Review

Caspase-independent programmed cell death with necrotic morphology

Chifumi Kitanaka^{*,1} and Yoshiyuki Kuchino^{*,1,2}

¹ Biophysics Division, National Cancer Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

² CREST (Core Research for Evolutional Science and Technology), Japan Science and Technology Corporation, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

* corresponding authors: Chifumi Kitanaka and Yoshiyuki Kuchino, tel: +81 03 3542 2511; fax: +81 03 3542 1369; e-mail: ckitanak@ncc.go.jp and ykuchino@ncc.go.jp

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Abstract

Cell death is generally classified into two large categories: apoptosis represents active, programmed cell death, while necrosis represents passive cell death without underlying regulatory mechanisms. Recent progress revealed that caspases, a family of cysteine proteases, play a central role in the regulation of apoptosis. Unexpectedly, however, caspase inhibition occasionally turns the morphology of programmed cell death from apoptotic into necrotic without inhibiting death itself. In this article, we review different models of caspase-independent programmed cell death showing necrotic-like morphology, including our Ras-mediated caspase-independent cell death. Based on these findings, we suggest the existence of a necrotic-like cell death regulated by cellular intrinsic death programs distinct from that of apoptosis. Even though type 2 physiological cell death, or autophagic degeneration, has been recognized as a necrotic-like programmed cell death for a long time, the underlying molecular mechanisms have not been identified despite its physiological significance. This has been in part due to the previous absence of adequate caspase-independent cellular models to study, recent efforts may now help to elucidate these mechanisms.

Keywords: apoptosis; necrosis; type 2 cell death; autophagic degeneration; caspase-independent; Ras

Abbreviations: mCCCP, m-chlorocarbonylcyanide phenylhydrazine; CTL, cytotoxic T lymphocyte; IGF, insulin-like growth factor; JNK, Jun N-terminal kinase; PI3K, phosphatidylinositol 3-OH kinase; PPIX, protoporphyrin IX; TNF, tumor necrosis factor

Introduction

In the course of morphological examination of cell death occurring under various physiological and pathological conditions, Kerr and his colleagues identified a distinct subgroup of cell death characterized by nuclear and cytoplasmic shrinkage, which they termed 'apoptosis'.¹ These authors in their pioneering work made sufficient and significant observations to indicate that apoptosis is an active, inherently programmed cell death involved in the regulation of cell population kinetics. This idea has been supported by ever increasing evidence, and it now seems established that apoptosis is regulated by a cell-intrinsic genetic program and plays critical roles in the development and maintenance of homeostasis of multicellular organisms. Subsequent to the original description of apoptosis, Kerr and colleagues further proposed that cell death can be classified by morphology into two large categories: apoptosis and necrosis, suggesting that necrosis, in contrast to apoptosis, is generally caused in a passive manner by environmental perturbation rather than by factors intrinsic to the cell itself.² This classification has made a crucial contribution, promoting the research of programmed cell death, and thus providing simple and clear-cut visual clues to predict the presence or absence of regulatory programs behind cell death. Unfortunately, however, excessive synonymy and schematism also arose in due course which may carry the risk of neglecting exceptions to a rigid classification, with the risk of oversimplifying.^{3,4} Do all active, programmed cell deaths actually occur by apoptosis? Isn't there active, programmed cell death with necrotic-like morphology having physiological or pathological significance? In this article, we review physiological cell deaths with necrotic morphology occurring *in vivo*, rapidly accumulating *in vitro* models of programmed cell death with necrotic-like morphology, and discuss the emerging significance and implications of recognizing and elucidating the regulatory programs operating in necrotic-like cell death.

Physiological cell death with necrotic-like morphology occurring *in vivo*

In order to understand the diversity of the mechanisms involved in physiological (or naturally occurring) cell death taking place during the embryogenesis of rat and mouse, Schweichel and Merker⁵ conducted a morphological evaluation of developmental cell death in rat and mouse embryo using an electron microscope. They identified three morphologically distinct groups of physiological cell death (Table 1).

