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News and Commentary

Does prothymosin-α act as molecular switch between apoptosis and autophagy?

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Under physiological and pathological settings, cell death in tissues can be classified into several morphological and biochemical subtypes, the most prominent being type-1 cell death (apoptosis) and type-2 cell death (autophagic cell death, ACD).[1–4] While the molecular mechanisms leading to apoptosis have been dissected to some extent during the past 15 years, ACD is not well characterized at the molecular level yet. An important issue that is under debate now is whether crosstalk between these two pathways exists and how a cell 'decides' to die from one or the other subroutine.[1–5]

Many physiological and pathogenic death signals trigger apoptosis through the induction of mitochondrial membrane permeabilization (MMP), an event that can stimulate the activation of the caspase cascade through the primary activation of caspase-9.[1,2,6] Activation of caspase-9 is mediated by a macromolecular complex, the apoptosome. The formation of the apoptosome is initiated upon liberation of cytochrome *c* from the mitochondrial intermembrane space.[2–7] Released cytochrome *c* binds to monomers of Apaf-1 in the cytosol, inducing a conformational change that enables stable association with (deoxy)adenosine triphosphate. Apaf-1 monomers then assemble into the heptameric apoptosome, which in turn binds to procaspase-9. Once recruited, procaspase-9 acquires catalytic activity, is proteolytically cleaved, and activates the effector caspase-3, -6, and -7, and consequently triggers caspase-dependent cell death.[1–7]

Wang and colleagues[8] recently identified two novel proteins involved in the regulation of the apoptosome. 'putative HLA-DR-associated' (PHAP) protein was found to facilitate the activation of caspases by the apoptososome. In contrast, prothymosin-α (ProT), a highly acidic protein widely expressed in mammalian cells, was found to negatively regulate caspase-9 activation by inhibiting the formation of the apoptosome.[8] This finding reveals the potential mode of action of ProtT, an oncoprotein required for cell proliferation. Knocking down of ProT by RNA interference facilitates apotposis induction, and a small molecule, α-(thicholomethyl)-4-pyridineethanol (PETCM), suppresses the negative effect of ProT on the apoptosome *in vitro,* while facilitating caspase activation in cellular extracts obtained from a variety of cancer cell lines.[8] This suggests that ProT may act as an oncogene by virtue of its capacity of inhibiting caspase activation via the mitochondrial pathway. Interestingly, human cells undergoing apoptosis can exhibit the caspase-dependent cleavage of ProT.[9] Caspase hydrolysis disrupts the nuclear localization signal of ProT and abrogates the ability of the truncated protein to accumulate inside the nucleus.[9] At present, it is elusive whether caspase-digested ProT loses its function to inhibit the apoptosome. If so, this would constitute a way of deinhibiting the caspase activation cascade once caspase activation has trespassed a critical threshold, thereby facilitating the explosive cascade-like activation of caspases. As we will discuss here, ProT could have additional pathogenic effects related to the switch between apoptosis and ACD.

It is well known that alterations of cell death regulation can contribute to the pathogenesis of many human disorders; for example, excessive cell death can lead to neurodegeneration, and deficient death programs can result into cancer.[1] Regarding neurodegeneration, it appears intriguing that PHAP can interact with the protein ataxin-1, suggesting that its proapoptotic function may contribute to the loss of Purkinje cells in spinocerebellar ataxia type 1, a polyglutamine repeat disorder.[7] Moreover, brains from mice transgenic for Huntington's disease (HD) mutation (caused by an expanded CAG repeat in exon 1 of the gene coding for the huntingtin protein) exhibit a drastic up-regulation of the ProT gene[10] (Figure 1). Thus, paradoxically, degenerating neurons are overexpressing the caspase-inhibitory protein, ProT. Accordingly, HD-transgenic mice fail to manifest signs of caspase-dependent apoptosis, at the biochemical or morphological levels.[11,12] Moreover, no variation in the expression of genes directly related to apoptosis (e.g. caspases, death receptors, Bcl-2 family members, etc.) was detected in the affected mouse brains.[10] Degenerating neurons from HD-transgenic mice exhibit features of neurodegeneration, including peculiar nuclear and cytoplasmic abnormalities, yet fail to show signs of apoptosis such as cytoplasmic fragmentation or chromatin clumping.[10] These morphological features are suggestive of a slow or delayed neuronal death, in which the apoptotic, caspase-dependent program is suppressed. Dying neurons frequently exhibit an increase in electron-dense lysosomes and autophagic granules, indicating that the mutant huntingtin gene exacerbates autophagy.[10–12]

