Chapter 12

Maspin Expression and its Metastasis Suppressing Function in Prostate Cancer

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Additional information is available at the end of the chapter

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Abstract

*Ma*mmary *S*erine *P*rotease *In*hibitor (Maspin) is a unique member of the serpin family with tumor suppressive properties. Maspin is a secreted protein encoded by a class II tumor suppressor gene, expressed in normal prostate luminal and basal cells but reduced or absent in prostate cancer. Currently, there is a consensus that maspin expression in prostate cancer is an indicator of a better prognosis and is a predictive marker for therapeutic response in prostate cancer. Experimental evidence consistently indicates that maspin suppresses tumor growth, invasion, and metastasis and promotes apoptosis in cancer cells. In this chapter, we discuss regulation of maspin expression, binding partners of maspin, and pathways through which maspin exerts its tumor suppressive properties. In addition, we summarize the progress that investigators have made in clarifying the role of maspin in prostate cancer biology and in assessing its role as a diagnostic marker and therapeutic agent.

Keywords: tumor suppressor, prognostic marker, SERPINB5, prostate cancer, maspin

1. Introduction

*Ma*mmary *S*erine *P*rotease *In*hibitor or Maspin (SERPINB5 or PI5 *Homo sapiens*) is a 42 kDa, non-classical, non-inhibitory member of the ovalbumin clade of serine protease inhibitors (serpins), encoded by the SERPIN5 gene [1]. Chromosome 18 encodes maspin along with gene cluster of other serpins in humans comprising squamous cell carcinoma antigens (SCCAs) 1 and 2 and plasminogen activator inhibitor type 2 (PAI-2) [1, 2]. Maspin has been characterized as a class II tumor suppressor gene, first recognized in 1994, in normal mammary tissue and breast

cancer cell lines through subtractive hybridization, comparing genes expressed in different stages of a biological or pathological process [2]. Maspin has been shown to be downregulated in many metastatic tumor cell lines, without evidence of an underlying mutation [3]. Maspin contains a reactive center loop (RCL), which is used to trap the target protease and inhibit its activity, a common characteristic of inhibitory serpins [1, 2]. Recent studies suggest that serpins are involved in cell adhesion and play a role in extracellular remodeling [2, 3]. However, maspin has been found to be more closely related to the non-inhibitory clade B serpins. As maspin's RCL is shorter than those of inhibitory serpins and unlike multiple other serpins, maspin does not undergo a stressed-relaxed conformational change to inhibit protease activity. Despite the reported activity as a serine protease inhibitor, several studies argue that the tumor suppressor activity of maspin is due to its ability to inhibit proteolysis. Some studies demonstrate the efficacy of maspin in the inhibition of activity of tissue-type plasminogen activator [4]. Furthermore, maspin has been shown to mediate the inhibition of urokinase-type plasminogen activator (uPA) on the surface of prostate cancer cells [5]. Although maspin might lack the ability to inhibit serine proteases, its biological function can be attributed to RCL, which can be derived from its crystal structure [6]. Recent studies of maspin have provided evidence for its ability to regulate cell adhesion, motility, apoptosis, and angiogenesis, which has been of utmost interest in medical field attempting to use maspin as a method of therapeutic intervention for prostate cancer and other forms of malignancies [3–7].

2. Expression of maspin in normal prostate and cancer

Maspin has been localized to the cell surface, nucleus, cytoplasm, and extracellular matrix of epithelial cells of different tissues [8]. In normal breast and prostate epithelial cells, maspin is highly expressed and found to be localized mostly in the cytoplasm but has also been detected in the nucleus, secretory vesicles, and occasionally at the cell surface [6–8]. Maspin expression is almost completely suppressed in the human prostate cancer LNCaP, DU145, and PC-3 cell lines [9]. At the tissue level, maspin's function seems to be directly correlated with its localization. In benign prostate epithelium, maspin expression is uniformly noted in basal cells at high levels. In contrast, maspin expression is predominantly absent in benign secretory cells and elevated in secretory cells at the transition site between benign prostatic hyperplasia and high-grade PIN lesions [9, 10]. Pierson et al. noted higher expression of maspin in HGPIN lesions, particularly within secretory cells [11]. Elevated maspin immunoreactivity in the secretory cells appeared at the transition area between benign prostate tissue and HGPIN, whereas less intense maspin staining was observed in neoplastic cells adjacent to HGPIN. No change in maspin expression was observed in the basal cells near HGPIN, compared to normal basal cells [12]. Moreover, an inverse relationship between maspin expression and tumor progression was noted in clinical specimens with gradual disappearance in primary prostate cancer. Due to the loss of basal layer during prostate cancer progression, strong immunohistochemical staining of maspin was lost in the basal cells [12, 13]. A progressive decrease in maspin expression was noted with increase in Gleason grade, with complete loss of maspin in high grade and metastatic tumors (**Figure 1**). Maspin expression was significantly higher in

