant immune response. Biochimica et Biophysica Acta 2006; 1757:1371-87.
15. Mizushima N. The pleiotropic role of autophagy: from protein metabolism to bactericide. Cell Death Differ 2005; 12:1535-41.
16. Dice FJ. Chaperone-Mediated Autophagy. Autophagy 2007; 4:295-9.
17. Baba M, Takeshige G, Baba N and Ohsumi Y. Ultrastrucmral Analysis of the Autophagic Process in Yeast: Detection of Autophagosomes and Their Characterization. J Cell Biol 1994; 6:903-13.
18. Bergmann A. Autophagy and cell death: No longer at odds. Cell 2007; 131:1032-4.
19. Lemasters JJ. Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction and aging. Rejuvenation Res 2005; 1:3-5.
20. Levine B and Yuan J. Autophagy in cell death: an innocent convict? J Clin Invest 2005; 115:2679-88.
21. Codongo P, Meijer AJ. Autophagy and signaling: their role in cell survival and cell death. Cell Death Differ 2005; 12:1509-18.
22. Kilonsky DJ. The molecular machinery of autophagy: unanswered questions. J Cell Sci 2005; 118:7-18.
23. Hamacher-Brady A, Brady NR, Gottlieb RA. The interplay between pro-death and pro-survival signaling pathways in myocardial ischemia/reperfusion injury: Apoptosis meets autophagy. Cardiovasc Drugs Ther 2006; 20:445-62.
24. Cuervo AM, Bergamini E, Brunk UT, Dröge W, Ffrench M, Terman A. Autophagy and aging: The importance of maintaining "clean" cells. Autophagy 2005; 3:131-40.
25. Denecker G, Vercammen D, Declereq W, Vandenabeele P. Apoptotic and necrotic cell death induced by death domain receptors. CMLS 2001; 58:356-70.
26. Pattingre S, Tassa A, Qu X, Garuti R, Liang XA, Mizushima N, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 2005; 122:927-39.
27. Saberi B, Shinohara M, Ybanez MD, Hanawa N, Gaarde WA, Kaplowitz N, et al. Regulation of H_2O_2-induced necrosis by PKC and AMP-activated kinase signaling in primary cultured hepatocytes. Am J Physiol Cell Physiol 2008; 295:50-63.
28. Ziporen L, Donin N, Shmushkovich T, Gross A, Fishelson Z. Programmed necrotic cell death induced by complement involves a bid-dependent pathway. J Immunol 2009; 182:515-21.
29. Maruyama R, Goto K, Takemura G, Ono K, Nagao K, Horie T, et al. Morphological and biochemical characterization of basal and starvation-induced autophagy in isolated adult rat cardiomyocytes. Am J Physiol Heart Circ Physiol 2008; 295:1599-607.
30. Edinger LE, Thompson CB. Death by design: apoptosis, necrosis and autophagy. Curr Opin Cell Biol 2004; 16:663-9.
31. Galluzzi L, Morselli E, Kepp O, Kroemer G. Targeting post-mitochondrial effectors of apoptosis for neuroprotection. Biochim Biophys Acta 2008; DOI: 10.1016/j.bbabio.2008.09.006.
32. Leber B, Lin J, Andrews DW. Embedded together: The life and death consequences of interaction of the Bcl-2 family with membranes. Apoptosis 2007; 12:897-911.
33. Galluzzi L, Zamzami N, de La Motte Rouge CL, Brenner C, Kroemer G. Methods for the assessment of mitochondrial membrane permeabilization in apoptosis. Apoptosis 2007; 12:803-13.
34. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. Nature Rev Mol Cel Biol 2008; 9:47-59.
35. Golbs A, Heck N, Luhmann HJ. A new technique for real-time analysis of caspase-3 dependent neuronal cell death. J Neurosci Methods 2007; 161:234-43.
36. Rami A, Bechmann I, Stehle JH. Exploiting endogenous anti-apoptotic proteins for novel therapeutic strategies in cerebral ischemia. Prog Neurobiol 2008; 85:273-96.
37. Kagan VE, Bayir A, Bayir H, Stoyanovsky D, Wipf P, Atkinson J, et al. Mitochondria-targeted disruptors and inhibitors of cytochrome *c*/cardiolipin peroxidase complexes: A new strategy in anti-apoptotic drug discover. Mol Nutr Food Res 2009; 53:104-14.