The first type (type 1), always found in isolated cells, was characterized by early condensation of the nucleus and cytoplasm. The dying cells were then fragmented and

Table 1 Morphological classification of physiological cell death

	Type 1 (apoptosis)	Type 2 (autophagic degeneration)	Type 3 (Non-lysosomal disintegration)
Nucleus	Nuclear condensation, pronounced pyknosis	Pyknosis in some cases, but is neither prevalent nor striking	Late disintegration
Cytoplasm	Reduction of volume	Abundant autophagic vacuoles	General disintegration: dilation of organelles
End stage	Fragmentation, rapid and prominent phagocytosis by neighboring cells	Fragmentation, late and occasional phagocytosis by neighboring cells	Fragmentation into very small pieces without detectable phagocytosis by neighboring cells
Incidence and location	Common, found in isolated dying cells	Common, found in regions where cells are removed <i>in toto</i>	Rare (?), found in vacuolated cartilage during mineralization

This table summarizes the salient features of the three main types of physiological, developmental cell death according to Schweichel and Merker⁵, Clarke⁶ and Zakeri *et al.*⁷

were phagocytosed by the neighboring cells. The second type (type 2) was characterized by the early appearance of large inclusions in the cytoplasm derived from autophagic vacuoles or autolysosomes. Later, swelling of cavities was observed and the dying cells were ultimately fragmented and were phagocytosed by the neighboring cells. This type of cell death was found in regions where tissue areas or anlagen of organs were removed *in toto*. The third type (type 3) was characterized by the swelling of cavities with a membrane border, such as mitochondria, followed by extensive fragmentation of the cells into fragments so small that cell debris can no longer be observed. This type of cell death occurred without the lysosomal system taking part and with recognizable reaction of the neighboring cells, and was observed in regions of vacuolated cartilage during mineralization. Apparently, type 1 physiological cell death is identical to apoptosis described by Kerr *et al.*¹ On the contrary, type 2 and type 3 physiological cell deaths, according to morphological criteria, would be classified as necrotic. The validity of this classification proposed by Schweichel and Merker was later confirmed in the worthy reviews of developmental cell death by Clarke⁶ as well as by Zakeri *et al.*⁷ A literature review revealed that type 1, 2 and 3 cell deaths do take place during normal development and that types 1 and 2 are by far the most common forms: similar to type 1 cell death (apoptosis), type 2 cell death characterized by formation of numerous autophagic vacuoles (hence alternatively called 'autophagic degeneration') and by relative paucity of nuclear changes occurred during developmental stages of various species ranging from invertebrates to mammals. There is no doubting the existence of developmental, physiological cell deaths with necrotic-like morphology and almost certainly, such 'developmentally programmed' cell deaths should be under genetic control in a broad sense. An important question to be addressed is whether genetic control of those 'developmentally programmed' cell deaths reside within the cell itself or outside the cell. For instance, assume cells within a tissue fed by a single blood vessel. If the survival of the cells constituting the blood vessel are under cell-intrinsic genetic control and commit suicide at a certain developmental stage, the cells fed by the vessel will die by ischemia closely following the collapse of the feeding vessel. In this case, the death of the cells fed by the vessel is 'developmentally programmed' in the sense that it

reproducibly occurs in the same place at the same developmental stage and is indeed genetically regulated, albeit indirectly. Nevertheless, the cell death is not apparently an active, programmed cell death in the sense that it is not regulated by a cell-intrinsic death program. Thus, the identification of type 2 and type 3 cell death provided compelling *in vivo* evidence of active, programmed cell death with necrotic-like morphology, but the evidence was not conclusive in the absence of direct demonstration that certain genes are involved in their regulation. Clearly, the absence of cellular models has limited the identification of the molecular mechanisms involved in these necrotic-like cell deaths.