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tumor specimens of patients treated with neoadjuvant androgen ablation therapy before radical prostatectomy [14]. Prostate cancer patients whose tumors expressed maspin had a significantly longer recurrence-free survival [6, 12].

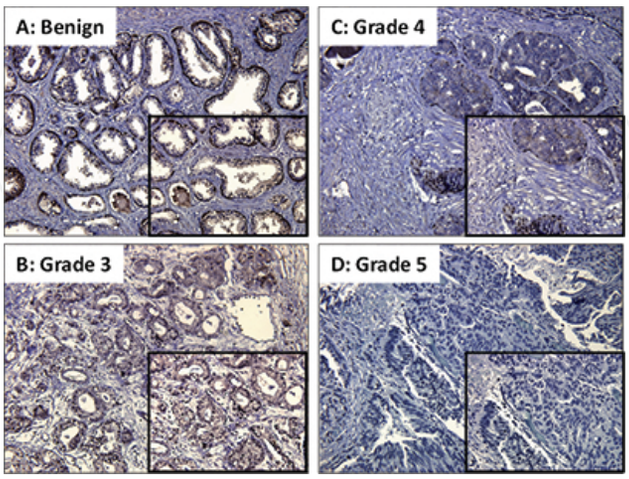

Figure 1. Expression of maspin in various representative human prostate specimens. Paraffin-embedded (4.0 μm) sections from benign *A*, and prostate cancer of various Gleason grades *B–D*, were used for maspin expression by immunohistochemistry. A strong nuclear and cytoplasmic staining was observed in benign tissue where the basal cell cytoplasm and nuclei were strongly and uniformly immunoreactive for maspin. Less intense cytoplasmic staining was noted in secretory cells. In low-grade cancer (Gleason grade 3), loss of nuclear maspin staining was observed and tumors progressively exhibited reduced maspin expression where the majority of high-grade tumors exhibit little or no cytoplasmic immunoreactivity. Magnified at ×20 and inset ×40.

3. Regulation of maspin expression

Maspin expression is regulated by a promoter with two response elements—Ets and a promiscuous hormone response element that binds glucocorticoid receptor and progesterone receptor [15]. Regulation of maspin by androgen receptor (AR) seems complex because the hormone response element of maspin's promoter appears to function as a negative regulator. Zou et al. have demonstrated that androgen-responsive LNCaP cells cultured in androgen-depleted medium exhibit induction of maspin promoter activity in a promoter luciferase reporter assay [16]. Furthermore, castration of nude mice induces maspin expression in LNCaP xenograft tumors. These data indicate that maspin may be transiently upregulated in early stages of prostate tumor development and remains sensitive to AR repression.

Another major regulatory mechanism for maspin is the involvement of p53 signaling pathway [16]. A consensus p53 site was identified in the maspin promoter, which induced its expression upon binding to p53. Maspin expression was increased in adenoviral-mediated expression of wild-type p53 in maspin-null prostate cancer cell lines. During cellular stress, p53-responsive pathways were induced in cells possessing wild-type p53, whereas mutant p53 failed to induce its expression. Purified p53 protein bound to regions within the promoter from −297 bp and p53 antibody supershifted maspin bands. In support of these data, a later study (using tissue

