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A subset of caspase substrates functions as the Jekyll and Hyde of apoptosis



Abstract

Cleavage of caspase substrates is believed to be the commitment point that will lead a cell towards apoptosis. While the cleavage of some caspase substrates participates directly in the dismantling of the cell, others regulate the extent of caspase activation. In this communication, we discuss some recent findings indicating that two caspase substrates, MEKK1 and RasGAP, change their functions from anti- to pro-apoptotic as caspase activity increases. MEKK1 is a MAPK kinase kinase regulating the JNK MAPK pathway. As a full-length protein,

MEKK1 generates protective signals (e.g. in cardiomyocytes), but potentiates apoptosis when cleaved by caspases. This switch is mediated by a translocation of the kinase activity from insoluble to soluble cellular structures. RasGAP is a regulator of Ras GTPase family members. As a full-length protein, RasGAP does not modulate apoptosis. However, low caspase activity readily induces the cleavage of RasGAP into an N-terminal fragment that generates potent anti-apoptotic signals. At higher caspase activity, the N-terminal fragment is further cleaved into two fragments that strongly potentiate apoptosis. RasGAP can, thus, be viewed as an 'apoptostat' because it allows the cells to determine when caspases have been mildly activated to fulfill functions other than apoptosis or when caspases are strongly activated to mediate apoptosis.

Full text

Introduction

Apoptosis is the process by which cells enter programmed cell death, a vital phenomenon that takes place during development, and is essential for the maintenance of homeostasis. The biochemical event that is believed to irreversibly commit a cell to apoptosis is the activation of caspases (cysteine proteases cleaving after aspartic residues). Cells undergoing apoptosis display characteristic morphological and biochemical changes, including membrane blebbing, cell rounding, chromatin condensation, DNA cleavage, expression of apoptotic markers at the cell surface and inhibition of anti-apoptotic signaling pathways. All these events can be blocked by specific caspase inhibitors. It is thus the cleavage of the caspase substrates that is responsible for most, if not all, of the characteristic changes observed during apoptosis.

Mitogen-activated protein kinase (MAPK) pathways have been shown to regulate apoptosis in a positive or negative manner [1, 2]. This could explain why the apoptotic caspases target some of the signaling proteins that regulate MAPK and/or are components of MAPK pathways [3]. These proteins include MEKK1, PAK2, RasGAP, and Mst1. Work from our laboratory and others has demonstrated the critical roles of two of these proteins, MEKK1 and RasGAP, in regulating the mechanisms that control apoptosis.

MEKK1

Protective functions of MEKK1

MEKK1 is a protein kinase of about 200 kDa that is an upstream component of the c-Jun N-terminal kinase (JNK) MAPK pathway. MEKK1 is implicated in JNK activation mediated by some, but not all, stimuli. For example, cells devoid of MEKK1 cannot stimulate the JNKs in response to microtubule disruption, while they can generate a perfectly normal JNK activation in response to anisomycin [4].

The observation that cells lacking MEKK1 are much more sensitive to stimuli inducing cell death [4, 5] indicates that MEKK1 fulfills protective functions. For example, MEKK1^{-/-} embryonic stem cells are extremely fragile and have enhanced sensitivity to apoptotic agents. MEKK1 also mediates critical protective functions in cardiomyocytes. It is possible to derive cardiac myocytes from embryonic stem (ES) cells by introducing into these cells, a drug-resistance gene under the control of a cardiomyocyte-specific promoter and incubating the cells in a differentiation medium containing the drug. Using this strategy, it has been shown that ES cell-derived cardiac myocytes (ESCM), lacking the MEKK1 gene, are extremely sensitive to hydrogen peroxide-induced apoptosis. Why are these cells more sensitive towards apoptosis? TNF- α is produced by cardiomyocytes in response to oxidative stress and this is mediated by the p38 MAPK pathway. ESCM lacking MEKK1 activate p38 MAPKs normally but fail to activate the JNK MAPKs. The inability of the MEKK1^{-/-} ESCM to stimulate the JNK MAPK pathway is the reason for their susceptibility to oxidative stress-induced apoptosis, because the JNKs antagonize the p38 MAPK pathways and consequently there is excessive TNF- α production. Thus, MEKK1 activation of the JNK MAPK pathway promotes the survival of cardiac myocytes by inhibiting the production of a pro-apoptotic cytokine.