38. Pacher P and Hajnóczky G. Propagation of the apoptotic signal by mitochondrial waves. EMBO J 2001; 20:4107-21.
39. Ferraro E, Pulicati A, Cencioni MT, Cozzolino M, Navoni F, di Martino S, et al. Apoptosome-deficient cells lose cytochrome *c* through proteasomal degradation but survive by autophagy-dependent glycolysis. Mol Biol Cell 2008; 19:3576-88.
40. Lemasters JJ, Nieminen AL, Qian T, Trost LC, Elmore SP, Nishimura Y, et al. The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. Biochim Biophys Acta 1998; 1366:177-96.
41. Klionsky DJ. Getting into the flow. Autophagy 2008; 2:139-40.
42. Clausen T, Gissel H. Role of Na^+,K^+ pumps in restoring contractility following loss of cell membrane integrity in rat skeletal muscle. Acta Physiol Scand 2005; 183:263-71.
43. Miyata S, Takemura G, Kawase Y, Li Y, Okada H, Maruyama R, et al. Autophagic cardiomyocyte death in cardiomyopathic hamsters and its prevention by granulocyte colony-stimulating factor. Am J Pathol 2006; 168:386-97.
44. Ramadass R, Bereiter-Hahn J. How DASPMI reveals mitochondrial membrane potential: Fluorescence decay kinetics and steady-state anisotropy in living cells. Biophys J 2008; 95:4068-76.
45. Li Y, Xing D, Chen Q. Dynamic monitoring of apoptosis in chemotherapies with multiple fluorescence reporters. Mol Imaging Biol 2009; DOI: 10.1007/s11307-008-0195-7.
46. Tyas L, Brophy VA, Pope A, Rivett AJ, Tavaré M. Rapid caspase-3 activation during apoptosis revealed using fluorescence-resonance energy transfer. EMBO Reports 2000; 1:266-70.
47. Shvets E, Fass E, Elazar Z. Utilizing flow cytometry to monitor autophagy in living mammalian cells. Autophagy 2008; 5:621-8.
48. Lockshin RA, Zakeri Z. Apoptosis, autophagy and more. IJBCB 2004; 36:2405-19.

49. Karantza-Wadsworth V, Shyam P, Kravchuk O, Chen G, Mathew R, Jin S, et al. Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. Genes Dev 2007; 21:1621-35.
50. Han W, Li L, Qui S, Lu Q, Pan Q, Gu Y, et al. Shikonin circumvents cancer drug resistance by induction of a necroptotic death. Mol Cancer Ther 2007; 6:1641-9.
51. Mizushima N, Yamamoto A, Matsui M, Yoshimori T, Ohsumi Y. In Vivo Analysis of Autophagy in Response to Nutient Starvation Using Transgenic Mice Expressing a Fluorescent Autophagosome Marker. Mol Biol Cell 2004; 15:1101-11.
52. Moore MN. Autophagy as a second level protective process in conferring resistance to environmentally-induced oxidative stress. Autophagy 2008; 2:254-6.
53. Hamacher-Brady A, Brady NR, Gottlieb RA. Enhancing Macroautophagy protects against ischemia/reperfusion injury in cardiac myocytes. J Biol Chem 2006; 281:29776-87.
54. Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, et al. Distinct roles of autophagy in the heart during ischemia and reperfusion. Roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. Circ Res 2007; 100:914-22.
55. Lam A, Lopaschuk GD. Anti-anginal effects of partial fatty acid oxidation inhibitors. Curr Opin Pharmacol 2007; 7:179-85.
56. Takagi H, Matsui Y, Hirotani S, Sakoda H, Asano T, Sadoshima J. AMPK mediates autophagy during myocardial ischemia in vivo. Autophagy 2007; 3:405-7.
57. Scherz-Shouval R, Shvets E, Elazar Z. Oxidation as a post-translatiobnal modification that regulates autophagy 2007; 4:371-3.
58. Zheng L, Roberg K, Jerhammar F, Marcusson J, Terman A. Oxidative stress induces intralysosomal accumulation of Alzheimer amyloid β-protein in cultured neuroblastoma cells. Ann NY Acad Sci 2006; 1067:248-51.
59. Kilonsky DJ. How selective is autophagy. Autophagy 2006; 3:151.
60. Bamber BA, Rowland AM. Shaping cellular form and function by autophagy 2006; 3:247-9.
61. Yu L, Wan F, Dutta S, Welsh S, Liu Z, Freundt E, et al. Autophagic programmed cell death by selective catalase degradation. PNAS 2006; 103:4952-7.
