Review

Apoptosis, autophagy, and more

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Abstract

Cell death has been subdivided into the categories apoptosis (Type I), autophagic cell death (Type II), and necrosis (Type III). The boundary between Type I and II has never been completely clear and perhaps does not exist due to intrinsic factors among different cell types and the crosstalk among organelles within each type. Apoptosis can begin with autophagy, autophagy can end with apoptosis, and blockage of caspase activity can cause a cell to default to Type II cell death from Type I. Furthermore, autophagy is a normal physiological process active in both homeostasis (organelle turnover) and atrophy. “Autophagic cell death” may be interpreted as the process of autophagy that, unlike other situations, does not terminate before the cell collapses. Since switching among the alternative pathways to death is relatively common, interpretations based on knockouts or inhibitors, and therapies directed at controlling apoptosis must include these considerations.

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1. Introduction

Cell death is a field that has attracted much deserved attention in recent times, leading to several new and important insights in cell biology, development, and pathology; but the recruitment of many new researchers to the field has led to some confusion in terms and sometimes overly precise dichotomies such as between apoptosis and necrosis, or between apoptosis and autophagic cell death. Often the biology is more complex or ambiguous. We therefore first define the terms and then summarize current understanding

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of their relationship. The field is moving so rapidly and it is so massive that it is not possible to cover everything in a small review. We therefore refer to several recent and more extensive summaries to which the interested reader should refer.

The terms used in the field have all evolved in the 30–40 years since their first use, and it is more useful to explain them than to attempt to preserve a definition, since efforts to constrain language ultimately succumb to popular understanding. In order to limit references to more current items, the reader is referred to several recent reviews that address older literature (Clarke & Clarke, 1996; Lockshin, 1997; Lockshin, Osborne, & Zakeri, 2000; Lockshin & Zakeri, 2001, 2002; Vaux, 2002) and more specific topics (Lockshin & Zakeri, 2004a). A summary of the relationships of cell deaths is given in Fig. 1.

The term “Programmed cell death” (Table 1) was from its inception an operational definition, referring to the fact that one could document, in cells that were doomed, a series of changes consistent with the impending failure but at a period at which one could experimentally prevent the death. The predictability of the death arose from examples in development, such as embryonic chick wings and the eponymous wing, programming was recognized by explantation of the posterior necrotic zone, in which case it would die on schedule in vitro, as opposed to transplantation to another locus, in which case it would heal in place and survive. For intersegmental muscles, programming was identified as early hormonal and neural signals affecting the muscles and finally metabolic changes, including expansion of the lysosomal compartment and activation of autophagic activities. When the cells begin to deteriorate rapidly, there is a generalized proteolytic loss of most major proteins, coupled with a rapid downregulation of most protein synthesis but the survival and even upregulation of a small number of proteins including ubiquitin (Haas, Baboshina, Williams, & Schwartz, 1995; Schwartz, Jones, Kosz, & Kuah, 1993; Schwartz, Kosz, & Kay, 1990; Schwartz, Myer, Kosz, Engelstein, & Maier, 1990; Wadewitz & Lockshin, 1988). Later, neurosecretory elements were identified, as was the activation of proteasomal proteases. For insect muscle, tadpole tail, and glucocorticoid-treated thymocytes, blockage of mRNA and protein synthesis was found to prevent or delay cell death. This observation is still valid, and is being reconfirmed today though, surprisingly, the requirement appears to persist even into an advanced stage of cell death (Myohara, 2004).