Pro-apoptotic functions of MEKK1

MEKK1 is a caspase substrate cleaved when cells undergo apoptosis [6, 7]. This cleavage event converts the full-length protein into a 91 kDa kinase-containing fragment that further stimulates the activation of caspases and consequently apoptosis [7]. While it is not totally understood why the cleavage of MEKK1 converts it into a pro-apoptotic molecule, we know now that this switch is mediated by sub-cellular translocation events [8]. The full-length MEKK1 protein is associated with insoluble cytoplasmic structures. Upon cleavage by

caspases, the kinase domain of MEKK1 is released into a soluble cytoplasmic compartment. Preventing the translocation of the kinase domain to this soluble compartment by tethering it to membranes abrogates the pro-apoptotic function of MEKK1. The effectors of MEKK1 that mediate its pro-apoptotic function need now to be identified. What we know already is that the soluble MEKK1 kinase fragment, whether membrane-tethered or not, equally activates p300, NFkB or JNK [8]. Therefore, the currently characterized MEKK1 effector pathways are either not sufficient to mediate apoptosis induced by MEKK1 caspase cleavage fragments or they are not involved in this response.

RasGAP

RasGAP bears a GTPase-activating domain at its C-terminus that is specific for Ras proteins. Therefore, RasGAP negatively regulates the activity of Ras. On the other hand, RasGAP is apparently required for an efficient Ras response and this effect is mediated by its N-terminally localized SH2 and SH3 domains. Remarkably, and in contrast to MEKK1, full-length RasGAP does not modulate apoptosis (Figure 1). As we will see, RasGAP only regulates the cell death response when it is cleaved by caspases and the way it does so is dramatically altered as the caspase activity increases.

Protective functions of RasGAP

The protective function of RasGAP is revealed when it is cleaved by caspases at position 455, generating an N-terminal fragment (fragment N) and a C-terminal fragment (fragment C). The 455 site is exquisitely sensitive to cleavage, occurring at caspase activities that are not high enough to induce cell death [9]. Expressed alone in cells, fragment C can induce apoptosis, but this effect is totally inhibited by fragment N (*Figure 1*, panel A). Therefore, the cleavage of RasGAP at position 455 does not lead to cell death. Fragment N is not only capable of inhibiting the pro-apoptotic activity of fragment C, it also blocks apoptosis induced by activated caspase 9 (provided that this caspase activity is not too high) [9]. We have recently demonstrated that fragment N protects cells by inducing the Ras-PI3K-Akt pathway [10]. Surprisingly, even though NF-kB can be activated by Akt, it plays no role in the anti-apoptotic functions of fragment N. This indicates that Akt effectors are differentially regulated when fragment N is generated [10].

There are situations where caspases are activated to fulfill other functions than apoptosis. Examples include the control of erythroblast differentiation by the caspase-mediated cleavage of GATA-1, and the activation and proliferation of T cells. In these situations, mechanisms must presumably be activated to prevent apoptosis following caspase stimulation. Generation of fragment N in response to low caspase activity may represent a safeguard mechanism to protect cells in such cases.

Pro-apoptotic functions of RasGAP

As caspase activity increases, fragment N is further cleaved into two fragments called N1 and N2. These, in contrast to fragment N, strongly potentiate the sensitivity towards genotoxin-induced apoptosis (Figure 1, panel B) [9]. Our data support a model where RasGAP functions as a sensor of caspase activity to determine whether or not a cell should survive. When caspases are mildly activated, the partial cleavage of RasGAP protects cells from apoptosis. When caspase activity reaches levels that allow the completion of RasGAP cleavage, the resulting RasGAP fragments turn into potent pro-apoptotic molecules.

CONCLUSION

In Robert Louis Stevenson's book "*The Strange Case of Dr. Jekyll and Mr. Hyde*", the good Dr. Jekyll, as his drug consumption increases, is little by little transformed into Hyde, the monster, and eventually the latter replaces the former. By analogy, as the caspase activity increases, MEKK1 and RasGAP are converted from protecting proteins to cell death promoters. The role played by these two proteins in the regulation of apoptosis reveals that the function of a subset of caspase substrates is not restricted to the promotion of cell dismantling. We anticipate that in the forthcoming years, caspases and their substrates will be found to fulfill more complex functions than originally thought.