62. Djavaheri-Mergny M, Amelotti M, Mathieu J, Besançon F, Bauvy C, Codogno P. Regulation of autophagy by NFκB transcription factor and reactives oxygen species. Autophagy 2007; 4:390-2.
63. Menzies FM, Ravikumar B, Rubinsztein DC. Protective roles for induction of autophagy in multiple proteinopathies. Autophagy 2006; 3:224-5.
64. Ravikumar B, Duden R, Rubinsztein DC. Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. Hum Mol Genet 2002; 11:1107-17.
65. Ravikumar B, Stewart A, Kita H, Kato K, Duden R, Rubinsztein DC. Raised intracellular glucose concentrations reduce aggregation and cell death caused by mutant Huntington exon 1 by decreasing mTOR phosphorylation and inducing autophagy. Hum Mol Genet 2003; 9:985-94.
66. Debnath J, Baehrecke EH, Kroemer G. Does autophagy contribute to cell death? Autophagy 2005; 2:66-74.
67. Yu L, Lenardo MJ, Baehrecke EH. Autophagy and caspases. Autophagy 2004; 9:1124-6.
68. Neufeld TP. Contribution of Atg1-dependent autophagy to TOR-mediated cell growth and survival. Autophagy 2007; 5:477-9.
69. Albeck JG, Burke JM, Aldridge BB, Zhang M, Lauffenburger DA, Sorger P. Quantitative analysis of pathways controlling extrinsic apoptosis in single cells. Mol Cell 2008; 30:11-25.
70. Boya P, Gonzalez-Polo RA, Casares N, Perfettini JL, Dessen P, Larochette N, et al. Inhibition of macroautophagy triggers apoptosis. Mol Cell Biol 2005; 1025-40.
71. Wattiaux R, Wattiaux-De Coninck S. Effects of ischemia on lysosomes. Int Rev Exper Pathol 1984; 26:85-106.
72. Kiselyov K, Jennings JJ Jr, Rbaibi Y, Chu CT. Autophagy, mitochondria and cell death in lysosomal storage diseases. Autophagy 2007; 3:259-62.
73. Gonzalez-Polo RA, Boya P, Pauleau A-L, Jalil A, Larochette N, Souquere S, et al. The apoptosis/autophagy paradox: autophagic vacuolization before apoptotic death. J Cell Sci 2005; 118:3091-102.
74. Maiuri MC, Criollo A, Tasdemir E, Vicencio JM, Tajeddine N, Hickman JA, et al. BH3-only proteins and BH3 mimetics induce autophagy by competetively disrupting the interaction between Beclin-1 and Bcl-2/Bcl-X_L. Autophagy 2007; 4:374-6.
75. Liang XH, Kleeman LK, Jiang HH, Gordon G, Goldman JE, Berry G, et al. Protection against fatal sindbis virus encephalitis by Beclin, a novel Bcl-2-interacting protein. J Virol 1998; 11:8586-96.
76. Levine B, Sinha S, Kroemer G. Autophagy in higher eukaryotes—A matter of survival or death. Autophagy 2008; 5:600-6.
77. Single B, Leist M, Nicotera P. Differential effects of Bcl-2 on cell death triggered under ATP-depleting conditions. Exper Cell Res 2001; 262:8-16.
78. Wei Y, Pattingre S, Sinha S, Bassik M, Levine B. JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. Mol Cell 2008; 6:678-88.
79. Zeng X, Overmeyer JH, Maltese WA. Functional specificity of the mammalian Beclin-Vps34 PI3-kinase complex in macroautophagy versus endocytosis and lysosomal enzyme trafficking. J Cell Sci 2006; 119:259-70.
80. Juhász G, Hill JH, Yan Y, Sass M, Baehrecke EH, Neufeld TP. The class III PI(3)K Vps34 promotes autophagy and endocytosis but not TOR signaling in Drosophila J. Cell Biol 2008; 4:655-66.
81. Backer JM. The regulation and function of Class III PI3Ks: novel roles for Vps34. Biochem J 2008; 410:1-17.
82. Amaravadi RK. Autophagy-induced tumor dormancy in ovarian cancer. J Clin Invest 2008; DOI: 10.1172/JCI37667.
83. Yu Y, Luo R, Lu Z, Feng WW, Badgwell D, Issa JP, et al. Biochemistry and biology of ARHI (DIRAS3), an imprinted tumor suppressor gene whose expression is lost in ovarian and breast cancers. Methods Enzymol 2006; 407:455-68.
