Protease nexin 1: A novel inducer of prostate tumor cell apoptosis

Yunchuan Ding¹, Chad M. McKee², Ruth J. Muschel², Danmei Xu¹

¹Dept of Internal Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Hubei, China
²Gray Institute of Radiation Oncology and Biology, Medical Science Division, University of Oxford, Oxford, United Kingdom

Correspondence: Danmei Xu
E-mail: xudanmei99@yahoo.co.uk
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Protease nexin 1 (PN1), a member of serine protease inhibitors (SERPINs) family, is known for its ability to bind and inhibit a wide range of proteases. Recently, we have found that PN1 is able to induce prostate cancer cell undergoing apoptosis through a distinct mechanism of engaging uPA-uPAR complexes and the regulation of two independent downstream signaling cascades, leading to the repression of X-linked inhibitor of apoptosis protein (XIAP). In this process, PN1 expression reduces the expression of NF-κB signalling component p65 and thereby lessens xiap transcription. Alternately, PN1 activity can prevent the stability of XIAP by reducing XIAP phosphorylation at serine 87 via a blockade of AKT signaling. A combination of exogenous PN1 and TRAIL leads to a substantial growth lag in prostate cancer xenografts, indicating the potential of PN1 as a promising target for improving prostate cancer therapy. Furthermore, human prostate tissue arrays show inverse levels of PN1 and XIAP in tumor and normal prostate. Hence, the PN1-uPA regulatory axis may serve as an inducer of tumor cell apoptosis by modulating survival pathways, and therefore delay the tumor growth of prostate cancer.

Keywords: PN1; XIAP; apoptosis; NF-κB; AKT


Aberrant apoptosis regulation is one of the primary causes of cancer development and progression. Thus, many cancer therapeutic strategies are designed to promote highly proliferative cancer cells undergoing apoptosis [¹]. Under many circumstances, failure of chemotherapy is attributed to the resistance of tumor cells to drug-induced cell death, as the result of defects of apoptotic regulators or by increased expressions of pro-survival proteins [²].

X-linked inhibitor of apoptosis protein (XIAP) belongs to the latter group, a survival molecule that actively promotes tumor cell resistance in response to a wide range of apoptotic stimuli and frequently found overexpressed in different types of malignancies [³]. XIAP effectively binds and neutralizes apoptosis effectors caspase-9, caspases-3 and-7 via their BIR3 and BIR2 domains respectively, therefore potently blocking the canonical cell death [⁴]. Using siRNA [⁵], antisense oligonucleotides [⁶], or morpholino-antisense [⁷] to reduce XIAP expression has been shown to increase the sensitivity of drug-resistant cancer cells to chemotherapy-induced apoptosis as well as induce spontaneous tumor cell death. Therefore, targeting XIAP becomes a promising therapeutic approach to combat tumor cell resistance [³].

In this research highlight we summarize our new findings that protease nexin 1 (PN1), an endogenous serine protease inhibitor (SERPIN), is capable of inducing tumor cell apoptosis through inhibiting XIAP [⁸]. PN1 exhibits a previously characterized ability to bind and neutralize the
activity of a variety of serine proteases, including urokinase plasminogen activator (uPA), tissue-type plasminogen activator (t-PA) and plasmin.\(^9\)

PN1 is secreted by various types of cells, including fibroblasts, macrophages, endothelial cells, astrocytes, as well as many cancer cells.\(^{10, 11}\) PN1 expression has been observed in a range of cancers including prostate, breast, colon, and pancreatic carcinoma, although the regulatory mechanisms underlying remained largely unidentified. It is not definitively known if PN1 levels are anti-tumorigenic. However, we have previously shown that PN1 can trigger distinct signaling pathways, one of which is to block the invasion of prostate metastatic cells through inhibiting uPA activity.\(^{12, 13}\) PN1 can also reduce tumor cell proliferation and delay the growth of tumor xenograft through regulating sonic hedgehog (SHH) levels.\(^{14, 15}\) Intriguingly, exogenous PN1 in combination with anti-Hedgehog molecules significantly promoted cell death in prostate cancer xenografts, suggesting its potential role on inducing tumor cell apoptosis.

Thus, to further investigate the pro-apoptotic aspects of PN1, we used an apoptotic array containing 35 pro- and anti-apoptotic factors to determine potential target molecules for PN1. Of the protein screened from PC3 cells treated with transient PN1 expression, XIAP level was the most remarkably affected, with a strong decrease in expression.\(^8\) We further proved that PN1 represses XIAP expression at the mRNA level, validated in both cell culture models and genetically modified mice. In a variety of tissues including prostate, seminal vesicles, brain and bladder, XIAP increased in PN1 knock-out mice as compared to that in the wild type.\(^8\) The inhibitory effect of PN1 upon uPA activity contributed to the repression of XIAP, consistence with a previous study that indicates the role of uPA in the transcriptional activation of XIAP in endothelial cells.\(^{16}\)

PN1 is known secreted to the extracellular matrix (ECM) to function.\(^9\) Both LRP-1 and uPAR have been reported as membrane receptors that bind protease-PN1 complexes to deliver cellular signals.\(^{17, 18}\) Interestingly, PN1-uPA complexes preferentially signal through the LRP-1 to control SHH signaling in prostate cancer while the complexes utilize the uPA receptor (uPAR) to control XIAP.\(^8\) The results suggest that down-regulation or blockade of uPAR reduces XIAP expression but not SHH levels.\(^8\) Therefore, through binding to different receptors by PN1-uPA complexes, distinct signaling pathways may be activated in prostate cancer cells. PN1, as an uPA inhibitor highly prevalent in the ECM, is capable of repressing XIAP expression and ultimately inducing apoptosis in cancer cells.