Caspase-independent programmed cell death with necrotic-like morphology

Genetic analysis of 'developmentally programmed' cell death in nematode *C. elegans* led to the identification of several genes essential for cell-autonomous death regulation,^{8,9} and was the first direct demonstration that 'developmentally programmed' cell death (apoptosis or type 1 cell death in this case) is indeed 'genetically programmed' within the cells. It subsequently turned out that these genes have mammalian counterparts,^{10–12} suggesting conservation of cell death program from worms to mammals. *ced-3* is one such gene essential for the execution of programmed cell death, and its protein product has homology to a family of mammalian cysteine proteases called caspases.^{10,13,14} The results of extensive studies at first seemed to support the idea that caspases play general and essential roles in mammalian programmed cell death.^{15,16} caspases were activated in virtually all programmed cell death with apoptotic morphology so far examined, except for a very limited number of cases.^{17,18} Furthermore, inhibition of caspase activities by the use of broad-spectrum (general) caspase inhibitors represented by zVAD-fmk and p35 resulted in the inhibition of apoptosis as assessed by the appearance of apoptotic nuclear morphology and oligonucleosomal DNA fragmentation; a biochemical hallmark of apoptosis. These findings established the idea that caspases are critical determinants of apoptosis at least in terms of morphology, but they did not necessarily imply that the process of caspase activation is the sole determinant of life and death decisions in programmed cell death. Indeed, an increasing number of cases are recently

reported where the inhibition of caspase activities in mammalian apoptotic systems fails to prevent cell death itself.

Perhaps the first clear demonstration of caspase-independent programmed cell death is Bax-induced apoptotic cell death. Xiang *et al.*¹⁹ demonstrated that, although Bax does induce caspase activation and apoptotic cell death in a human leukemic cell line Jurkat, the inhibition of caspase activities inhibited only apoptotic morphology and not cell death itself. Bax-induced Jurkat cell death in the presence of a broad-spectrum caspase inhibitor was characterized by dramatic cytoplasmic vacuolation and only partial chromatin condensation and hence looked 'necrotic'. A similar observation was made using cerebellar granule cells.²⁰ Cerebellar granule cells undergo apoptotic cell death in Bax-dependent manner when cultured in medium with low potassium concentration. Again, caspase inhibitors did inhibit typical apoptotic nuclear changes associated with this potassium deprivation-induced cell death, but not cell death itself. Since Bax-induced cell death is inhibitable by broad-spectrum caspase inhibitors at least in some other cell types,^{21,22} these findings suggest that Bax can activate a caspase-independent program that results in non-apoptotic form of cell death, in a cell type-dependent manner. The ability to trigger non-apoptotic form of cell death was not unique to Bax, as demonstrated by the subsequent observation made by McCarthy *et al.*²³ In their report, another Bcl-2 family member Bak, oncogene products such as Myc and E1A, induce typical apoptotic cell death with the evidence of caspase activation. However, in the presence of a broad-spectrum caspase inhibitor, they all induced cell death accompanied by cytoplasmic vacuolation and partial chromatin condensation. Importantly, both cell death and associated morphological changes were inhibited by insulin-like growth factor (IGF)-1 and Bcl-2, indicating that cell death is under the regulation of intracellular survival signaling rather than a result of non-specific perturbation of cellular homeostasis. Adding to these observations, it was also demonstrated that, in cytotoxic T lymphocyte (CTL) granule exocytosis-mediated apoptotic cell death of target cells, broad-spectrum caspase inhibitors inhibit apoptotic nuclear morphological changes but not target cell lysis itself.²⁴ Furthermore, Hirsch *et al.*²⁵ demonstrated that a glucocorticoid receptor agonist dexamethasone, a topoisomerase II inhibitor etoposide, and permeability transition (PT)-inducers such as mCCCp and PPIX induce apoptotic cell death associated with caspase activation in murine thymocytes but, in the presence of a broad-spectrum caspase inhibitor, cell death with a morphology indistinguishable from necrosis (vacuolization, mitochondrial hydrops, lack of pronounced chromatin condensation). Brunet *et al.*²⁶ also confirmed using a lymphoblastoid cell line that dexamethasone induce, in the presence of a broad-spectrum caspase inhibitor, cell death with only partially condensed chromatin, which was inhibitable by Bcl-2 overexpression. Amarante-Mendes *et al.*²⁷ showed that staurosporine, a protein kinase inhibitor, induces caspase activation and apoptotic cell death in Jurkat cells which can be inhibited by the overexpression of Bcl-2 or Bcr-Abl, but that it induces in the presence of a broad-