![Fig. 1. Cell deaths fall into several categories, the boundaries of which are not always distinct. Deaths are controlled (physiological) or not. The controlled deaths frequently display substantial caspase-independent autophagy) or they are predominantly apoptotic. Most apoptotic deaths are caspase-dependent, but there are claims of apoptotic morphology in situations in which caspase activity is equivocal. Caspase activation can occur by means of ligation of a membrane-bound receptor or by means of metabolic changes resulting in depolarization of mitochondria and release of cytochrome c and APAF-1. Other metabolic means of activating apoptosis include “ER stress,” usually interpreted as overloading of the ER with misfolded proteins.](BC 1772 1–15)
Table 1

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Programmed cell death</td>
<td>Development A sequence of (potentially interruptible) events that lead to the death of the cell Now recognized to require the activity of specific genes (but often activation of pre-existing proteins, not necessarily transcription at time of death)</td>
</tr>
<tr>
<td>Necrosis</td>
<td>Apparently uncontrolled cell death Loss of ATP or membrane pumps Most commonly osmotic swelling of cell membrane and organelles, with extraction of contents and precipitation of proteins Inflammatory response</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>A specific morphology, cell shrinkage and blebbing; organelles (other than ER) do not swell; nucleus fragments; chromatin marginates; no inflammation Degradation of DNA by 3′ cleavage to nucleosome-sized fragments Exteriorization of phosphatidylserine Activation of caspases such as caspase 3</td>
</tr>
<tr>
<td>Lysosomal/Type II/autophagic cell death</td>
<td>Death characterized by formation of many large autophagic vacuoles Caspase activation very late if at all Primary proteases are cathepsins or proteasomal proteins DNA fragmentation very late if at all Exteriorization of phosphatidylserine No inflammation</td>
</tr>
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This latter function proved not to be necessary in most instances of cell death not directly associated with development (see below). Nevertheless, the observations provided a hint toward what became the most important discovery and change in the concept of “programmed cell death,” the recognition that there were a handful of genes that controlled substantially all cell deaths in embryonic Caenorhabditis elegans. Today “programmed cell death” carries the overtone that cells possess the genes and hence the proteins for their own destruction, and that almost all physiological and most pathological cell deaths are managed and ritualistic rather than chaotic. “Necrosis” is today the catch-all term for any deaths that do not fit in the other categories described here. Typically, cells entering necrosis lose control of their ionic balance, imibe water, and lyse. Intracellular proteins in new ionic milieu, often in the presence of high ionic calcium and acid or other abnormal pH, often precipitate (Fig. 2). The lysis releases many intracellular constituents, attracting (in vertebrates) Mast cells and provoking an inflammatory response. Consequently, the morphology of necrosis is variable and poorly defined. Many of these deaths may have some physiological basis. For instance, the death of osteocytes in bone is usually described as more necrotic in style, but it is not clear to what extent the osteocyte participates in its own death. Osteocytes may undertake a fair amount of self-destruction before decaying, inaccessible to phagocytes (Cerri, Boabaid, & Katchburian, 2003). Similarly, between fertilization and the maternal-zygotic transition vertebrate eggs are considered to be incapable of undergoing apoptosis. In our hands, early zebrafish eggs exposed to cycloheximide indeed die a necrotic death rather than the apoptotic death of an older embryo, but they activate caspase 3. We consider that these freshwater eggs lyse before they can complete apoptosis (Negrón & Lockshin, in press). In severely inflammatory situations, the number of phagocytes clearing dead cells is likely to be limiting, with apoptotic cells lying around like rotting corpses until they lyse. When strong toxins are administered to animals, for instance hepatotoxins, many cells are identified that appear to have begun apoptosis but failed to complete it before becoming necrotic (Ledda-Columbano et al., 1991). In tissue culture the fate of a cell is always necrosis, because if a cell is...
not consumed by phagocytes it will ultimately lyse.