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Figures

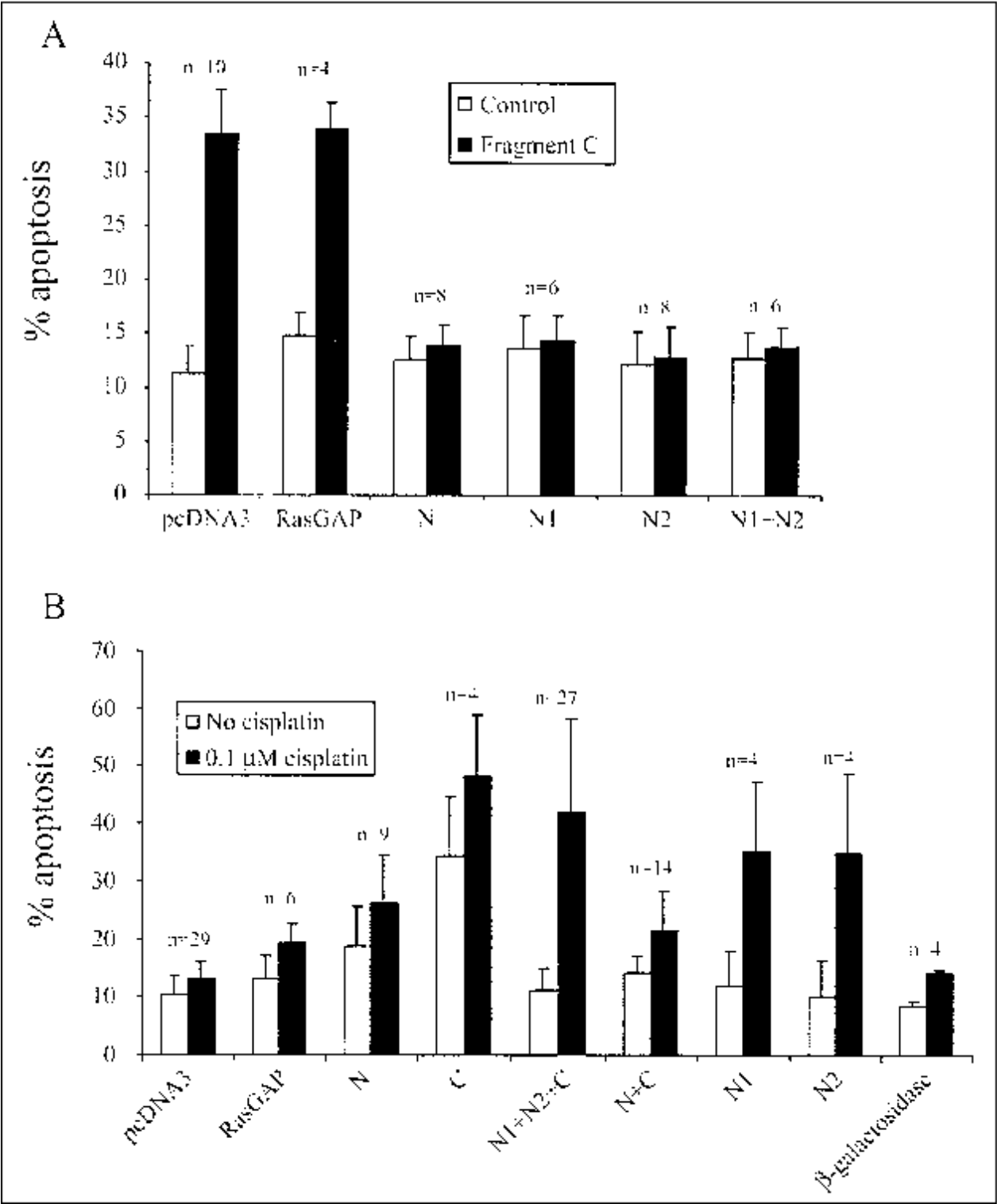


Figure 1 : Full-length RasGAP does not regulate apoptosis.
HeLa cells cultured in RPMI 1640 containing 10 % newborn

calf serum (NBCS) (GIBCO/BRL) at 37°C and 5 % CO₂ were transfected as described [9] with a GFP-encoding plasmid together with empty pcDNA3 vector or with pcDNA3-encoding full-length RasGAP (RasGAP), fragment N (N), fragment N1 (N1), fragment N2 (N2), fragment C (C), b-galactosidase, alone or in the indicated combinations. Apoptosis was determined by scoring the transfected cells (as assessed by the expression of GFP) displaying pycnotic nuclei (visualized with Hoechst 33342) [9]. In panel B, the cells were incubated overnight with or without 0.1 mM cisplatin. Results are expressed as the mean ± SD of the indicated numbers of independent experiments. The N-terminal fragments of RasGAP (N, N1 and N2) were each able to inhibit the apoptotic response induced by fragment C (panel A). In contrast, only fragments N1 and N2 strongly potentiated cisplatin-induced apoptosis (panel B). Remarkably, the full-length RasGAP protein did not modulate the cell death response, either positively or negatively.

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