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Surviving the kiss of death

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Abstract

Executioner caspases induce the biochemical and cellular changes characteristic of apoptosis. Activation of caspases is therefore regarded as ''the kiss of death'' resulting in the cell's demise. Recent reports indicate however that in some situations, caspase activation may induce other responses than apoptosis. These findings raise the question of how cells manage to counteract the killing activities of executioner caspases. Experiments performed in our laboratory have unraveled a mechanism that allows cells to survive in the presence of activated executioner caspases. This mechanism is based on the partial cleavage of RasGAP into an N-terminal fragment that activates the Ras–PI3K–Akt survival pathway. This protective pathway may be activated to allow cells to use executioner caspases for other purposes than inducing apoptosis.

Keywords: Apoptosis; Caspases; RasGAP; Stress; Survival pathways

''Caspases: the executioners of apoptosis'', ''Caspases: killer proteases'', ''Caspases: enemies within'' sound like titles of detective stories but are in fact those of reviews on caspases published at the end of the 1990s [1–3]. These titles were carrying the message that activation of caspases is a lethal event that cannot be avoided by the cell. Indeed, most, if not all, of the biochemical and cellular features of apoptosis are induced when executioner caspases (i.e. caspase-3, -6, and -7) cleave their substrates. For example, DNA fragmentation seen in apoptotic cells is a consequence of the inactivation by proteolysis of the inhibitor of CAD, the DNAse that cuts the genome into 200 bp multimeric fragments [4,5]. Consistent with the crucial role of caspases in the induction of apoptosis are the numerous observations that inhibition of caspases with chemical compounds or with natural inhibitors (e.g. IAPs or Bcl2 family members) efficiently blocks the death of cells in most situations. But does activation of the ''killer'' caspases always condemn cells? The answer appears to be ''not always''.

1. Involvement of caspases in non-apoptotic processes

Initially welcomed with skepticism, the notion that the very same caspases that participate in the induction of apoptosis can, in certain conditions, fulfill other cellular functions is now accepted by many researchers. Killer caspases have been shown to be implicated in the differentiation and function of several cell types including hematopoietic cells, muscle cells, skin cells and neurons [6–8]. For example, inhibition of caspase activity prevents CD3-induced T cell proliferation and interleukin 2 production [9], inhibition of caspase 3 prevents myotube formation and expression of muscle-specific proteins [10], inhibition of caspase-3 also hampers chemotropic responses in neurons [11], and terminal differentiation of keratinocytes is blocked by caspase inhibitors [12]. An important question raised by these studies is how can cells having activated their ''killer'' caspases survive? Obviously, the extent of activation of the caspases is a critical factor that will surely determine whether a cell should survive or commit suicide. But what is the mechanism that allows a cell to withstand mild caspase activation? Are the cells intrinsically resistant to low levels of executioner caspase activity or is there a survival mechanism that is turned on in response to mild caspase activation? The latter possibility is supported by experiments performed in our laboratory showing that a

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protective pathway is activated by the cleavage of a peculiar caspase-3 substrate called RasGAP.

2. The amino-terminal RasGAP caspase-generated fragment induces the activation of the Ras–PI3K–Akt survival pathway

RasGAP is a 120 kDa protein bearing a GTPase-activator protein (GAP) domain at its C-terminal end and several SH domains at its N-terminus. The GAP domain of RasGAP amplifies the intrinsic GTPase activity of Ras, thereby favoring the inactivation of Ras [13]. In contrast, the SH domains of RasGAP positively participate in the induction of Ras effector pathways [14]. RasGAP, therefore, regulates the function of Ras in a complex manner, but the way it does so is still poorly understood.

In 1998, two independent laboratories discovered that RasGAP is a caspase target that is cleaved in apoptotic cells [15,16]. RasGAP bears two caspase-3 consensus cleavage sites that are sequentially targeted by the caspases as their activity augments [17]. The first cleavage site, located at position 455, is used at very low caspase activity, while the second, located at position 157, is only used at higher caspase activity and only if the first cleavage event has occurred [17] (Fig. 1). It appears that caspase-3 is the only caspase whose presence is critical for the cleavage of RasGAP because fibroblasts derived from mouse lacking caspase-3 are unable to process RasGAP in response to apoptotic stimuli.