spectrum caspase inhibitor a 'necrotic-like' cell death characterized by extensive cellular vacuolization. Deas *et al.*²⁸ showed that anti-CD2 treatment induces apoptotic cell death in human peripheral T lymphocytes but induces, in the presence of broad-spectrum caspase inhibitors, cell death characterized by cytoplasmic vacuolization due to dilated endoplasmic reticulum and by partial condensation of nucleus. Also Fas can induce 'necrotic-like' cell death, as demonstrated by Vercammen *et al.*²⁹ Treatment with an agonistic Fas antibody induced apoptotic cell death in L929 murine fibrosarcoma cell line in Fas-dependent manner. Surprisingly, pretreatment of the cells with a caspase inhibitor suppressed apoptotic morphology but facilitated Fas-mediated cell death, and the dying cells assumed a necrotic morphology characterized by intact nucleus and cytoplasmic swelling. Consistent with this, Kawahara *et al.*³⁰ reported that enforced oligomerization of FADD induces cell death with necrotic morphology (swelling of cells without nuclear condensation) in Jurkat cells lacking caspase-8 or in the presence of a broad-spectrum caspase inhibitor, although in Jurkat cells, Fas-induced cell death was completely inhibited by caspase inhibition in contrast to the Fas-induced cell death in L929 cells.

Collectively, these cases demonstrate that quite a broad range of classical (or established) triggers of apoptosis and programmed cell death induce necrotic-like cell death characterized by cytoplasmic vacuolation and by minimal nuclear changes when caspase activities are inhibited. This implies that triggers of programmed cell death can activate caspase-independent cell death programs encoding necrotic (at least non-apoptotic) morphology together with caspase-dependent apoptotic program, and the apparently growing list of caspase-independent programmed cell death strongly suggests that this is not an exceptional, but rather a common event. The demonstration that what has been believed to be programmed cell death has a 'necrotic' face under the mask of 'apoptosis' is quite an impact on current cell death concepts and brings home the inevitable necessity of reassessing 'necrotic-like' cell death as programmed cell death.

Although the above instances were events occurring under a very specific condition whereby caspase activities are artificially inhibited, there are also reports of programmed cell death that originally assumes necrotic (or non-apoptotic) morphology. One such representative case may be tumor necrosis factor (TNF)-induced cytotoxicity. It has been well documented that TNF induces not only apoptotic cell death but also cell death with necrotic morphology depending on the cell type.³¹ Apparently, TNF-induced necrotic-like cell death is not a passive one occurring as a result of non-specific perturbation of cellular homeostasis, because TNF-induced necrotic-like cell death is dependent on the expression of TNF receptors and on the intact downstream intracellular signaling pathway most likely involving generation of reactive oxygen species.³¹ It has been recently demonstrated that this TNF-induced necrotic-like programmed cell death occurs independently of caspase activation.³² Another example is glutathione depletion-induced cell death of a neuronal cell line which takes the form of necrosis and yet can be inhibited by Bcl-