Thus, the distinction between apoptosis and necrosis may be simply one of timing and severity of insult. There is evidence that necrosis may not be completely chaotic. There may even be defined pathways for necrosis (Yuan, Lipinski, & Degterev, 2003). "Apoptosis" was first used to describe a particular morphology of death, common to the vast majority of physiological deaths, that was not readily explicable by the assumption of loss of ionic control: shrinkage and blebbing of cells, rounding and blebbing of nuclei with condensation and margination of chromatin, slight shrinkage or morphologically undetectable changes in organelles, and phagocytosis of cell fragments without accompanying inflammatory responses (Fig. 2). Later, active exteriorization of phosphatidylserine was identified as one of the signals for phagocytosis. The margination of the chromatin was associated with a controlled internucleosomal cleavage of DNA detectable by electrophoresis and in situ end labelling. Many of the other changes derived from activation, in apoptotic cells, of one or more specific proteases called caspases. In contrast to the developmental situations, protein synthesis was not required and in fact apoptosis was often experimentally induced by administration of cycloheximide. Today the morphology and behaviour of apoptotic cells is largely explained by activation of caspases, and apoptosis is considered to be nearly synonymous with caspase activation. In most cells the machinery for killing
the cell is present but inactive long before the cell is induced to die, and death appears to be a release from inhibition. Here we assume that classical apoptosis is a caspase-dependent form of cell death, whether triggered by extrinsic (cell surface receptor) or intrinsic (mitochondrial depolarization) means, and manifesting any of several other markers including DNA laddering as determined by electrophoresis; DNA fragmentation as determined by TUNEL or similar techniques; sub-2N DNA as seen by FACS analysis; blebbing and rounding of the cell; fragmentation of nuclei with condensation and margination of chromatin; and exteriorization of phosphatidylserine as detected by annexin V binding (Table 1). There is also a profound implication to our understanding of apoptosis. This is that, in contrast to the developmental situations described above, all or the vast majority of maturing or mature cells possess the machinery for self-destruction in the form of inactive proenzymes (pro-caspases) as well as machinery for regulating or adjusting the level at which the proenzymes can be activated. Cells normally hold the machinery in abeyance, and default to its activation when any of numerous conditions define an imperfect situation for the cells. The fact that cells are programmed to self-destruct should inform our interpretations of sequences leading to death.

In some situations, the machinery can be used in partial or targeted fashion, in which parts of apoptotic cells are preserved for other physiological purposes. These situations are described as "partial apoptosis" and include maturation of lens fibres, keratinocytes, spermatocytes, and mammalian erythrocytes. Here major organelles are discarded, usually in a process that involves one or more components of apoptosis, but other parts of the cell persist or survive. The means by which apoptosis is rendered selective can potentially teach us much, but our current understanding is limited (Allombert-Blaise et al., 2003; Cerri et al., 2003; Gandarillas, Goldsmith, Gschmeissner, Leigh, & Watt, 1999; Ishizaki, Jacobson, & Raff, 1998; Lippens et al., 2000; Mammone et al., 2000; Weil, Raff, & Braga, 1999).

2. Lysosomal/Type II/autophagic cell deaths

In 1980, apoptosis became a centrepiece of attention and within a few years apoptotic cell death and activation of caspases dominated our understanding of cell death. However, before the discovery of the caspase family of proteases, most cell deaths were considered to be lysosomal (Lockshin, 1969), or "Type II" (Schweichel & Merker, 1973) requiring activation of the lysosomal compartment. The term "autophagic cell deaths" was applied later, as the relationship between primary lysosomes, autophagic vacuoles, and autophagosomes became more apparent. In insect larval muscles and salivary glands, the bulk of the cytoplasm is removed. In salivary gland (Drosophila) or the homologous labial gland (Manduca, Lepidoptera) (Lockshin & Zakeri, 2001) the formation of autophagic vacuoles is most prominent. In insects, cell death at metamorphosis is typically autophagic, and blocking autophagy is a pupariation lethal (Juhasz, Csikos, Sinka, Erdelyi, & Sass, 2003). In insect muscles, the myofilaments are removed by a predominantly proteasomal mechanism, while organelles such as mitochondria are last seen in autophagic vacuoles.