The initial assumption was that cleavage of RasGAP, like the cleavage of the dozen other caspase substrates described to date [8], would generate fragments with pro-apoptotic properties. Unexpectedly, the N-terminal fragment generated after the first cleavage of RasGAP (thereafter called fragment N) protected cells from apoptotic stimuli when over-expressed in cells [17]. Fragment N ectopically expressed in cells activates the Ras–PI3K–Akt pathway [18]. Blocking the activity of any protein of this pathway with the corresponding dominant-negative mutants abrogated the protection conferred by fragment N [17]. These findings raised the possibility that caspase-3, by producing fragment N, is able to induce a survival pathway in cells. But if this is true, there must be situations where caspase-3 is activated and fragment N generated in cells that do not undergo apoptosis. As we will now see such situations do indeed exist.

3. Surviving the presence of activated "killers"

In response to mild stress (e.g. low concentrations of FasL or cisplatin), cells very weakly activate caspase-3.

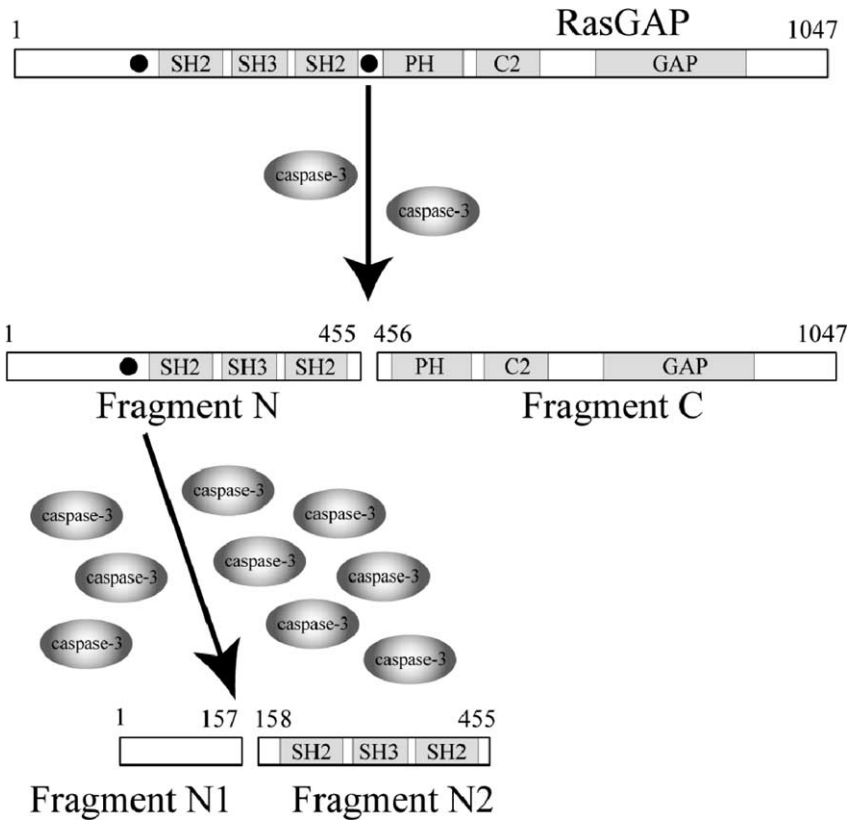

Fig. 1. Schematic representation of RasGAP cleavage by caspases. RasGAP bears two caspase cleavage sites (indicated by the black dots). At low caspase activity, RasGAP is cleaved into fragment N and fragment C. At higher caspase activity, fragment N is further cleaved into fragments N1 and N2. Note that the cleavage at positions 455–456 is mandatory for cleavage at positions 157–158 to occur [17]. SH, Src homology domain; PH, plekstrin homology domain; C2, Calcium-dependent phospholipid binding domain; GAP, GTPase-activating protein domain.

Detection of active caspase-3 in cells subjected to these mild stress stimuli is not due to a low percentage of cells that are undergoing apoptosis because the clonogenicity potential of the cells is not affected by the presence of the mild stress stimuli (a cell able to form a clone is obviously not undergoing apoptosis). Therefore, in response to a mild stress, cells can activate caspase-3 to a limited extent without committing suicide. But what about the caspase substrates? Are they cleaved when caspase-3 is mildly activated? The answer is yes for some and no for others. Cleavage of the inhibitor of CAD in mildly stressed cells is not detectable for example. If it were the case, it would be difficult to comprehend why cells survive the cleavage of their DNA by CAD. In contrast, RasGAP is cleaved in mildly stressed cells but this cleavage is partial because fragment N is not further processed despite bearing a caspase-3 consensus site at position 157. Formation of fragment N in mildly stressed cells depends on caspase activity because caspase inhibitors block its generation. In certain situations, therefore, cells can activate caspase-3 inducing the cleavage of RasGAP into a fragment displaying an anti-apoptotic activity. But is this important for the survival of cells subjected to adverse conditions?

