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Maspin: The New Frontier

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Abstract

Maspin (mammary serine protease inhibitor) was identified in 1994 by subtractive hybridization analysis of normal mammary tissue and breast cancer cell lines. Subsequently, emerging evidence portrays maspin as a multifaceted protein, interacting with diverse group of intercellular and extracellular proteins, regulating cell adhesion, motility, apoptosis, and angiogenesis and critically involved in mammary gland development. The tissue-specific expression of maspin is epigenetically controlled, and aberrant methylation of maspin promoter is closely associated with *maspin* gene silencing. Identification of new tissue sites expressing maspin and novel maspin-binding partners has expanded the horizon for maspin research and promises maspin-based therapeutic approaches for combating cancer. This perspective briefly outlines the past and present strides in deciphering this unique molecule and speculates on new frontiers in maspin research and prospects of maspin as a diagnostic/prognostic indicator in cancer.

The serine protease inhibitor superfamily (serpins) is categorized to inhibitory and noninhibitory serpins (1). Inhibitory serpins use their reactive center loop to trap the target proteinase and inhibit its activity (1). The noninhibitory serpins have shorter NH₂ and COOH termini, and they also lack the classic serpin secretory signal peptide (1). Recent studies indicate serpins function beyond their serpin properties: they are involved in cell adhesion and play a role in extracellular matrix remodeling (2).

Maspin (mammary serine protease inhibitor) shares sequence homology with inhibitory serpins, such as plasminogen activator inhibitors 1 and 2, α -1 anti-trypsin, and non-inhibitory serpin ovalbumin (3), and several recent sequence comparisons seem to suggest that maspin is more closely related to noninhibitory clade B serpins. The reactive center loop of maspin is significantly shorter than that of most inhibitory serpins, and maspin does not undergo conformational rearrangement required to inactivate target protease(s) (4). However, the reactive center loop of maspin is clearly important for its function; studies using synthetic maspin reactive center loop peptides and maspin/ovalbumin chimeras reveal that this region is important for promoting cell adhesion (5).

In the past decade, with the expansion of studies on maspin, novel protein-binding partners have been identified and provided insight into the molecular aspects of its regulation and its divergent mechanism of action. More importantly, they have presented new prospects for therapeutic interventions for breast, prostate, and many other cancers.

Maspin Gene Regulation, DNA Methylation, and Histone Methylation/Deacetylation

Cloning maspin promoter led to identification of Ets, activator protein 1, hormone-responsive element, HIF, and p53 binding sites within the 1-kb promoter region (6). Further studies indicated sufficiency of the 1-kb upstream region for activating transcription in normal breast epithelial cells and determined the activity to be due to the Ets and its synergy with the activator protein 1 site (6). The hormone-responsive element is a negative regulator, acting through the androgen receptor in prostate (6). Nonadjuvant androgen ablation and antiestrogen (tamoxifen) therapies resulted in the induction of maspin in prostate cancer cell line (7) and breast cancer cell line MCF-7 (8), respectively. Later studies identified p53 as another regulator of the expression of maspin in both breast and prostate cancer cell lines (9).

It is now established that maspin is epigenetically regulated, and its tissue-specific expression is closely associated with DNA methylation (10). Epigenetic changes of *maspin* expression occur in the 5' regulatory region of the *maspin* gene and involve cytosine methylation, histone deacetylation, and chromatin accessibility (11). DNA methylation and histone lysine methylation and/or deacetylation are stable chromatin modification defining epigenetic programs. The epigenetic deregulation frequently participates in tumorigenesis by inactivation of tumor suppressor genes, and the association of promoter hypermethylation and gene silencing is an established oncogenic process in cancer (12). The promoter methylation of the *maspin* gene leads to gene silencing in cancers, such as breast, thyroid, skin, and colon (refs. 13–15; Table 1).