This autophagic type of death, which was typically seen in large, cytoplasm-rich post-mitotic or only slowly mitotic cells, was characterized by autophagic capture of organelles and particles, substantial expansion of the lysosomal compartment including primary lysosomes, autophagic vacuoles, and secondary lysosomes, and belated collapse of the nucleus. Often organelles appeared to be eliminated in waves, for instance one wave in which mitochondria were seen in autophagic vacuoles and afterwards nearly eliminated, and another in which ribosomes or glycogen particles were the primary occupants of autophagic vacuoles (Locke & McMahon, 1971) (Fig. 3). Interest in autophagic cell deaths has recently revived and is now recognized to occur in many situations. In some instances the lysosomes that had been detected proved to reside in attacking phagocytes. In others, most notably insect intersegmental muscles and silk glands, post-lactational mammary glands, and post-castration prostate, the sequestration and digestion of cell organelles such as mitochondria was well documented, with any apoptotic morphology delayed until the cytoplasm was nearly completely destroyed. This death appeared to be distinct from apoptosis and is now generally called autophagic cell death. It is far more common than most researchers recognize, and is consequently under-investigated.
Insect cells, which figure heavily in this definition, are in many ways different from mammalian cells, including the prominence of remodelling of surviving cells during metamorphosis and the greater effectiveness of the barrier effect of the basement membrane (Locke, 2003), and one could argue that insects are a special case. However, in many tissues, autophagy is a means of reducing cell mass prior to apoptosis.
autophagy, evidence from both genetically long-lived Caenorhabditis and dietary-restricted mammals suggests that an active autophagic system is essential for maintenance of youthful characteristics and extended life.

3. Autophagy is a normal physiological process that does not necessarily lead to cell death

The biggest constraint to the theory of autophagic cell death is the realization that most cells manifesting substantial autophagy do not die. Autophagy is a well-known physiological process involved in routine turnover of cell constituents. It is an evolutionarily ancient process, well documented in species as simple and diverse as Dictyostelium (Cornillon et al., 1994; Levraud et al., 2004; Olie et al., 1998) and yeast (Klionsky & Emr, 2000) and has often been described in metamorphosing insects. It functions in normal physiology (Mizushima, Yamamoto, Matsui, Yoshimori, & Ohsumi, 2004) and is a major mechanism regulating turnover of many proteins and organelles (Yoshimori, 2004). The autophagic pathway is used for bulk proteolysis, and the ubiquitin for fine control (Kadowaki & Kanazawa, 2003). It is likewise used for recycling of materials during starvation; autophagy is induced by starvation and by catabolic hormones (Wang & Klionsky, 2003). Autophagy eliminates abnormal proteins, such as regulating processing of N terminal huntingtin fragments (Qin et al., 2003). Blocking autophagy leads to accumulation of small mitochondria, suggesting that these are normally processed by autophagy. Such results suggest that changes in autophagy probably determine changes in aging post-mitotic cells (Terman, 1995; Terman, Dalen, Eaton, Neuzil, & Brunk, 2003). Though overall metabolism slows with aging, entraining a slowing of specific elements of metabolism such as apoptosis and autophagy, evidence from both genetically long-lived
as neurons or muscle fibres) and the organism. The result is that there is considerable controversy as to whether autophagy protects cells or is a means to their destruction. The most reasoned arguments suggest that the role of autophagy depends on the status or history of the cell, that autophagy (which can be subdivided into macroautophagy, microautophagy, and chaperone-mediated autophagy) is initially protective but ultimately results in the accumulation of indigestible materials (Cuervo, 2003, 2004) or the destruction of vital components of the cell (Fig. 4).