microarray analysis) reported an inverse relationship between mutated p53 and maspin in human tumors [17]. The implication of maspin involvement in the p53 pathway demonstrated a potential hierarchy of tumor suppressor pathways. Studies suggest that maspin may act as an effector molecule downstream of the p53 stress-induced pathway. Interestingly, other proteins and signaling pathways related to maspin regulation were reported to be dependent on p53. Transforming growth factor β (TGFβ) was also found to increase maspin expression and required wild-type p53 activity [18]. This work demonstrated two p53 binding sites in the maspin promoter that were either in close proximity or overlapped with a Smad binding element, leading to recruitment of Smad2/3 and p53 to the maspin promoter following TGFβ signaling. In addition, Smad2/3 increased the binding of p53 to the maspin promoter demonstrating transcriptional co-regulation. Reports suggest that there are other factors that regulate maspin expression, independent of p53 function. Expression of the antioxidant manganese superoxide dismutase in prostate cells led to an increase in stability of maspin mRNA, and this effect persisted in the presence of wild-type or mutant p53 [19]. Furthermore, the activating transcription factor (ATF-2) was shown to induce maspin expression independently of p53 by binding to a CRE-like sequence downstream of the transcription start site [20]. In addition, other members of the p53 family, specifically the p63 isoform TAp63γ, induce expression of maspin by binding to the same consensus p53 promoter element and can substitute for activation in the absence of p53 [21]. Other transcription factors have also been noted to bind to maspin promoter and regulate its expression as shown in **Figure 2**.

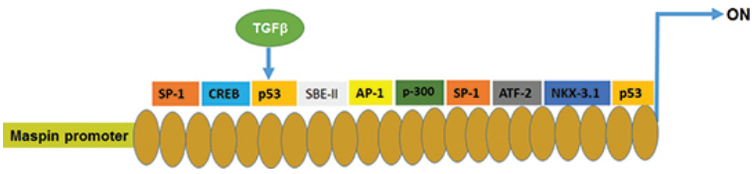

Figure 2. Regulation of maspin by various transcription factors. Maspin promoter has several transcription factor binding sites that regulate its expression and function. Most of maspin regulation by the transcription factors is unclear, but the regulation by p53 is widely studied and involves the TGF-β signaling.

Recent evidences suggest that maspin can be epigenetically regulated and that its expression in relationship with tissue specificity directly correlates with DNA methylation [2, 22]. Treatment of maspin-null cancer cell lines with 5-aza-2′-deoxycytidine resulted in the induction of maspin expression [22]. Furthermore, promoter methylation was found to serve as a mechanism for tissue and cell-specific expression of maspin [2, 22]. Epigenetic changes regulating maspin expression have been demonstrated to occur at the 5′ regulatory region of the maspin gene that involves methylation of cytosine, histone deacetylation, and the accessibility of chromatin. Two defining epigenetic events are DNA methylation and histone lysine methylation or deacetylation, which are categorized as chromatin modification events. In support of this, we and others have shown that treatment of prostate cancer cell lines with histone deacetylase (HDAC) inhibitors, namely sodium butyrate and trichostatin A, led to induction of maspin at mRNA and protein level [23]. Re-expression of maspin using demethylating agents and HDAC inhibitors in combination has been confirmed in additional studies. Furthermore, studies using prostate cancer cells and clinical specimens, we have

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further demonstrated that maspin expression was only induced by inhibition of class I histone deacetylases regardless of promoter methylation status, highlighting that chromatin condensation alone may determine its transcriptional activity [23].

Maspin is a non-glycosylated protein; however, phosphorylated forms have been identified and detected in various tumors [24]. Early studies demonstrate abundance of tyrosine phosphorylation in both endogenously expressed maspin in normal epithelial cells and after induction of maspin in transfected tumor cells [18]. Although the kinases responsible for this phosphorylation have yet to be identified, incubation of rMaspin with the TKD38 EGFR kinase domain led to tyrosine phosphorylation in a cell-free system. In addition, serine and threonine phosphorylation sites have been recently identified on maspin secreted from cornea cells using a mass spectrometry approach [25]. Whether this phenomenon is cell type specific requires further studies. In addition to phosphorylation studies, maspin also contains eight cysteine residues; however, intramolecular disulfide bonding had not previously been observed nor predicted from maspin crystallography. Under oxidative stress, maspin adopted an oxidized, disulfide-bonded structure, which was analyzed under non-reducing conditions in epithelial cells [26]. In this state, maspin was no longer able to bind glutathione *S*-transferase (GST), a binding partner for maspin, which suggested a potential difference in protein functionality.

4. Protein binding partners of maspin

Through the yeast two-hybrid assays, screening studies identified possible protein-protein interactions with maspin [27]. A short list of candidate intracellular partners of maspin include heat shock proteins (Hsp90 and Hsp70), glutathione *S*-transferase (GST), interferon regulatory factor 6 (IRF6), histone deacetylase 1 (HDAC1), early growth response protein 1 (Egr-1), and GC-binding factor 2 (GCF2). These studies provide new dimensions in understanding the role of maspin from that of a serine protease inhibitory serpin to that of a stress-responsive chaperone and role of maspin in tumor suppression.