It is noteworthy that in somatic tissues, the majority of CpG islands are methylated, and tumor cells have global DNA hypomethylation compared with their normal counterparts (16). Hypomethylation is involved in the progression from the premalignant to a fully developed malignancy (17) and leads to activation of genes important for cancer development. Studies have indicated that overexpression of maspin in gastric, pancreatic, and ovarian cancers results from promoter CpG demethylation (refs. 11, 18, 19; Table 1). This clearly indicates that both methylation and demethylation of maspin promoter could regulate *maspin* gene expression. Interestingly, pharmacologic approaches for DNA demethylation often fail to activate gene expression, indicating that re-expression of certain genes requires supplementary factors and interacting partners (20) in addition to DNA hypomethylation.

The “histone code” (constituting a combination of modifications, including acetylation, methylation, and ubiquitination) dictates chromatin structure and function during development, growth, differentiation, and homeostasis of cells (21). Recently, quantitative proteomic approach has revealed that treatment of human cell cultures with histone deacetylase inhibitor PXD101 leads to the expression of genes regulating growth arrest, differentiation, and apoptosis (21). In line with these studies, treatment of maspin-deficient breast cancer cell lines with trichostatin A (a histone deacetylase inhibitor) induced maspin expression in breast and bladder cancer cell lines (22, 23), thus indicating that histone deacetylation may suppress *maspin* gene activation in these cancers.

Collectively, the methylation (and/or histone acetylation) status of the *maspin* gene is tissue specific; thus, epigenetic regulation of this tumor suppressor can lead to either activation or silencing of maspin. However, what determines the tissue-specific methylation of maspin remains to be elucidated. Interestingly, in pregnant women, differential maspin DNA methylation has been observed between the fetus and maternal blood cells (methylated in mother, unmethylated in fetus; ref. 24). This intriguing difference might assist in

deciphering the regulatory mechanism(s) governing tissue-specific methylation of maspin and perhaps certain other genes.

As both DNA methylation and histone deacetylation are reversible biological modifications, they represent novel targets for cancer therapy in general and for targeted re-expression of maspin in specific tissues.

Tissue Distribution and Subcellular Localization of Maspin and Its Protein-Binding Partners

Maspin was originally identified in prostate, thymus, testis, intestine, tongue, lung, and thymus (25). However, expanded studies have identified new tissue sites expressing maspin (Table 1).

Maspin is predominantly cytoplasmic, with some membrane association, partial secretion, and nuclear localization (26, 27). The partitioning of maspin into different subcellular location(s) is indicative of different functions, thus distinct binding partners. To date, an impressive list of biological functions has been attributed to both intercellular and extracellular maspin, which includes promoting cell adhesion and apoptosis and inhibiting cell motility, invasion, and angiogenesis (27–33). Our laboratory and others have used a yeast two-hybrid approach to identify the protein partners of maspin. This has uncovered an imposing list of proteins with potential of defining new biological functions and has provided new perspectives key to understanding the already established functions of this unique protein. In our laboratory, IFN regulatory factor 6 has been identified in mammary epithelial cells, and maspin/IFN regulatory factor 6 interaction regulates cellular phenotype (34), and IFN regulatory factor 6 plays a role in transfer of maternal immunity to offspring.¹ In prostate, maspin in association with glutathione *S*-transferase inhibits oxidative stress–induced reactive oxygen species generation, whereas through interaction with histone deacetylase 1, HP70, and HP90, maspin regulates androgen signaling (35, 36).

Interaction of maspin with the components of extracellular matrix, collagens type I and III, has also been reported (37). This interaction could contribute to the tumor-suppressive property of maspin, either by direct adherence between cell surface maspin and extracellular matrix collagen, or by altering the ability of maspin to interact with other proteins.