When does autophagy, used to reduce cell volume, become autophagic cell death, in which the cell becomes non-recoverable? In insects this conundrum is quite common: one can augment autophagy in the salivary gland of Drosophila by starving the larva, in which case the cells atrophy; but they die by autophagy at metamorphosis. In the blood-sucking Hemipteran Rhodnius prolixus the intersegmental muscles atrophy after each moult but are destroyed at metamorphosis. Our technology provides new challenges. Although we have genetic markers or inhibitors of autophagy, particularly in yeast, we have very poor markers of autophagy (Mizushima, Ohsumi, & Yoshimori, 2002), and little ability to measure the origin of autophagy and its metabolic control. Likewise, we need to improve our analysis of the means by which isolation membranes recognize and target organelles for ingestion, or the consequences of blockage or failure of autophagy. An effort to identify genes associated with autophagic cell death, as opposed to autophagy, has begun (Inbal, Bialik, Sabanay, Shani, & Kimchi, 2002; Kimchi, 2001; Lee et al., 2003). For instance, the molecular interactions of the autophagy-promoting DAP kinase that connect it to phosphorylation of the myosin light chain and membrane blebbing as well to calmodulin activity are being established. As for other mechanisms of cell death, autoinhibition keeps these

Fig. 4. Suggested physiological maintenance mechanisms: a cell under stress can determine that maintenance is not possible, and activate apoptosis machinery (right branch). If the stress is initiated by extracellular ligands such as Fas or TNF, it will use the extrinsic (caspase 8) pathway; if the stress derives from metabolic changes, it will use the intrinsic (mitochondrial, caspase 9) pathway. If the stress is initially less severe, the cell will attempt to cope by activating the autophagic or proteasomal salvage pathways (left branch). This may suffice (dotted line) and the cell recovers. If not, autophagy may continue in a cyclical fashion (leftmost curved arrow). Ultimately, if the condition is not compensated, the cell will be too severely drained and will die. It may elect, belatedly, to exit via the metabolic route to apoptosis, or it may simply destroy all of its contents to disappear via autophagic cell death. These latter two options may not be readily distinguishable.
death-promoting kinases silent in healthy cells (Inbal et al., 2002; Kimchi, 2001).

We also need to design experiments to establish whether there is any difference between autophagy as physiological regulation and autophagy leading to cell death, and if it is possible to reverse "autophagic cell death" by supplying nutrients, energy sources, or other materials. We also need to understand more thoroughly what activates autophagy. Current impressions are that organelles are targeted by some failure in their metabolism. In other words, in a situation such as obtains in insect metamorphosis, a remarkable stage-specific removal of organelles such as mitochondria reflects a stage-specific injury to or failure of mitochondria, rather than an aggressive attack on healthy mitochondria by isolating membranes.

In Parkinsonism, dysfunctional mitochondria are destroyed by autophagy, resulting in activation of ERK signalling pathways and eventually activating apoptosis (Zhu, Guo, Shelburne, Watkins, & Chu, 2003).

The question nevertheless remains, what change has activated the autophagy, and what brought about this change?

4. There is overlap between autophagic and apoptotic cell deaths

It has long been clear that not all deaths can be neatly categorized, and that different types overlap (Lockshin & Zakeri, 2004b, Fig. 5). Recently, several laboratories have reported that molecules previously defined as intermediaries in the activation of apoptosis also function as intermediaries in the activation of autophagy, thus calling into question the primacy of the roles of both apoptosis and autophagic cell death in these situations as well as our ability to distinguish the processes by use of inhibitors. For instance, in a model of the formation of lumens in mammary acini, the pro-apoptotic TRAIL mediates autophagy