4. Formation of fragment N is a prerequisite for cell survival in mild stress conditions

It is not trivial to assess the importance of the cleavage of a given caspase substrate in the regulation of apoptosis because of the difficulty of selectively blocking the cleavage of one substrate without affecting the cleavage of all the others. One of the best approaches is to replace the wild-type form of the substrate with a mutant bearing a point mutation in the consensus caspase cleavage site so as to prevent its processing. Surprisingly, despite the existence of more than 280 caspase substrates [8], this approach has only been used for two of them: Rb and PARP [19,20]. The results of such experiments indicated that cells unable to cleave Rb or PARP were more resistant to some, but not all, apoptotic stimuli. Cleavage of Rb and PARP is therefore required for optimal death in response to specific apoptotic stimuli.

We have recently generated cells in which the wild-type RasGAP protein was replaced by a mutant that cannot be cleaved by caspases. When subjected to a mild stress, and in contrast to control cells, cells expressing the uncleavable form of RasGAP could not activate Akt, could not prevent an amplification of the caspase-3 activity, and eventually underwent apoptosis. Ectopic expression of fragment N in these cells restored their resistance towards mild stresses. Formation of fragment N in response to low caspase-3 activation is therefore critical for survival of stressed cells. An important conclusion drawn from these experiments is that even if other anti-apoptotic fragments are generated by mild activation of caspases, they cannot compensate for the lack of RasGAP cleavage. Remarkably therefore, the negative feedback control of caspases following their mild activation cannot operate if only 1 protein among the 280 described caspase substrates is not cleaved [8]. The other conclusion is that cells are not intrinsically resistant to mild caspase activation. Rather, executioner caspases control the extent of their own activation by a feedback regulatory mechanism initiated by the partial cleavage of RasGAP. This allows cells to withstand the presence of activated caspases, which have then the potential to fulfill other functions than the induction of apoptosis.

5. Puzzling results that make sense now

In 1998, the laboratory of Peter Vandenabeele published an article demonstrating that, in contrast to the accepted dogma, inhibition of caspases can be detrimental for some cells. They used L929 fibrosarcoma cells that rapidly die by necrosis in response to tumor necrosis factor (TNF). Necrosis is a form of cell death distinct from apoptosis that may not require caspases to occur. However, inhibition of caspases, either with chemical inhibitors or with CrmA, a caspase inhibitor of viral origin, rendered L929 cells 1000-fold more sensitive to TNF-induced death [21].

Importantly, similar observations were gathered in an in vivo mouse model. Injection of TNF in mice induces damages in multiple organs resulting in a shock response that can cause the death of the animal. This TNF toxicity is greatly exacerbated when the cytokine is co-injected with caspase inhibitors, resulting in hyperacute hemodynamic collapse, kidney failure and rapid death [22].

The importance of caspase in cell survival has also been suggested in another in vivo model of preconditioning in neurons. In this model, mice exposed to a 10 min middle cerebral artery occlusion, followed by a permanent occlusion 1 day later, show reduction of at least 50% in the infarct volume compared to mice that were not subjected to the brief ischemic episode. The protection conferred by the 10 min middle cerebral artery occlusion was found to be associated with induction of caspase-3 activity. In vitro, stressing neurons with KCN, an inhibitor of oxidative phosphorylation, rendered the cells more resistant to subsequent excitotoxic doses of NMDA. The protective response induced by KCN was blocked if caspases were inhibited [23].