2.^{33,34} It remains to be shown whether caspases play a role in this necrotic-like cell death, however. Lowering of intracellular pH has been shown to effectively kill tumor cells^{35,36} and Zanke *et al*³⁷ recently demonstrated that this intracellular acidification-induced cell death occurs without any morphological changes characteristic of apoptosis or any evidence of caspase activation and that it is resistant to caspase inhibition and Bcl-2 overexpression. Importantly, however, intracellular acidification-induced Jun N-terminal kinase (JNK) activation was essential for this cell death, indicating that it is regulated by JNK-mediated intracellular signaling. It has been recently reported that the expression of PML (promyelocytic leukemia) cellular gene product can induce cell death either with apoptotic or non-apoptotic nuclear morphology depending on the cell type.^{38,39} PML-induced cell death of rodent fibroblasts³⁹ was accompanied by cellular shrinkage, but the dying cells showed neither chromatin condensation nor nuclear fragmentation. There was no evidence of caspase activation, and caspase inhibition failed to protect cells from PML-induced death.

Altogether, these observations from *in vitro* experimental systems strongly argue for the existence of caspase-independent programs for necrotic-like cell death, and that they can be activated either alone or in concert with the caspase-dependent apoptotic program.

Ras-mediated activation of intracellular signaling leads to necrotic-like cell death with characteristic features of physiological cell death

As described so far, morphological analysis of developmental cell death *in vivo* suggested that cell death with necrotic morphology may be active and programmed, and recent reports of caspase-independent cell death in *in vitro* systems suggested that genetically-regulated programmed cell death may assume necrotic morphology. However, pieces are lacking which would definitively link these *in vivo* and *in vitro* lines of evidence for 'necrotic-like' programmed cell death, and consequently, informative correlation has not yet been established between them. In other words, it is not yet clear from the currently available data whether the 'necrotic-like' programmed cell deaths observed in *in vitro* systems have characteristic features of the necrotic-like physiological cell deaths occurring *in vivo*, or whether the genes activating necrotic-like programmed cell *in vitro* also have a role in the regulation of necrotic-like cell deaths *in vivo*. Here, our recent demonstration that the activation of Ras signaling pathway leads to type 2 physiological cell death (autophagic degeneration), the most common form of developmental cell death with necrotic morphology, may serve as one such important piece.⁴⁰

That activated Ras signaling pathway causes cell death with a unique morphology was found while we were exploring the reason why activating mutation of Ras, which is very common in other tumor types, is extremely rare in glioma.⁴¹ In an attempt to elucidate the reason, we transiently transfected oncogenically activated H-ras into human glioma cells, and found that activated ras-transfected but not control-transfected glioma cells degenerate accompanied by cytoplasmic vacuolation. The results

of the transient transfection assays were confirmed by the use of stable glioma transfectants that inducibly express wild-type (wt), activated, and dominant-negative H-Ras proteins by tetracycline removal. Induction of activated Ras (RasV12) in the stable glioma transfectants caused cell death accompanied by cytoplasmic vacuoles as in transient transfection assays. Wt-Ras also induced cell death albeit much less efficiently, and cell death was not induced in control transfectants or transfectants inducibly expressing dominant-negative form of Ras. These results clearly indicated that Ras-mediated activation of intracellular signaling pathway is responsible for cell death induction. In support of this, we have recently found that phosphatidylinositol 3-OH kinase (PI3K), a physiological effector of Ras,⁴² plays an essential role in this Ras-mediated cell death (unpublished observation). Morphological analysis under phase-contrast microscope revealed that, in the early stage of degeneration, the nucleus is intact and that vacuoles and/or granules appear in the perinuclear region (Figure 1B). With the progression of degeneration, the vacuoles grow larger (Figure 1C), and in the late stage, degenerating cells may either remain attached (Figure 1D) or become rounded up or fragment into small pieces (Figure 1E and F). The nuclear morphology is relatively well preserved compared to the prominent cytoplasmic change. These morphological changes associated with activated Ras-mediated cell death were clearly distinct from those of apoptosis. The morphological changes were further examined by electron microscopy. The degenerating cells in the advanced stage showed prominent cytoplasmic change characterized by numerous vacuoles but minimal change in nuclear morphology. Upon closer inspection, some vacuoles appeared to contain cytoplasmic components suggesting that those vacuoles were derived from autophagosomes, and there was no evidence of mitochondrial swelling (Figure 2, Tc (–) Day 5).