![Fig. 5. Overlap of types of cell death. Deaths are frequently clearly physiological or uncontrolled (necrotic) but because of temporal or other factors the distinction may not be precise. Among the physiological deaths, programming involving protein synthesis is most clearly seen in developmental situations, whereas most pathological or induced cell deaths are pre-programmed and can activate apoptosis without protein synthesis. Cell turnover is for the most part not documented but is presumed to be apoptotic in nature.](BC 1772 1–15)
In neural precursor cells, deprivation of growth factors leads to an autophagic cell death, which can be blocked by the anti-apoptotic Bcl-2 (Cardenas-Aguayo et al., 2003), an involvement that has also been recognized in autophagic cell death induced by HSPin1, a molecule first identified as interacting with Bcl2/Bcl-xL (Yanagisawa, Miyashita, Nakano, & Yamamoto, 2003). Ceramide, which has been considered by many researchers to participate in the activation of apoptosis, is effective in establishing macroautophagy (Scarlatti et al., in press). The endosome–lysosome system appears to be activated early in Alzheimer’s disease, in which death is ultimately apoptotic (Cataldo, Hamilton, Barnett, Paskevich, & Nixon, 1996). In Drosophila salivary glands, caspase-like enzymes are needed for needed for autophagy (Baehrecke, 2003; Martin & Baehrecke, 2004); the same is true for death of neurons in Manduca at metamorphosis (Weeks, 2003).

One of the means of interaction between autophagy and apoptosis is the possibility that lysosomal activity can activate apoptosis. Taking advantage of newer inhibitors and fluorescent markers, Kroemer and co-workers have established that lysosomes can activate classical apoptotic pathways (Boya et al., 2003a,b; Ferri & Kroemer, 2001). Several laboratories have observed that damage to the lysosomal compartment, like other serious injuries to cells, can activate apoptosis. Their findings, as discussed below, indicate that lysosomal misbehaviour can trigger apoptosis, operating through the mitochondrial pathway. Others have argued that autophagy is a precursor and even initiator of apoptosis (Uchiyama, 2001). This adds a new question to those listed above: How does activation of the lysosomal system, or damage to the lysosomal system, activate apoptosis (Boya et al., 2003b)?

Alternatively, since release of materials from mitochondria triggers the intrinsic pathway to apoptosis, sequestration of mitochondria in autophagic vacuoles might protect cells against Type I cell death. At least one laboratory has postulated that sequestration of mitochondria may delay the release of cytochrome c (Bauvy, Gane, Arico, Codogno, & Ogier-Denis, 2001) and therefore interfere with apoptosis; autophagy would therefore become anti-apoptotic and protective. Surprisingly, but similar to the findings for caspase 3, it appears that limited release of lysosomal enzymes into the cytoplasm is not necessarily lethal (Boya et al., 2003a,b; Perfettini & Kroemer, 2003). Typically, lysosomal enzymes function at very acid pH and would be expected to be ineffective in the cytoplasm, but the pH curves for cathepsins B and H extend into ranges that might be approached in hypoxic cells. As for caspase 3, these proteases are potentially extremely damaging, especially to proteins required for cell structure or enzymes at key points of metabolism, and the preponderance of cell effort is to constrain their activities. It appears that the activation of most cellular proteases is a very carefully orchestrated release from inhibition.

5. Perhaps autophagic cell death is open-ended autophagy

It is common today to contrast apoptosis with “autophagic cell death” but there are compelling reasons to question whether this is truly a qualitative difference. It is currently not possible to distinguish among autophagy as a routine mechanism of turnover of organelles, autophagy as a response to organelle injury or cell starvation, and autophagic cell death. Until we can, it remains conceivable that, if autophagy is a means by which a cell can temporize in difficult times, then autophagic cell death may be the result of the inability of the cell, for whatever reason, to terminate the autophagy. The question may therefore turn to the issue of why the cell cannot recover—ability of the cell to recognize or respond to growth factors, lack of a crucial substrate, biochemical or physical impediment to its ability to process oxygen, or other reason. One clue might be the following: during the programmed cell death of metamorphosing labial glands in Manduca sexta or of the salivary gland of Drosophila, the first 90% of the collapse is autophagic, with no sign of activation of caspases or other indicator of cell death. Finally, once virtually all the cytoplasm has been removed by autophagy, the cell manifests many of the criteria of apoptosis including exteriorization of phosphatidylinerse, mitochondrial depolarization, and, more equivocally, activation of a caspase-like enzyme. One question that deserves far more attention is whether the end of autophagic cell death is ultimately apoptosis, with the autophagy being the prelude that leads to apoptosis (Fig. 3).
6. Other proteolytic processes in cell death