The molecular basis of the protection induced by executioner caspases is not clear at the present time. However, based on our work on RasGAP, we can now provide a plausible mechanism that can explain the paradoxical role of caspases in cell survival. In the case of TNF toxicity, we suggest that TNF can induce two cellular responses in the cell types that are more sensitive to TNF-induced death when caspases are inhibited: induction of necrosis and weak stimulation of caspases (but the activation of these proteases is not required for necrosis to occur). What the

caspases would do however is to induce the generation of the anti-apoptotic RasGAP fragment N. Blocking caspases in this system would therefore abrogate the survival pathway and render cells extremely sensitive to TNF-induced death. Other stresses, such as the brief ischemia mentioned above, induces caspases activation, favoring the formation of the anti-apoptotic fragment N derived from RasGAP. We therefore postulate that some preconditioning events critically require activation of caspases to generate fragment N that will induce an anti-apoptotic survival signal.

A series of diseases (e.g. amyotropic lateral sclerosis and Alzheimer's disease) appear to result from inappropriate cell death in specific organs. Blocking caspases in this context could be beneficial for the patients. However, the likelihood that executioner caspases are protective in some situations indicates that the use of their inhibitors in clinical applications may have detrimental effects. Evaluation of the anti-apoptotic and pro-apoptotic functions of caspases in a given disease should therefore be critically evaluated before treating patients with caspase inhibitors.

6. Role of RasGAP cleavage fragments in the induction of apoptosis

So far we have mainly discussed the anti-apoptotic function of fragment N that is generated when RasGAP is partially cleaved by low levels of caspase activity. But what is the function of the second cleavage event occurring when caspase activity increases inducing the formation of two smaller N-terminal fragments named N1 and N2 [17] (Fig. 1). To address this question, we have generated cells in which the wild-type RasGAP protein was replaced with a RasGAP mutant that cannot be cleaved at position 157 and therefore can be processed into fragment N and fragment C but not further into fragments N1 and N2. These cells were found to be significantly more resistant to a series of apoptotic stimuli, including cisplatin, FasL, staurosporine and UV. The second cleavage of RasGAP therefore fulfills the function of the cleavage of most caspase substrates, which is to promote the efficient dismissal of cells. How the second cleavage of RasGAP sensitizes cells towards apoptosis is not fully understood. It seems not to be solely due to the disappearance of the anti-apoptotic N fragment, because the two polypeptides (fragments N1 and N2) resulting from the cleavage of fragment N at position 157 have the capacity to potentiate apoptosis when over-expressed in cells [17].

7. Potential therapeutic applications

Some of the properties of the RasGAP fragments generated by caspases could be exploited in clinical applications. For example, the ability of fragment N to render cells more resistant to stresses could be useful in the context of transplantation. There are indeed some organs that experience high cell death rate after the surgical procedure (e.g. pancreas transplantation). Introducing fragment N in the cells of an organ could reduce cell loss when the organ is transplanted and improve its functionality in its new host.

The pro-apoptotic activities of the smaller N-terminal fragments of RasGAP (e.g. fragment N2) can also potentially be exploited to generate interesting therapeutic tools. Expression of fragment N2 in cells does not by itself induce apoptosis. However, tumor cells expressing fragment N2 are more sensitive to genotoxin-induced death [17]. Importantly, fragment N2 does not sensitize non-tumor cells towards genotoxin-induced death. Therefore, if a chemical compound bearing the activity of fragment N2 could be administered to patients bearing tumors, this could augment the efficacy of chemotherapies. We have narrowed down the genotoxin-sensitizing property of fragment N2 to a 10 amino acid sequence. This sequence when fused to a cell permeation sequence efficiently entered tumor cells and sensitized them to the action of various genotoxins, including cisplatin, adriamycin and mitoxantrone. Satisfactorily, this peptide did not render human primary cells more sensitive to genotoxin-induced death. We are currently testing the ability of this peptide to increase the antitumorigenic property of genotoxins in tumor mouse models.

8. Conclusion

As sometimes occurs in science, seemingly well-understood proteins turn out to fulfill additional unanticipated functions. A famous example is cytochrome c, a protein known for its role in oxidative phosphorylation in mitochondria, which was found to be a critical inducer of cell death when released in the cytosol. As discussed in this commentary, the functions of the downstream caspases are being revisited. Initially considered as the executioner swords that were inducing irreparable modifications in cells inducing their death, they now appear to show mercy when it is appropriate to protect cells from adverse or stressful conditions. These caspases not only allow cells to survive in some conditions but they seem also to be crucially required for non-apoptotic cell responses including differentiation and proliferation. Our work shows that the protective functions of executioner caspases rely on their ability to cleave RasGAP, a protein discovered more than 15 years ago [24] that slowly reveals its multifaceted talents.

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