Hsp90 is one of the most abundant stress-responsive chaperones that shuttle between the cytoplasm and nucleus to protect its client proteins from degradation [28]. Hsp90 binds and protects the native conformation of AR. AR attains activation and becomes functional as a consequence of agonists binding to its receptor, or when phosphorylated by Akt or due to mutation is ensued by its nuclear translocation. In the nucleus, AR employs co-activators that facilitate its binding to the promoter sequence of responsive genes to activate their expression [29]. Inhibition of Hsp90 destabilizes both wild type and mutant AR, presumably by releasing AR from the chaperone complex, to be subjected to degradation by the proteasome. Once in the nucleus, AR is acetylated, which may be mediated by its co-activators such as p300. Acetylated AR has been shown to specifically interact with and be deacetylated by HDAC1 [30]. Reports suggest that molecular interactions of maspin with HDAC1 and/or Hsp90 may underlie the positive correlation between nuclear maspin and better prognosis of cancer [31]. Inhibition of HDAC specifically upregulates genes that promote cell differentiation, cell cycle arrest, or cell death and downregulates genes that promote tumor survival and epithelial-

mesenchymal transition, which correlate with higher levels of maspin, whereas both Hsp90 and HDAC have been implicated as key regulators of AR activity and stability. Lockett et al. have proposed a model where maspin may negatively regulate AR-dependent survival/proliferation of both hormone-sensitive and hormone-refractory prostate epithelial cells [32]. This model proposes that genetic engineering approaches to induce maspin expression may prove to be effective in blocking Hsp90-mediated stability, and/or HDAC1-mediated transcriptional activation, of AR in prostate cancer cells.

The maspin/GST interaction was initially characterized [27]. Endogenous maspin has been shown to correlate with increased cellular GST activity, even though purified maspin does not affect the activity of GST *in vitro*. Furthermore, maspin transfected tumor cells exhibit markedly lower basal levels of ROS, compared to the control transfected cells. In contrast, siRNA knockdown of maspin in prostate cancer PC-3 cells increased the basal ROS level. Tahmatzopoulos et al. have shown that treatment of human prostate cancer DU145 cells with H_2O_2 (or PMA) but not with TRAIL further increases the maspin/GST interaction and significantly attenuated H_2O_2-induced ROS generation and VEGF expression [33]. This study further demonstrates that maspin transfected tumor cells produced less VEGF than the transfection control cells. Interestingly, a single point mutation at the RSL p1 position of maspin (MasR340A) greatly reduced the affinity for GST. Consistently, treatment with purified wild-type maspin, but not MasR340A, significantly increased cellular GST activity.

Studies by Bailey et al. reported specific interaction between maspin and interferon regulatory factor 6 (IRF6) [34, 35]. IRF6 is a member of the IRF family associated with epithelial-to-mesenchymal transition through increase in N-cadherin. The interaction between maspin and IRF6 appears to be regulated by IRF6 phosphorylation and may negatively regulate the IRF6 activity. Other maspin-binding proteins identified by yeast two-hybrid approach include transcription factors, such as early growth response protein 1 (Egr-1), GC-binding factor 2 (GCF2), and RNA-binding protein KHDRBS3 and FBX032, which are involved in ubiquitin protein ligase reactions [25]. Additional studies to understand how these proteins interact with maspin and their role during prostate cancer progression are needed.

5. Biological functions of maspin

Maspin downregulation correlates with increased tumor growth and metastasis [36]. Several published studies using cell lines and animal models underscore the critical role of maspin in tumor growth and invasion. Treatment with recombinant maspin protein was found to inhibit tumor invasion and motility of prostate cancer cells (LNCaP, DU145, and PC-3) in culture by binding specifically to the cell surface. This surface action was further supported by the ability of maspin to block urokinase-type plasminogen activator on cell surface of prostate cancer DU145 cells [5]. Cher et al. used a SCID human intraosseous tumor model that ectopically overexpresses maspin in prostate cancer DU145 cells to demonstrate maspin's ability to abrogate bone matrix remodeling, repress bone tumor growth, and prevent angiogenesis [37]. In another study, Hall et al. using maspin overexpressed PC-3 tumor cells injected to athymic

nude mice demonstrated a decrease in bone metastasis but failed to suppress the ability of tumor cells spread/metastasize to distant sites providing new insight in the underlying inhibitory role of tumor cell homing into the bone [38]. Interestingly, in genetically engineered mouse model of prostate cancer, TRAMP the mechanism of maspin repression occurred through association of receptor activator of NF-kB (RANK) with ligand RANKL that facilitates IkB kinase α (IKKα) nuclear translocation, which in turn suppresses maspin transcription allowing progression of prostate cancer [39].