In addition to lysosomes and caspases, other means exist to destroy cells. The maintenance of cell viability is heavily dependent on its shape, ability to conduct intracellular trafficking, and its communication with extracellular matrix and neighbouring cells. Evidence exists that ubiquitination–proteasome system and of matrix metalloproteases can function in cell death. Proteasomes were first connected to cell death when Schwartz et al. documented their prominent role in the destruction of myofilaments during the programmed cell death of the intersegmental muscles of moths (Grimm, Goldberg, Poirier, Schwartz, & Osborne, 1996; Schwartz et al., 1990). Several laboratories have made similar observations. However, ubiquitination can also destroy pro-apoptotic proteins, and, in a convoluted way, become protective. In Drosophila, the inhibitor DIAP can be degraded by a caspase, which necessarily binds to it. The caspase activity however creates a substrate for ubiquitination, entraining the caspase as well into ubiquitination and destruction and thereby limiting apoptosis. Such a mechanism potentially applies also IAPs in neurons (Ditzel et al., 2003; Varshavsky, 2003). In healthy cells, the balance favours anti-apoptotic complexing partners (IAPs) over pro-apoptotic complexing partners and the modest levels of spontaneously-activated caspases are not sufficient to induce apoptosis. Finally, three laboratories have argued that a cell’s interaction with its matrix helps define its survival. Joining more theoretical and conceptual arguments related to anoikis, Tenniswood and colleagues several years ago identified, using differential display, a few genes that were upregulated in both autophagic death and apoptosis. One of these was a matrix metalloprotease (Guenette, Mooibroek, Wong, Wong, & Tenniswood, 1994). More recently, in two gene screens using very different systems, different laboratories found that among the most prominent upregulated genes in autophagy and apoptosis were matrix metalloproteases (Hu, Fink, & Mata, 2002; Lee et al., 2003). These interesting findings suggest that release of the affected cell from its environment (if this is the function of the MMPs) is a central feature of many types of cell death. However, as intriguing as these studies are, one must remember that many events of different types of cell death, including activation of caspases and formation of isolation membranes, are not controlled at the transcriptional level and will not be identified in gene screens using microarrays.