These microscopic findings suggested that activated Ras may induce programmed cell death by autophagic degeneration (type 2 cell death) and not by apoptosis. To establish this point, we next examined by electron microscopy the early stage of cell degeneration caused by activated Ras, and we identified primary activation (increase in number and size) of lysosomal apparatus containing electron-dense material (Figure 2, Tc (–) Day 1.5), a characteristic feature distinguishing autophagic degeneration from other types of physiological cell death.^{5,7} We also examined the degenerating cells by immunocytochemistry using lysosomal membrane markers and confirmed that most of the vacuoles are derived from lysosome.⁴⁰ Furthermore, when involvement of caspases was examined, this activated Ras-induced cell death was neither accompanied by caspase activation nor inhibited by broad-spectrum caspase inhibitors.⁴⁰ Collectively, the results indicated that: (i) activation of Ras signaling induces a caspase-independent programmed cell death, (ii) the cell death corresponds to autophagic degeneration (type 2 cell death) observed during normal development. These results therefore provide evidence that autophagic degeneration is under a cell-intrinsic genetic control, molecularly distinct from that of apoptosis.

Recognizing active, programmed cell death among cell death with necrotic morphology: its necessity and significance

Under 'apoptosis vs necrosis' classification, cell death is generally divided into two groups: (i) active, programmed cell death having apoptotic morphology, and (ii) passive,

uncontrolled cell death having necrotic morphology. However, accumulating *in vivo* and *in vitro* findings presented in the literature as well as in this article, apparently suggest the existence of exceptions to this classification, dictating reassessment of 'necrotic cell death' as a heterogeneous entity containing both active and passive cell deaths (Figure 3). At least, type 2 cell death (autophagic degeneration),

Figure 1 Morphology of Ras-mediated U251 human glioma cell death under phase-contrast microscope. U251TA-RasV12 cells are a stable U251 transfectant which inducibly expresses activated H-Ras (RasV12) by tetracycline removal. (A) U251TA-RasV12 stable glioma transfectant cultured in the presence of tetracycline (activated Ras expression suppressed). (B–F) U251TA-RasV12 cells were cultured in the absence of tetracycline (activated Ras expression induced) for 2 days (B), 3 days (C), and 4 days (D–F). Scale bar=25 μ m

Figure 2 Transmission electron microscopic analysis of Ras-mediated cell death. U251TA-RasV12 cells were cultured in the absence of tetracycline (activated Ras expression induced) for 1.5 days (Tc(-), Day 1.5), 5 days (Tc(-), Day 5), or in the presence of tetracycline (activated Ras expression suppressed) (Tc(+)). Scale bar=1 μ m

which would be categorized into 'necrosis' under 'apoptosis-necrosis' classification, needs to be distinguished from other cell deaths showing necrotic morphology. The existence of type 2 cell death itself was once questioned, and it was claimed that what appears to be type 2 cell death is actually a cell phagocytosing apoptotic cells (bodies).² But now, our *in vitro* data indicate that type 2 cell death does exist as an active, programmed cell death having a distinct regulatory mechanism from that of apoptosis. On the other hand, whereas most of programmed cell deaths with necrotic morphology seem to be independent of caspase activation as shown above, there is also a report of cell death with necrotic morphology in which caspases play a regulatory role.^{43,44} Thus, programmed cell death with necrotic morphology may be further divided into two subgroups according to the involvement of caspases in cell death regulation.