The anti-angiogenic effects of maspin were demonstrated by Zhang et al. using endothelial cells [40]. Increasing concentrations of rMaspin inhibited both the growth and migration of endothelial cells toward vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) *in vitro*. *In vivo* experiments using human prostate cancer LNCaP cells grown in immunodeficient xenograft model, tumor growth and neovascularization were reduced following rMaspin treatment [40]. A chimeric bone cancer model in which human cancer cells were injected into human bone that had been implanted into SCID/SCID mice demonstrated that DU145 human prostate cancer cells transfected with maspin exhibited less tumor neovascularization from murine endothelial cells compared to controls. This effect was associated with a decrease in tumor growth and bone destruction [36, 37]. Another study demonstrated that conditioned media (CM) from maspin-expressing human keratinocytes inhibited the ability of human endothelial cells to migrate toward angiogenic factors, namely VEGF, bFGF, and interleukin-8 (IL-8), in a dose-dependent manner [40]. Using maspin neutralizing antibody to the CM, the cells resumed their ability to migrate, providing evidence for a paracrine anti-angiogenic role of maspin.

Increased cell adhesion and cell-cell contact are negative factors for cell cycle progression. Increased cell adhesion to ECM is shown to cause certain cell types to arrest in G1 phase [41]. Studies suggest that maspin has ability to increase prostate epithelial cell adhesion to different matrix proteins and inhibit prostate tumor progression through increased cell adhesion to matrices. Recent study provides evidence that maspin controls cell adhesion through its interaction with integrin β1 [42]. Interestingly, loss of one copy of maspin gene in *Maspin*$^{+/-}$ heterozygous knockout mice leads to the development of prostate hyperplastic lesions, accompanied with a changed pattern of matrix deposition and a loss of epithelial cell polarity [41]. It was also demonstrated that maspin may be able to inhibit surface-bound urokinase plasminogen activator in prostate tumor cells. However, the aforementioned results were not easily observed *in vitro* or *in vivo*. *In vitro*, tumor suppression was not observed with the use of maspin, while *in vivo*, only 50% of tumors that expressed maspin showed a significant reduction [36, 37].

Maspin has been observed to be involved in the regulation of apoptosis. Pro-apoptotic effects from maspin have been demonstrated in prostate cancer cells. Studies by Mckenzie et al. underline maspin as a pertinent therapeutic target to overcome hypoxia in prostate cancer. Overexpression of maspin in DU145 cells leads to apoptosis by abrogating AKT activation induced by hypoxia. According to recent studies, it has been shown that maspin expression (endogenously) is able to sensitize prostate cancer LNCaP and DU145 cells to apoptosis [9]. Watanabe et al. used adeno-associated virus (AAV, serotype 2) vector encoding maspin as a

means for introducing *in vivo* gene therapy subcutaneously formed human prostate cancer LNCaP or DU145 tumors in nude mice [43]. In this study, intratumoral AAV-mediated maspin expression significantly upregulated the number of apoptotic cells when compared with AAV-LacZ treatment. Moreover, significantly fewer CD31-positive micro-vessels were observed in AAV-maspin-treated tumors when compared with the control tumors, which correlated with persistent maspin expression. Studies on mechanics have demonstrated that maspin could possibly lead to apoptosis of tumor cells through the manipulation of mitochondrial permeability and the initiation of degradation through apoptosis [44]. Maspin has also been shown to induce prostate tumor cell dedifferentiation and to increase tumor cell sensitivity to drug-induced apoptosis. Suppression of maspin may partly be attributed to the involvement of AR in prostate tumorigenesis and cancer progression. Maspin's role as a tumor suppressor in prostate cancer cells irrespective of their AR status makes it an ideal candidate with therapeutic capabilities against hormone-refractory prostate cancer. Other biological functions of maspin are listed in **Figure 3**.