84. Lu Z, Luo RZ, Lu Y, Zhang X, Yu Q, Khare S, et al. The tumor suppressor gene ARHI regulates autophagy and tumor dormancy in human ovarian cancer cells. J Clin Invest 2008; DOI: 10.1172/JCI35512.
85. Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T, et al. Growth factor regulation of autophagy and cell survival in the absence of apoptosis. Cell 2005; 120:237-48.
86. Sun Q, Fan W, Chen K, Ding X, Chen S, Zhong Q. Identification of Barkor as a mammalian autophagy-specific factor for Beclin 1 and class III phosphatidylinositol 3-kinase. PNAS 2008; 105:49:19211-6.
87. Apel A, Herr I, Schwarz H, Rodemann HP, Mayer A. Blocked autophagy sensitizes resistant carcinoma cells to radiation therapy. Cancer Res 2008; 5:1485-94.
88. Tasdemir E, Maiuri MC, Galluzzi L, Vitale I, Djavaheri-Mergny M, D'Amelio M, et al. Regulation of autophagy by cytoplasmic p53. Nat Cell Biol 2008; 6:676-87.
89. Phang JM, Donald SP, Pandhare J, Liu Y. The metabolism of proline, a stress substrate, modulates carcinogenic pathways. Amino Acids 2008; 35:681-90.
90. Morselli E, Tasdemir E, Maiuri MC, Galluzzi L, Kepp O, Criollo A, et al. Mutant p53 protein localized in the cytoplasm inhibits autophagy. Cell Cycle 2008; 19:3056-61.
91. Karni-Schmidt O, Friedler A, Zupnick A, McKinney K, Mattia M, Beckerman R, et al. Energy-dependent nucleolar localization of p53 in vitro requires two discrete regions within the p53 carboxyl terminus. Oncogene 2007; 26:3878-91.
92. Jones RG, Plas DR, Kubek S, Buzzai M, Mu J, Xu Y, et al. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Mol Cell 2005; 18:283-93.
93. Crighton D, Wilkinson S, O'Prey J, Syed N, Smith P, Harrison PR, et al. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. Cell 2006; 126:121-34.
94. Abida WM, Gu W. p53-dependent and p53-independent activation of autophagy by ARF. Cancer Res 2008; 2:352-7.
95. Codogno P. Autophagy and caspase-independent cell death: $p19^{ARF}$ enters the game. Dev Cell 2006; 10:688-9.
96. Pimkina J, Humbey O, Zilfou JT, Jarnik M, Murphy ME. ARF induces autophagy by virtue of interaction with Bcl-x_L. JBC 2008; DOI: 10.1074/jbc.M804705200.
97. Kunchithapautham K, Rohrer B. Apoptosis and autophagy in photoreceptors exposed to oxidative stress. Autophagy 2007; 5:433-41.
98. Artal-Sanz M, Tavernarakis N. Proteolytic mechanisms in necrotic cell death and neurodegeneration. FEBS Letters 2005; 579:3287-96.
99. Sybers HD, Ingwall J, DeLuca M. Autophagy in cardiac myocytes. Recent Adv Stud Cardiac Struct Metab 1976; 12:453-63.
100. Morgan MJ, Kim YS, Liu ZG. TNFalpha and reactive oxygen species in necrotic cell death. Cell Res 2008; 18:343-9.
101. Samara C, Syntichaki P, Tavernarakis N. Autophagy is required for necrotic cell death in *Caenorhabditis elegans*. Cell Death Differ 2008; 15:105-12.
102. Leist M, Single B, Castoldi AF, Kuhnle S, Nicotera P. Intracellular adenosine triphosphase (ATP) concentration: A switch in the decision between apoptosis and necrosis. J Exp Med 1997; 185:1481-6.
103. Sipkens J, Krijnen P, Meischl C, Cillessen S, Smulders Y, Smith D, et al. Homocysteine affects cardiomyocyte viability: concentration-dependent effects on reversible flip-flop, apoptosis and necrosis. Apoptosis 2007; 12:1407-18.
104. Leist M, Single B, Naumann H, Fava E, Simon B, Kuhnle S, et al. Inhibition of mitochondrial ATP generation by nitric oxide switches apoptosis to necrosis. Exper Cell Res 1999; 249:396-403.
105. Grover G, Atwal K, Sleph P, Wang FL, Monshizadegan H, Monticello T, et al. Excessive ATP hydrolysis in ischemic myocardium by mitochondrial F_1F_0-ATPase: effect of selective pharmacological inhibition of mitochondrial ATPase hydrolase activity. Am J Physiol Heart Circ Physiol 2004; 287:1747-55.