Our research demonstrates that PN1 could regulate XIAP in two arms, firstly via control of RNA levels by altering NF-κB pathway and secondly through stabilization of the XIAP molecule itself.\(^8\) NF-κB signaling is known to regulate XIAP expression transcriptionally.\(^9\) We have shown that PN1 remarkably reduced the levels of certain NF-κB sub-units, in particular p65/p50, followed by the decrease of XIAP levels.\(^8\)

Previous literature suggests that uPA is able to activate AKT signaling through uPAR.\(^{20, 21}\) AKT signaling is also known related to stabilizing XIAP at serine 87 via phosphorylation.\(^{22}\) Thus we further investigate whether PN-1 is able to affect AKT signaling and thereby XIAP stability. Indeed, in both PN1 knockout mice and tumor xenografts, we observed that the absence of PN-1 significantly promotes the phosphorylation of AKT as well as the levels of total and phosphorylated XIAP, all of which are associated with resistance to cell death. Thus, if the repression of XIAP mediated by PN1 is blocked, tumor
cell survival is more likely to be engaged. Importantly, blocking the AKT signaling by specific inhibitors MK-2206 does not appear to impact the NF-κB pathway, as evidenced by the unchanged p65 levels, indicating the independence of these two signaling pathways [8].

Resistance of tumor cells to conventional chemo- and radiotherapy remains a significant challenge in cancer therapy. As the most potent cellular inhibitor of caspases, XIAP serves an essential role on promoting cell survival. Aberrant expressions of XIAP have been shown to correlate with reduced survival rate and poorer prognosis [23, 24]. So far most pharmacological approaches have focused on designing small-molecule inhibitors that could displace XIAP from its target caspases [25]. XIAP expression has also been found correlated with the sensitivity to the chemotherapy drug such as cytarabine [26]. PN1, as a newly discovered endogenous inhibitor of XIAP, may be used to promote cell death in conjunction with chemical compounds against XIAP. In a similar vein, our apoptotic array also revealed that PN1 promoted TRAIL-R1 (DR4) and TRAIL-R2 (DR5) expression simultaneously with reducing XIAP. Treatments of PC3 cells by a combination of recombinant PN1 and TRAIL proteins further delayed xenografts growth than using single agent [9], suggesting that PN1 might be a potent pro-apoptotic factor, especially if in combined with other molecules involved in apoptosis. Further study on the efficacy and mechanisms of PN1 mediated tumor suppressive role is warranted in order to develop new therapeutic approaches.

SERPINs are defined by their capability to inhibit serine proteases, but they may play wider roles, ranging from embryological development to synaptic plasticity, and tumor progression [27]. Accordingly, several SERPINs have been shown regulating cellular proliferation through apoptosis [27, 28]. Such function of SERPINs has been linked with their inhibitory effects on the uPA/uPAR complex, which induces focal proteolysis of ECM as well as the activation of cell survival pathways [28]. Blockade of Plasminogen activator inhibitor-1 (PAI-1) has recently been shown to reduce cancer cell migration, proliferation and survival through modulating the function of uPAR [28, 29]. Other non-inhibitory SERPINs may have effects on cancer growth, apoptosis, invasion or angiogenesis through diverse mechanisms. For example, Maspin sensitizes cancer cells to apoptosis as well as inhibits their migration [30]. Pigment epithelium derived factor (PEDF) promotes cancer cell apoptosis via the FasR/FasL pathway [31] and regulation of the Bcl-2 family of proteins [32]. Therefore, the novel findings of PN-1 mediated apoptosis show the versatility of SERPIN in the promotion of programmed cell death.

Prostate cancer is the second leading cause of male tumor-related mortality in the western world [33]. Increased XIAP levels have been found in this malignancy [34] and correlated with apoptosis resistance and increased metastatic foci in vivo [35]. Tissue staining revealed that the XIAP level is dramatically higher in the prostate cancer tissue than in normal or benign prostate hypertrophy (BPH) [36]. Our results of human prostate tissue microarrays (TMAs) confirmed the expression pattern of XIAP and further showed that PN1 levels declined in a step-wise fashion from their high expression in normal prostate tissue to near ablation in the most advanced cancer (Gleason 8-10) [8]. Interestingly, an inverse correlation between PN1 expression and XIAP and a concomitant expression of p65 with XIAP were evident in TMAs, matching observed regulation found in cell culture and pn1-/- mice [8]. Thus PN1 in line with other biomarkers may serve as a predictive panel in individualized therapy of prostate cancer.

In summary, our results reveal novel regulatory targets of PN1 and suggest a greater potential in hindering tumor growth and metastasis. By engaging to different cell surface receptors, PN1 chooses to initiate distinct signaling to regulate tumor proliferation, apoptosis and metastasis. It is capable of substantially repressing XIAP levels. PN1 mediated neutralization of uPA-uPAR complexes leads to a downstream cascade of events that contribute to the transcriptional activation and stabilization of XIAP in prostate cancer cells. Combination of PN1 and TRAIL treatment significantly induces growth lag in prostate cancer xenografts, suggesting that PN1 may serve as a suitable target for improving prostate cancer therapy.

Conflicting interests

The authors have declared that no competing interests exist.

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