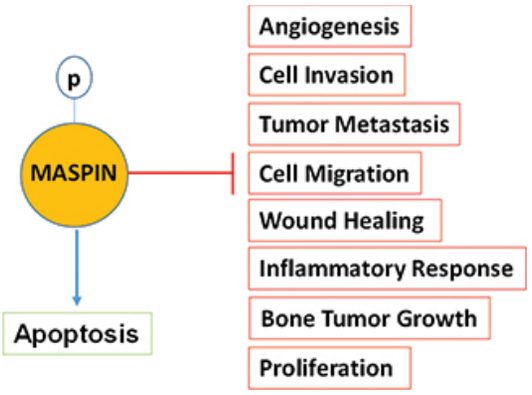

Figure 3. Biological functions of maspin. Post-translational modification of maspin and its nuclear localization suppresses angiogenesis, tumor metastasis, cell invasion, and migration and inhibits bone tumor growth and proliferation, while inducing apoptosis.

6. Therapeutic application of maspin

Recent studies have investigated the anticancer effects of maspin expression and the use of maspin as a therapeutic agent against cancer. Tumors with LNCaP cells expressing adenoviral maspin (AAV-GFP) resulted in a higher percentage of apoptotic cell death and a decrease in the number of CD31 positive vessels when compared with tumors with empty vector (GFP), thereby substantiating the role of maspin in gene therapy [44]. These results confirm maspin as a therapeutic agent/target in inhibiting prostate cancer progression. Furthermore, animal studies using targeted delivery of maspin by liposome/DNA and/or adenoviral constructs to tumor and/or tumor vasculature have supported a viable approach in cancer treatment [45].

Several reports suggest that exogenously added rMaspin, or maspin-derived peptide fragments, can act at the surface of numerous migratory cell types to suppress cell motility and

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movement by enhancing cellular adhesion to the extracellular matrix components laminin, fibronectin, and collagen [43, 45]. McGoven et al. have shown that purified recombinant maspin produced in baculovirus-infected *Spodoptera frugiperda* Sf9 insect cells [rMaspin] binds specifically to the surface of human prostate cancer DU145 cells, inhibits the DU145 cell surface-bound uPA, and forms a stable complex. Similar results with rMaspin were observed in *in vivo* studies transplanted with human prostate cells [5]. In fact, biological assays of invasion by prostate tumor cells through Matrigel membranes and of motility have shown that rMaspin inhibits invasion and migration of these cells [46].

Maspin expression in tumor cells can also be increased by the use of various phytochemicals. He et al. have demonstrated that gum mastic, a natural resin, can upregulate maspin expression in prostate cancer cells [47]. Gum mastic induced maspin mRNA and protein expression, as well as maspin promoter activity in LNCaP and DU145 cells by suppressing ARE binding activity and enhancing Sp1 binding activity, and the increased activity in the maspin promoter. Sheth et al. have demonstrated that resveratrol (trans-3,49,5-trihydroxystilbene), a polyphenolic antioxidant found in peanuts, grapes, and red wine inhibits cell proliferation, induces apoptosis of human prostate cancer cells mediated by increase in maspin levels through Akt/miR-21 pathway [48]. Natural compound curcumin, a hydrophobic polyphenol derived from turmeric (the rhizome of the herb *Curcuma longa*), has been shown to inhibit the AR gene activity in androgen-responsive human prostate cancer LNCaP cells suppressing AR transaction, thereby affecting AR-regulated genes including maspin [49]. As AR negatively regulates maspin expression, curcumin-mediated inhibition of AR may induce the expression of maspin through a mechanism, which is presently unclear. Nevertheless, increased expression of maspin occurs only in prostate cancer cells, which harbor wild-type p53. As p53 activates maspin promoter by binding directly to its p53 consensus-binding site [16], we studied the effect of apigenin, a natural plant flavone in potentially restoring p53-mediated maspin levels. Exposure of LNCaP and 22Rv1 cells, harboring wild-type p53, with apigenin resulted in dose dependent increase in maspin expression and p53 activation through acetylation at the Lys305 residue by inhibiting class I HDACs. Apigenin withdrawal in LNCaP cells caused loss of maspin and p53 acetylation. Furthermore, proteasome inhibitor MG132 inhibited apigenin-mediated proteasomal degradation of class I HDACs in these cells. The increased apigenin-mediated p53 acetylation enhanced its binding on maspin promoter, which was associated with decrease in tumor cell invasion and migration. Apigenin treatment also caused accumulation of acetylated histone H3 in total cellular chromatin, increasing accessibility to bind with the promoter sequences of maspin, consistent with the effects elicited by HDAC inhibitor, trichostatin A. Similar observations were noted after feeding apigenin to 22Rv1 tumor xenograft implanted in nude mice [50]. Other natural compounds that have been reported to enhance prostate cancer suppression and inhibit invasion and migration through upregulation of maspin are tanshinone IIA and apple peel extract [51, 52].