There are examples in the literature in which the suppression of caspase activation can lead to a switch from apoptosis to APC. Thus, in newly isolated sympathetic neurons, two independent apoptotic stimuli, addition of cytosine arabinoside or deprivation of the obligate growth factor NGF, cause a

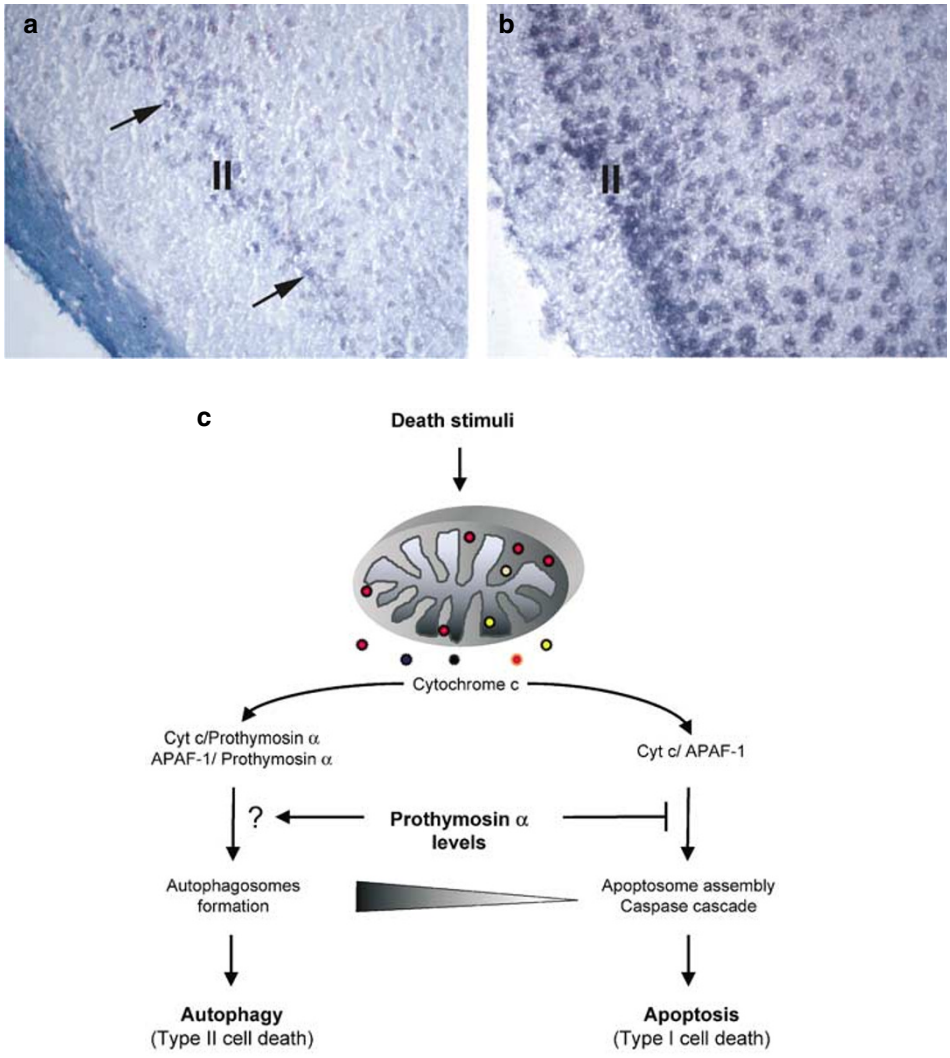

Figure 1 Prothymosin-α is a potential molecular switch in neuronal cell death. For the *in situ* hybridization of wild-type and HD-transgenic mouse brain sections, digoxigenin (DIG)-labeled RNA was obtained by using the DIG RNA labeling kit (Boehringer-Manheim), the antisense prothymosin-α robe was revealed by alkaline phosphatase reaction according to the manufacturer's directions. (**a**) Wild-type mouse secondary motor cortex. Prothymosin-α labeling is preferentially expressed in some neurons (arrows) of the layer 2 (II). (**b**) Transgenic HD mouse secondary motor cortex. Neurons localized in all layers of the cortex are intensely positive. (**c**) Following cytochrome *c* release as a consequence of apoptotic stimuli, both classic type I apoptosis or autophagy programs can be activated depending on the cell environment. In keeping with this, by inhibiting the assembly of the apoptosome and thus caspase activation, prothymosin might act as a switch between the two kind of PCD, and high levels of ProTα might induce ACD. Although ProTα is able to modify cytochrome *c* changing its oxidising properties, it is not yet clear whether the cytosolic cytochrome *c* might have a regulatory role in this cell death pathway too

30-fold increase in the number of autophagosomes per cell, a phenomenon that manifests well before DNA fragmentation.[13] When these two apoptotic stimuli are applied in the presence of chemical pan-caspase inhibitors, the neurons manifest cytochrome *c* release and fail to undergo immediate (apoptotic) cell death, yet develop an increased level of autophagy that eventually leads to cellular atrophy and death.[13]

It is indeed possible that mitochondrial dysfunction elicited by an apoptotic stimulus can activate autophagy when caspase activation is inhibited. Thus, mitochondria undergoing a specific type of MMP (the permeability transition) have been found to associate with autophagosomes, at least in liver cells, in condition in which caspases are not activated.[14] Moreover, a Bcl-2 antisense oligonucleotide can trigger MMP with subsequent ACD in myelomonocytary leukemia cells.[15]