106. Tatsumi T, Shiraishi J, Keira N, Akashi K, Mano A, Yamanaka S, et al. Intracellular ATP is required for mitochondrial apoptotic pathways in isolated hypoxic rat cardiac myocytes. Cardiovasc Res 2003; 59:428-40.
107. Nicotera P, Leist M, Single B, Volbracht C. Execution of apoptosis: Converging or diverging pathways? Biol Chem 1999; 380:1035-40.
108. Mistiaen WP, Somers P, Knaapen M and Kockx M. Autophagy as mechanism for cell death in degenerative aortic valve disease. Autophagy 2006; 3:221-3.
109. Nixon RA. Autophagy in neurodegenerative disease: friend, foe or turncoat? TRENDS in Neuroscience 2006; 29:528-35.
110. Ferraro E, Cecconi F. Autophagic and apoptotic response to stress signals in mammalian cells. Arch Biochem Biophys 2007; DOI: 10.1016.

111. Huber H, Rehm M, Plchut M, Dussmann H, Prehn J. APOPTO-Cell-a simulation tool and interactive database for analyzing cellular susceptibility to apoptosis. Bioinformatics 2007; 23:648-50.
112. McMullen JR, Sherwood MC, Tarnavsaki O, Zhang L, Dorfman AL, Shioi T, et al. Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by pressure overload. Circulation 2004; 109:3050-5.
113. Amaravadi RK, Yu D, Lum JJ, Bui T, Christophorou MA, Evan GI, et al. Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. J Clin Invest 2007; 117:326-36.
114. Ravikumar B, Berger Z, Vacher C, O'Cane CJ, Rubinsztein DC. Rapamycin pre-treatment protects against apoptosis. Hum Mol Genet 2006; 7:1209-16.
115. Camarata PJ, Heros RC, Latchaw RE. "Brain attack": The rationale for treating stroke as a medical emergency. Neurosurgery 1994; 34:144-58.
116. Ross J. Energy transfer from adenosine triphosphate. J Phys Chem B 2006; 110:6987-90.
117. Hiratsuka T. Fluorescent and colored trinitrophenylated analogs of ATP and GTP. Eur J Biochem 2003; 270:3479-85.
118. Cumming RC, Simonsen A, Finley KD. Quantitative analysis of autophagic activity in drosophila neural tissues by measuring the turnover rates of pathway substrates. Methods Enzymol 2008; 451:639-51.
119. Klionsky DJ. Monitoring autophagy in yeast: the Pho8Delta60 assay 2007; 390:363-71.
120. Manfredi G, Yang L, Gajewski CD, Mattiazzi M. Measurement of ATP in mammalian cells. Methods 2002; 26:317-26.
121. Golstein JC, Munoz-Pinedo C, Ricci J-E, Adams SR, Kelekar A, Schuler M, et al. Cytochrome *c* is released in a single step during apoptosis. Cell Death Differ 2005; 12:453-62.
122. Yang AJ, Chandswangbhuvana D, Margol L, Glabe CG. Loss of endosomal/lysosomal membrane impermeability is an early event in amyloid Aβ1-42 pathogenesis. J Neurosci Res 1998; 52:691-8.
123. Schilling WP, Snyder D, Sinkins WG, Estacion M. Palytoxin-induced cell death cascade in bovine aortic endothelial cells. Am J Physiol Cell Physiol 2006; 291:657-67.
124. Medeiros D. Assessing mitochondria biogenesis. Methods 2008; 46:288-94.
125. Zhdanov AV, Ward MW, Prehn JHM, Papkovsky DB. Dynamics of intracellular oxygen in PC12 cells upon stimulation of neurotransmission. JBC 2008; 9:5650-61.
126. Will Y, Hynes J, Ogurtsov VI, Papkovsky DB. Analysis of mitochondrial function using phosphorescent oxygen-sensitive probes. Nat Protoc 2006; 6:2563-72.
127. Marin S, Lee WNP, Bassilian S, Lim S, Boros LG, Centelles JJ, et al. Dynamic profiling of the glucose metabolic network in fasted rat hepatocytes using [1,2-$^{13}C_2$]glucose. Biochem J 2004; 381:287-94.
128. Srinivas V, Bohensky J, Zahm AM, Shapiro IM. Autophagy in mineralizing tissues: Microenvironmental perspectives. Cell Cycle 2009; 4:8.