A study by Jiang et al. revealed differential expression patterns of maspin mRNA and protein expression following treatment of cancer cells with essential fatty acids (EFAs) [53]. Addition of omega-6, EFAs arachidonic acid and α-linolenic acid had no effect on maspin expression, while treatment with γ-linolenic acid led to rapid increase in maspin mRNA [53]. Consumption

of γ-linolenic acid has been linked to many beneficial effects in humans. Interestingly in the same study, another omega-6 EFA, linoleic acid, resulted in decreased maspin expression. This study highlighted specific effects of EFAs on maspin that may have significant implications in cancer biology as well as a role for maspin in lipid signaling and processing. Not surprisingly, *in vitro* promoter activity study showed that, in addition to p53 [16], a list of stress-related signals including DNA-damaging agents, cytotoxic drugs [16], peroxisome proliferator-activated receptor-gamma [54], nitric oxide [55], and manganese superoxide dismutase (MnSOD) [56] activates maspin expression. Consistently, several stress signals that induce maspin expression also induced more differentiated phenotypes [16, 54–56]. Furthermore, epigenetic treatment of prostate cancer cell lines with trichostatin A, a histone deacetylase (HDAC) inhibitor, led to induction of maspin mRNA [20, 50]. Re-expression of maspin using demethylating agents and HDAC inhibitors has been confirmed in additional studies [50, 57].

7. Conclusions and future directions

Much remains to be understood about the molecular mechanism(s) of action of maspin in the normal prostate and during cancer progression. Studies in our laboratory are ongoing to determine the post-translational modifications of maspin, which may help elucidate the role of maspin in signaling pathways relevant to cell adhesion and angiogenesis. The ability of maspin and its post-translationally modified forms to act as effective therapeutic agents in prostate cancer is also being pursued. Additional studies are required to unravel the multi-faceted interrogation of how maspin expression can alter the malignant phenotype of some cell types and not others. Nevertheless, recent findings and ongoing studies should encourage researchers to continue to explore the molecular mechanisms underlying maspin's biological effects in prostate cancer.

Clinical studies performed this far in prostate cancer demonstrate the significance of maspin expression as a useful prognostic and possibly predictive marker for patients undergoing definitive therapy. Focusing on the malignancies in which maspin exhibited a positive prognostic value, therapeutic approaches studied so far aimed to re-activate this dormant tumor suppressor gene by transcription factors that regulate its expression and/or to identify natural substances that can determine the activation and the expression of maspin or possibly deliver this molecule in tumor cell through gene therapy capable of upregulating maspin in an attempt to reduce invasiveness and the risk of metastasis. Maspin packaged as an adenoviral construct or a liposomal DNA has been utilized to reduce tumors and tumor vasculatures in *in vivo* studies corroborates as a feasible approach toward cancer therapy. Nevertheless, the approach using viral vectors or liposomal DNA complexes is accompanied with several safety and efficacy issues that modify their association upon interaction with serum components, which is a subject of rigorous studies, and further evaluation is required through clinical trials. The usefulness of rMaspin in targeted therapy is debatable because proteins undergo rapid clearance from the body through proteolysis, liver clearance and filtered through the kidneys. These issues can be resolved by the use of nanotechnology, which has provided a valuable option for targeted delivery of genes, drugs, and proteins.

Recently performed studies highlight reversible nature of epigenetic silencing of maspin in prostate cancer, which offers a unique opportunity for therapeutic intervention by several epigenetic modifiers. Considering the anti-angiogenic and pro-apoptotic properties of nuclear maspin shown by recent studies, re-activated nuclear maspin in association with anti-angiogenic or chemotherapeutic drugs may be effective in the treatment of advance-stage prostate cancer. Undoubtedly, the challenges are numerous, but the prospects for improved therapeutic approaches through maspin application for this debilitating disease could be immense.

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Conflict of interest

The authors have no competing interest.

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