7. When the death of a cell is inevitable, a cell will take any available route to death

Many of our interpretations of the role of autophagy or its role vis-à-vis apoptosis depend on experiments involving the blockage of one or more pathways. However, it is not sufficient, for instance, to block caspase 3 and measure cell survival using markers of apoptosis, since a cell with a severe defect or deficit may still die by other means. Four common overlapping fallacies often produce conflicting claims concerning the role of particular pathways in cell death. The first is that inhibiting or knocking out a given pathway prevents cell death when (particularly in the case of effector caspases) it prevents the development of apoptotic morphology or another marker of apoptosis, but not the death itself (which may be evaluated by various tests of function or reproduction). The second is that if upregulating a specific pathway results in apoptosis, then the pathway is part of the functional sequence of apoptosis. The third is the assumption that if one blocks a single pathway and the cell dies by another pathway, that the connection between the pathways is meaningful. Finally, the assumption is risky that the response of a cell under extremely arduous conditions reflects its normal biology. Many comments about apoptosis and autophagy fail to acknowledge that a cell under extreme conditions—penurious medium, lack of growth factors, exposure to cycloheximide, staurosporine, or other toxic media—is likely to die. For most cells, the preferred means is the controlled death for which it is predisposed and prepared, the activation of pre-existent procaspases and apoptosis. If the apoptotic pathway is blocked for any reason, for instance interference with effector caspases or caspase activation, upregulation of antiapoptotic factors such as bcl-2, or protection of mitochondria, the cell can still die, perhaps using autophagic pathways or others. Autophagy is most likely, in evolutionary terms, more ancient than apoptosis (Cormillon et al., 1994; Klionsky & Emr, 2000; Levraud et al., 2004; Olie et al., 1998) and is a function possessed by almost all cells. It is likely that, although activation of
apoptosis takes precedence for a cell in trouble, if
apoptosis fails the cell can fall back on autophagy.
For instance, though mammary epithelium normally
can undergo apoptosis, in vivo milk-filled epithelium
can become heavily autophagic, and MCF-7 cells,
which lack caspase 3, die by autophagy (Bursch et al.,
2004; Zakeri, Bursch, Tenniswood, & Lockshin,
1995). Autophagy likewise becomes an alternative
pathway to death when caspases are inhibited in neu-
rons (Lang-Rollin, Rideout, Noticewala, & Stefanis,
2003) and when protein synthesis is inhibited in de-
veloping retina (Guimaraes, Benchimol, Amarante-
Mendes, & Linden, 2003). In aging, both apoptosis
and autophagy generally decrease, though apoptosis
may increase in cells of hematopoietic origin (Ahn
et al., 2003; Bergamini, Cavallini, Donati, & Gori,
2003; Birge, 2004; Bossy-Wetzel, Barsoum, Godzik,
Schwarzbach, & Lipton, 2003; Brunk & Terman,
2002; Cho et al., 2003; Cuervo & Dice, 2000; Del
Roso et al., 2003; Dirks & Leeuwenburgh, 2004; Donati
et al., 2001; Ermak & Davies, 2002; Fraker
& Lill-Elghanian, 2004; Nixon, Cataldo, & Mathews,
2000; Terman et al., 2003; Warner, 2004). Mainte-
nance of autophagy extends the life of Caenorhabditis
(Melendez et al., 2003; Riddle & Gorski, 2003).

8. Conclusions

The threshold at which a cell commits to die is set
by many metabolic and structural features of cells.
The permeabilization or depolarization of mitochon-
dria can release to the cytoplasm cytochrome c, but
the threshold at which it does so is surely adjusted
by metabolic and respiratory factors, which are them-
selves adjusted by other activities of the cell. As the
lung commits to death, there is considerable crosstalk
among metabolic pathways. Cytoplasmic or lysoso-
mal proteases other than caspases can affect the ac-
tivation of effector caspases, most frequently proba-
bly contributing to the activation. Matrix metallopro-
teinases are often upregulated in dying cells, assuring
the release of the dying or apoptotic cell from its at-
tachments, and perhaps playing other important roles
in the death of the cells. In some cells there is consid-
erable autophagy prior to or instead of apoptosis. This
autophagy might be defensive and protective, reducing
metabolic demand, generating maintenance resources,
or even sequestering mitochondria and preventing re-
lease of cytochrome c; it could remove enough mito-
ochondria to commit a cell to death; or it could be an
automatic, mechanical response to building failures in
a cell already condemned and for which maintenance
mechanisms have been shut down. Most commonly,
cells follow an apoptotic route to death, but they have
many options can divert to or accentuate autophagic
or other catabolic pathways. We also do not fully un-
derstand the extent to which cells can switch among
pathways. If apoptosis is blocked, can a sufficiently
challenged cell default to autophagic cell death, or
vice versa? When, if at all, is autophagy protective, as
opposed to being the prodromal phase of an apoptotic
or other cell death? To what extent can the metabolic
history of a cell, including nutritional reserves, ac-
cumulated oxidative damage, and prior and current
stress to the endoplasmic reticulum—in other words,
the conversation among organelles—affect the thresh-
old at which the cell commits to death, or the pathway
that it follows to death? The cell operates more as an
ecosystem than as a collection of individual enzymatic
pathways. Efforts to establish therapies that are based
on the control of apoptosis will eventually incorporate
these considerations.

Uncited reference

Gorski et al. (2003).

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1335–1341.

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