MORPHOLOGICAL DIVERSITY OF PROGRAMMED CELL DEATH: DECIPHERING OF TRIGGERING SIGNALS NEEDS COMPREHENSIVE CLASSIFICATION

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ABSTRACT

Programmed cell death was defined as cell death normally occurring in embryogenesis at defined steps of the ontogeny. The term apoptosis was proposed when remarkably similar morphological features, such as marked chromatin condensation, were found in cells of various tissues dying during normal embryogenesis or cell turn-over in adult. When the expression of genes specifically involved in cell death was demonstrated in cells dying through apoptosis, it was almost universally admitted that apoptosis and programmed cell death were designating the same phenomenon. However, parallel works, performed by embryologists, evidenced different types of programmed cell death with characteristics not found in apoptosis, such as cell autolysis. Hence, confusion, still subsisting nowadays, appeared in the classification of the different types of cell death. This short comment presents a brief summary of the successive classifications of the various types of cell death, in order to draw attention on the need of agreement concerning their definitions. A clarification might help to address almost unexplored questions, such as elucidation of factors that determine the type of cell death appearing in degenerating cells.

The Relation between Programmed Cell Death and Apoptosis Still Needs Clarification

Do all programmed cell deaths occur via apoptosis? This question was the title of a report written by Schwartz *et al.* (13). Surprisingly, nearly 30 years before, it had been already shown that the disappearance of intersegmental muscles during metamorphosis of the silkworm *Antheraea pernyi*

occurred through autodigestion of cytoplasm by lysosomes (8, 9), a process that is not associated with apoptosis, following the definition of Kerr *et al.* (7). This mode of physiological cell death, now called autophagic degeneration (for review, see 4), was described subsequently in various studies made during development or after cell intoxication (3, 4, 10). Moreover, Lockshin and Williams (9) proposed to call 'programmed cell death' the

process of cell death associated with insect metamorphosis: "... There can be little doubt that the death of specific cells and tissues is a part of 'the construction manual' for the insect as a whole. The cells that will die have been programmed to do so ...". Thus, it appeared that apoptosis was not the universal mode of programmed cell death. Then, what has led Schwartz *et al.* (13) to reexamine this apparently established idea, in a study comparing the cell death process occurring during moth metamorphosis with experimentally induced apoptosis in immature thymocytes?

Apoptosis, Defined by Morphological Criteria, Is One among Several Modes of Programmed Cell Death

The term apoptosis was introduced by Kerr *et al.* (7) to designate the programmed and controlled cell deletion occurring in embryogenesis, renewal of adult tissues and tumor regression. These authors, after careful examination of various tissues, in physiological and experimental conditions, became aware that the ultrastructural features of cell death taking place during normal cell turnover were similar to those observed during development. These morphological features indicated that controlled cell deletion occurs through a sequence of events including condensation of chromatin and cell shrinkage, formation of membrane-bound fragments with intact cytoplasmic organelles and final absorption and digestion by neighbouring cells. Thus, it appeared that physiological cell death was a basic phenomenon, not only present in embryos, as already well described, but also in adults in both normal and tumorous tissues. This controlled cell death revealed itself essential for an organism to maintain its proper functioning, in contrast with necrosis, caused by noxious insult resulting in inflammatory reaction and tissular disorganization. The article of Kerr *et al.* (7) was certainly an important and stimulating step in the research concerning cell death. However, almost unavoidable confusion emerged from the proposition of this new concept. These authors observed, in development, tissue renewal and tumor regression, one type of cell death with well-defined ultrastructural criteria. Thus, they suggested that these morphological criteria were common to all cells undergoing programmed cell death, which is not surprising since at that time, ultrastructural studies on cell death process were still sparse. However, other types of natural cell death can be found, for exam-

ple during development. In particular, cell death associated with autodigestion of cytoplasm by lysosomes, which is not a characteristic found in apoptosis, was described in embryogenesis by Lockshin and Williams (8, 9) and Schweichel and Merker (14). It cannot be excluded that, in some situations, such autophagic process may act as a protective mechanism by removing altered organelles from injured cells (4). However, the observation of the bulk of degenerating cells in autolysosomes during the regression of various embryonic tissues indicates that autophagy plays in most cases a major role in cell destruction (4, 6). Biochemical analysis, performed in the regressing tadpole tail, have demonstrated that this process of cell autolysis may require new RNA and protein synthesis, probably associated with increased synthesis and activity of lysosomal or cytosolic hydrolases (4-6, 15). Thus, such specific synthesis of macromolecules, found frequently but not always in apoptosis (2), confirms that in autophagic degeneration cells play an active role in their own demise.

Identification of the Main Types of Cell Death Did not Result in Clear Classification

Identification and definition of all the types of physiological cell death (Table 1) were lacking until the ultrastructural analysis of Schweichel and Merker (14). These authors, based on observations made in mouse and rat embryos, described three different types of physiological cell death (referred as necroses) and a fourth type of cell death, observed after the use of embryotoxic substances (referred as induced necrosis). Type 1 necrosis corresponded to apoptosis such as described by Kerr *et al.* (7). In type 2 necrosis, the prominent feature was the presence of numerous autophagic vacuoles in the cytoplasm. Type 3 necrosis was described as cytoplasmic disintegration without participation of lysosomal digestion. This classification has been generally accepted by the other authors with some modifications concerning the definition of type 3 necrosis. Indeed, whereas cytoplasmic degradation without striking lysosomal reaction was subsequently observed in different tissues, total disintegration of cytoplasm was proved to be an exceptional process (4). Moreover, the use of the word necrosis was restricted to cell death occurring after loss of cell homeostasis and leading to inflammatory reaction, which is generally caused by toxic agents. Therefore, Clarke (4) proposed the following classification: type 1 cell death corre-

