The emerging role of serine proteases in apoptosis

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Abstract
Unregulated apoptosis can be due to a disruption in the balance and control of both intra- and inter-cellular proteolytic activities leading to various disease states. Many proteases involved in apoptotic processes are yet to be identified; however, several are already well characterized. Caspases traditionally held the predominant role as prime mediators of execution. However, latterly, evidence has accumulated that non-caspases, including calpains, cathepsins, granzymes and the proteasome have roles in mediating and promoting cell death. Increasingly, research is implicating serine proteases within apoptotic processing, particularly in the generation of nuclear events such as condensation, fragmentation and DNA degradation observed in late-stage apoptosis. Serine proteases therefore are emerging as providing additional or alternative therapeutic targets.

Apoptosis
Apoptosis is a process by which cells undergo programmed suicide. Cellular proliferation and apoptosis play a major role in maintaining homeostasis. However, in many instances, the rate of apoptosis either exceeds or is much reduced in comparison with proliferation and this can trigger or exacerbate various diseases and clinical states. Apoptosis is the culmination of various signalling pathways within the cell and it is known that proteases are involved within these pathways, particularly in the cleavage of zymogens, necessary for the activation or maturation of apoptotic enzymes. Unregulated apoptosis, either as an increased or a decreased rate, can be due to a disruption in the balance and control of both intra- and inter-cellular proteolytic activities.

It is important to have an understanding of apoptotic processes and the role that proteases play in its execution. Controlled blockade or initiation of apoptotic processes via inhibition of key proteases could have major clinical and therapeutic implications.

The role of proteases in apoptosis
Several proteolytic systems have been implicated in apoptosis and its associated processes. These are variable, depending upon both the origin of apoptotic stimuli and the cell type involved [1]. The cysteine protease family of caspases have traditionally been considered to play the predominant role as prime mediators of the execution of the apoptotic programme. However, evidence has accumulated that non-caspases, including the cysteine proteases calpain and cathepsin B, and the proteasome (a threonine protease) also have roles in mediating and promoting cell death [2]. In addition, there is emerging evidence to suggest that serine proteases are also involved in the execution of apoptotic programmes [3,4]. These serine proteases may function independently within the apoptotic signalling pathways or interact with other mediators such as the caspases or Bcl-2 family proteins.

Serine proteases and apoptosis
Implication of serine proteases in apoptosis was suggested as early as 1987, where serine protease inhibitors prevented apoptosis induced in SK-MEL-109 melanoma cells [5]. By 1994, the relationship between serine proteases and apoptosis was established further when it was demonstrated that introduction of chymotrypsin and trypsin into tumour cells caused a concentration-dependent toxicity in a macrophage cell line, which was completely inhibited by the serine protease inhibitors tosylphenylalanylchloromethane (‘TPCK’) and tosyl-lysylchloromethane (‘TLCK’). These workers made no attempts to isolate or identify the apoptotic serine proteases. In 2003, a novel trypsin-like serine protease isolated from macrophages was identified by Chen et al. [7] and was designated EOS. This discovery led to the suggestion that other serine proteases may be involved in macrophage apoptosis. More recently, it has been suggested that serine proteases are required for the generation of nuclear events such as condensation, fragmentation and DNA degradation observed in late-stage apoptosis [8].

Other serine proteases with apoptotic functionality have already been well documented, and include Omi/HtrA2, granzyme B, thrombin and AP24 (apoptotic protease of 24 kDa). It is noted that serine proteases have been implicated as functioning both up- and down-stream of caspase 3.