The significance and importance of recognizing active, programmed cell deaths among cell deaths with necrotic morphology and elucidating the death programs involved (which is most likely caspase-independent) will be most apparent in the medical aspect of cell death research. Reflecting the fact that programmed cell death plays a critical role in the maintenance of tissue homeostasis, dysregulation of programmed cell death has been demonstrated to result in various pathological conditions.⁴⁵ For instance, neurodegenerative disorders are regarded as a representative of diseases caused by excessive programmed cell death. Great efforts have been made to

identify apoptotic processes in neurodegenerative disorders with the intention to control disease progression by inhibiting apoptosis. However, conclusive evidence is yet to be obtained that apoptosis is solely responsible for the excessive cell death observed in these diseases.^{46,47} Intriguingly, postmortem electron-microscopic analysis of human brain affected with neurodegenerative diseases such as Alzheimer's and Parkinson's diseases revealed that the dying neurons showed morphological changes compatible with autophagic degeneration.⁴⁸⁻⁵⁰ Such observations suggest the possibility that caspase-independent cell death program may be operating in those diseases, and in that case, elucidation of the caspase-independent cell death program is essential for rescuing neurons from pathological cell death. Even in diseases where the affected cells have been shown to die with 'apoptotic' morphology, one cannot exclude the possibility that caspase-independent cell death program is operating in concert with caspase-dependent apoptosis program, as demonstrated by the instances of caspase-independent cell death reviewed in this article. In such cases, inhibition of apoptosis by caspase inhibition would only delay, but not prevent, the progression of diseases. On the other hand, cancer is a representative of diseases caused by inappropriate survival of preneoplastic cells that would be normally disposed through activation of cell death program. Activation of a suicide program in tumor cells should therefore be a straightforward approach of cancer therapy, and attempts have been made to kill tumor cells via

Figure 3 Morphological and functional classifications of cell death proposed in this article. Cell death is in general divided into two groups according to their morphology, 'apoptotic' and 'necrotic'. It can be further classified into 'programmed' and 'unprogrammed' according to the presence or absence of underlying regulatory mechanisms, and 'programmed' cell death is subdivided according to its dependence on caspases

apoptosis. However, it needs to be remembered that cancer is so often made up of selected population of cells that have gained resistance against apoptosis.^{51,52} In this respect, if tumor cells have cell death programs other than 'apoptotic' program, activation of such programs would be quite beneficial and become a promising therapeutic approach that complements apoptosis-based therapies. Thus, identifying active, programmed cell deaths among cell deaths with necrotic morphology that would otherwise have been dismissed as passive, unprogrammed ones, together with the elucidation of their underlying mechanisms, is not only important for the comprehensive understanding of programmed cell death itself but has potential practical implications in the treatment of human diseases.

Concluding remarks

With the recognition of 'necrotic-like' programmed cell death, molecular study of 'necrotic death program' has just emerged with only a handful of information but with plentiful possibilities. Much remains to be done before this field is widely accepted as the current apoptosis research is. For instance, it would be important to demonstrate that factors which have been shown to induce necrotic-like cell death in *in vitro* systems are involved in necrotic-like cell deaths occurring under physiological and pathological conditions. Similarly important is further identification of genes or factors that are essential for necrotic-like cell death *in vivo* as well as in *in vitro* systems. Although we have mainly discussed necrotic-like programmed cell death in mammalian systems, no one would cast doubt on the power of genetic approaches utilizing worms and flies for the above purposes, as exemplified by the apoptosis research. Indeed, there are some worm models of necrotic-like cell death,^{53–58} and it has been recently reported that adenylyl cyclase is essential for G protein hyperactivation-induced necrotic-like neuronal degeneration in *Caenorhabditis elegans*.^{59,60} Developing animal models of necrotic-like cell death would thus be of great help in advancing our knowledge of molecular regulation of necrotic-like cell death as it was for apoptotic cell death.

Finally, what is most vital for the promotion of this new-born research field is that researchers become fully aware of the existence of 'necrotic-like' programmed cell death and have keen intention to elucidate the regulatory mechanisms behind their 'necrotic' faces which might have been neglected for a long time just because they look 'necrotic'.

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