Table 1 *Main Types of Cell Death in the Diverse Morphological Classification*

	Lockshin and Williams (1964, 1965)	Kerr <i>et al.</i> (1972)	Schweichel and Merker (1973)	Pilar and Landmesser (1976)	Clarke (1990)	Schwartz <i>et al.</i> (1993)	Pollard <i>et al.</i> (1994), Charriaud-Marlangue <i>et al.</i> (1996)
Physiological cell death: «active cell death»	Programmed cell death (autolysis by lysosomes)	Apoptosis (cell condensation without autolysis and heterophagy)	Type 1 necrosis (cell condensation without autolysis and heterophagy)	Nuclear cell death	Apoptosis (type 1 cell death)	Type 1 degeneration (apoptotic)	Apoptosis type 1
			Type 2 necrosis (autolysis by lysosomes)			Type 2 degeneration (non apoptotic)	
			Type 3 necrosis (non lysosomal auto-disintegration)			Type 2 degeneration (non apoptotic)	
Passive cell death (only pathological)	—	Coagulative necrosis	Induced necrosis	—	Necrosis	Necrosis	Necrosis

Fig. 1 Besides normal cells (A and B), cells undergoing apoptosis (C and D) and autophagic degeneration (E and F) can be detected in the population of the nigral dopaminergic neurons in Parkinsonian patients. The nigral dopaminergic neurons are identified by neuromelanin granules (nm) in the cytoplasm. A: Nigral dopaminergic neuron with normal morphological features. The chromatin of the nucleus (n) formed a fine network with small patches. B: Enlargement of the area marked by a star in A. Despite the postmortem delay, the mitochondria (small arrows) and the endoplasmic reticulum (er) do not show any striking morphological abnormalities. n, nucleus. C: Nigral dopaminergic neuron with characteristics of apoptosis (type 1 cell death). The cell is shrunken compared to A. Condensed chromatin is visible in the nucleus (n), darkened and markedly convoluted. D: Higher magnification of the area indicated by a star in C. Chromatin condensation is observed in the nucleus (n), whereas mitochondria (arrows) and

sponding to apoptosis, type 2 cell death to autophagic degeneration, type 3 cell death to non-lysosomal vesiculate degradation. Nowadays, this classification is currently used, though entailing the confusing use of apoptosis to designate either the concept of programmed cell death or one type of programmed cell death with well-defined morphological characteristics. This probably explains the proposition to term the three types of programmed cell death that have been described until now, as apoptosis of types 1, 2 and 3 (3, 12). At the end, despite all the successive attempts of clarification, analyses of cell death process in various tissues illustrate that such useful classification may be sometimes insufficient. One example is the embryonic cell death normally occurring in chick ciliary ganglia described by Pilar and Landmesser (11). In this case, though the primary changes in the dying neurons were observed in the cytoplasm (ruling out type 1 cell death), the description made by these authors—"...While typical autophagic vacuoles and lysosomes were not more common than normal, the Golgi-associated smooth endoplasmic reticulum and vacuoles were greatly increased in number, and may possibly a source of lytic enzymes, resulting in initial intracellular digestion..."—makes difficult to decide whether it is a cell death of type 3 or a variant of type 2. Thus, it is worth noting that Pilar and Landmesser (11) proposed a classification of cell death types in only two categories, nuclear type (corresponding to type 1) and cytoplasmic type (prominent changes occurring first in cytoplasm).

Towards Elucidation of Factors Triggering Selection of the Different Types of Cell Death?

The meanders followed by the classification of the different modes of cell death emphasizes need of a general agreement on the use of the word apoptosis. More basically, it suggests the necessity to decipher what underlies the specificity of each of the types of programmed cell death, all remarkably conserved during the phylogenetic evolution. Some guidelines have been already provided, such as the observation that the same neurons may undergo

different modes of cell death depending on the presence or absence of contacts made with neighbouring cells, i.e. probably their differentiation by trophic factors (11). Another clue is the fact that, during development, autophagic degeneration is often found during rapid regression of a whole tissue or organ, whereas apoptosis seems to be preferentially involved in fine adjustment in cell numbers or cell-cell relations, although it is not always the case (4, 14). Hence, it has been hypothesized that autophagy might occur when the number of dying cells overwhelms the ingestion capacity of heterophagic system (4). However, the factors that determine the type of cell death selected by degenerating cells remain to be elucidated. One obstacle to their identification seems to be the quasi-general absence of reliable correlation between morphological and biochemical data, as already stressed by Clarke (4): "...In the large number of studies devoted to the chemical signals that initiate cell death, the type of cell death is rarely known or discussed..." Then it can be hoped that better communication between molecular biologists and morphologists will overcome this problem. Indeed, these questions are relevant not only in developmental biology, but also in other fields, such as neuropathology, since at least two types of cell death (apoptosis and autophagy) were observed in degenerating neurons in experimental ischemia and epilepsy (3) and Parkinson's disease (1) (Fig. 1).

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endoplasmic reticulum (er) have no striking abnormal features, although they appear slightly compacted, in comparison with B. E: Nigral dopaminergic neuron with features of autophagic degeneration (type 2 cell death). The nucleus (n) has a moderate chromatin condensation. Numerous vacuoles are detected in the cytoplasm filled with neuromelanin (nm). F: Vacuoles indicated by arrows in E viewed at higher magnification. The vacuoles (arrows) contain remains of cytoplasmic fragments (stars). Bars A, C and E: 2 μ m; B, D and F: 0.5 μ m

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