The crosstalk between mitochondria and the lysosomal/autophagic system may be bilateral. Indeed, it appears that a variety of different agents which destabilize lysosomal membranes and/or stimulate macroautophagy can induce MMP. This applies to the addition of lysosomotropic agents such as chloroquine, hydroxchloroquine, ciprofloxacine and norfloxaxine, which first induce lysosomal membrane destabilization and then cause Bax/Bak-dependent MMP, which is required for cell death induction.[16] In addition, in several paradigms of cell death induction, the so-called death-associated protein (DAP) kinase has been shown to be a rate-limiting factor.[17] In fact, HeLa cells stimulated with interferon-g undergo ACD, and this can be prevented by blocking DAP kinase. Overexpression of constitutively active DAP kinase suffices to trigger cell death accompanied by

MMP,[17,18] and overexpression of Bcl-2 (or knockout of Bax and Bak) can prevent DAP-kinase-induced cell death, presumably through its capacity to interfere with MMP.[17,18] These findings further underscore the existence of an intimate crosstalk between apoptosis and ACD, suggesting that MMP might constitute a rate-limiting event in both cell death modalities. Along these lines, recent findings indicate that multiple genes involved in apoptosis are also acting during developmental ACD,[19,20] supporting the notion that these two processes can utilize common pathways or pathway components.

We propose here that ProT could be one of the molecules mediating the physiological switch between apoptosis and ACD, in Huntington's disease. Speculatively, terminally differentiated cells such as neurons may have developed a particular strategy of suppressing caspase-dependent death in response to stress – by overexpressing ProT perhaps along with other endogenous caspase inhibitors – while potentiating repair mechanisms allowing for the elimination of mutated proteins via ubiquitinization or their segregation into intracellular aggregates. If the level of endogenous stress due to intranuclear aggregation of huntingtin becomes too important, then the resulting cytochrome *c* release (secondary to MMP) might fail to cause rapid caspase-mediated apoptotic cell death. Rather, it might stimulate ACD. Intriguingly, it has been found that cytochrome *c* interacting with truncated ProT strongly changes its redox properties,[21] and it might be speculated that the pro-oxidant features of the cytochrome *c* interacting with ProT might signal for the activation autophagy. Alternatively, mitochondria that have lost their physiological function themselves and/or other mitochondrial proteins than cytochrome *c* could stimulate autophagy in conditions of caspase inhibition, a problem that needs to be addressed in the near future. This appears particularly important in view of the fact that pharmacological blockade of caspases has been proposed as a novel therapeutic tool for the prevention of cell loss, including in neurodegenerative diseases.[1]

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