Omi/HtrA2
The mature serine protease Omi (also known as HtrA2) has been identified as being a mitochondrial direct BIR-3 (baculoviral inhibitor of apoptosis protein repeat 3)-binding...
protein and caspase activator [9]. BIR motifs bind directly to caspases, thus inhibiting their activity. Ordinarily, Omi is sequestered within the intermembrane space of the mitochondria, preventing proteolytic damage to healthy cells. Cytosolic translocation occurs upon loss of mitochondrial integrity following apoptotic stimulation. Overexpression of Omi/HtrA2 within the cytoplasm of mammalian cells induces apoptosis, indicating a functional role for the protease within the mitochondrial apoptotic pathway [9]. Omi can induce apoptosis in human cells in a caspase-independent manner through proteolytic activity and in a caspase-dependent manner through its ability to disrupt caspase–IAP (inhibitor of apoptosis protein) interaction [9]. The IAP-binding motif of Omi begins at residue 134; this requires proteolytic processing at residue 133 to remove its N-terminal leader sequence [9]. This leader sequence also contains a typical mitochondrial targeting sequence within its first 60 residues, which, upon Omi’s localization within the mitochondria, is removed by mitochondrial processing peptidases [9]. Omi disrupts interaction of caspase 9 with XIAP (X-linked IAP), thus permitting the promotion of procaspase 3 activation. Furthermore, Omi participates with other apoptotic factors in the overall sensitivity of cells to apoptosis [9].

**Granzyme B, thrombin and AP24**

Granzyme B is a member of a family of serine proteases expressed exclusively by CTLs (cytotoxic T-lymphocytes) and NK (natural killer) cells. Upon receptor-mediated fusion of a CTL or NK cell with an infected target cell, granzymes are extruded into the target cell and induce apoptosis [10]. Granzyme subfamilies have trypsin-like, chymotrypsin-like and elastase-like specificities and are expressed as zymogens contained within cytolytic granules. The major role for granzymes is the destruction of virus-infected or other potentially harmful cells. Granzyme B possesses the strongest apoptotic activity of the family. It triggers increased mitochondrial membrane permeability, cytochrome c release and downstream caspase activation by cleaving BID. Moreover, granzyme B cleaves at aspartate residues and activates the caspase cascade through direct activation of several procaspases. In addition, it has the ability to cleave some of the caspase substrates, e.g. PARP [poly(ADP-ribose) polymerase] and BID, as does the less effective granzyme A. This characteristic may be due to granzyme B possessing active sites that are similar structurally to those of the cysteine proteases of the caspase family [11].

At high concentrations, thrombin, a trypsin-like protease, impairs tumour cell growth by cell-cycle arrest and caspase-dependent apoptosis [12]. This observation is not observed in normal cells, indicating an important role for thrombin as a carcinogenic therapeutic tool.

AP24, a chymotrypsin-like serine protease, is activated by a variety of apoptotic stimuli and is implicated in initiating internucleosomal DNA fragmentation. This occurs indirectly through the inactivation of LEI (leucocyte elastase inhibitor) via translational modification. Inhibition of AP24 is observed by overexpression of Bcl-2 in HL-60 cells [13].

**The role of the serpins in regulating apoptosis**

Studies demonstrating the effect of the naturally occurring serine proteinase inhibitors (serpins) in regulating apoptosis also provide indirect evidence for the role of serine proteases in this process. The serpin family, known for displaying broad-spectrum anti-serine proteinase activity, are reported to be involved in cell death regulation [14]. Although several members of the serpin family have been shown to inhibit apoptosis such as CrmA (cytokine response modifier A), SPI-1 (serine protease inhibitor 1), PI-9 (proteinase inhibitor 9), PAI-2 (plasminogen activator inhibitor 2), PN-1 (protease nexin 1); intriguingly, SPI-2 (serine protease inhibitor 2) has been implicated in the induction of neural apoptosis [15].

**Conclusions**

To date, much of the focus on the role of proteases as key apoptotic regulators has tended to concentrate on the caspase family of cysteine proteases. However, serine proteases are now emerging as key players within certain apoptotic processes, thus providing potential additional or alternative therapeutic targets for a range of pathologies and diseases that are triggered or exacerbated by the dysregulation of apoptotic programmes. What is now required is the development of methodologies and approaches that will permit the precise identification of serine protease species involved in apoptosis, in order that these potentially novel therapeutic targets can be validated completely and exploited fully.

**References**


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