The function of maspin in the nucleus is less investigated and more complex; in the absence of a nuclear localization signal, maspin must either be chaperoned to the nucleus or cross the nuclear membrane by passive diffusion. Studies from our laboratory¹ have indicated that dihydrotestosterone and, to a lesser extent, 17 β -estradiol treatment of breast epithelial cells (which express minimal levels of androgen receptor) causes a portion of maspin to translocate into the nucleus, and this translocation is in association with β -catenin. Although the nuclear association of maspin and β -catenin has been established by coimmunoprecipitation approach,¹ further studies are required to confirm these observations and examine if the action of both dihydrotestosterone and 17 β -estradiol is mediated via binding their respective receptor and interacting with hormone-responsive element in maspin promoter. It is possible that in normal mammary epithelial cells, maspin could function as a transcription factor regulating gene expression. In the prostate tissue, identification of histone deacetylase 1 as the binding partner of maspin has led to the hypothesis that association of maspin with androgen receptor–interacting proteins heat shock protein 90 and histone deacetylase 1 may play an important role in the regulation of androgen receptor stability and function (36). However, it is important to note that the

¹Khalkhali-Ellis, unpublished observations.

presence of maspin in the nucleus observed in ovarian and/or pancreatic cancers (tissues that do not express maspin under normal conditions) might have a distinct function compared with that of mammary or prostate tissues, and further studies are required to clarify this issue.

In addition, there is an impressive list (Table 2) of identified maspin-binding partners from our laboratory and several others (34, 35, 37), which includes numerous enzymes and transcription factors. However, it is premature to contemplate maspin association with any of these proteins in the context of function of maspin until their interaction is established. We propose that emerging evidence is illuminating maspin as the central component of epithelial cells, engaged in a network of interactions with a wide variety of proteins, regulating the homeostasis of breast, prostate, and most probably many other tissues.

Clinical Relevance of Maspin

Based on the differential expression of maspin in normal mammary epithelial cells and breast carcinoma cell lines, a tumor-suppressive property for maspin was proposed (3, 38). This property of maspin has been the most widely studied *in vitro* in animal models and by cancer patient survival studies (for reviews, see refs. 27, 32, 38). *In vitro* studies have revealed that the function of maspin as tumor suppressor is a combination of increased cell adhesion and apoptosis and decreased motility, angiogenesis, and pericellular proteolysis (27–33). Indeed, a shotgun proteomic approach has indicated that restoring the expression of maspin in invasive carcinoma cells alters the expression of proteins regulating cell death, cytoskeletal architecture, and protein turnover, resulting in increased rate of spontaneous apoptosis, more prominent actin cytoskeleton, reduced invasive capacity, and altered proteasome function (39). Animal studies have verified *in vitro* observations and assisted in determining usefulness of maspin as a therapeutic agent in cancer.

The most compelling data regarding the clinical significance of maspin in cancer progression and metastasis emerges from cancer patient survival studies. Although the original observations pointed to the association of reduced maspin expression with cancer progression, ensuing studies have revealed this correlation to be far more complex than originally concluded. Factors contributing to this complexity include, but are not limited to, genetic background, type of cancer, the organ where tumorigenesis originated, the expression of maspin (or lack of it) in the original corresponding normal tissue, subcellular distribution of maspin (which can potentially affect the prediction of patient survival; review refs. 27, 40, 41), and use of cytotoxic drugs for cancer therapy. It is also imperative to consider that both methylation and demethylation processes could, at least in part, determine the “presence or absence” of maspin in the tumor and most probably its subcellular expression (i.e., cytoplasmic versus nuclear). Inevitably, further studies are required to shed further light on the usefulness of maspin as a prognostic marker in different types of cancer and to assist in tailored therapeutic approaches for specific cancers.

Noteworthy is the emerging association of the inflammatory response and hyperplasia with changes in maspin. For example, maspin is significantly overexpressed in inflammatory bowel disease (42) but reduced in synovial tissue of rheumatoid arthritis patients (43), which is suggestive of a role in disease “flare” in the inflammatory bowel disease, and synovial hyperplasia/cartilage invasion in the rheumatoid arthritis patients. Similarly, aberrant maspin expression in intestinal metaplasia of the gallbladder may predispose to gallbladder carcinoma (44).

Collectively, these studies, although limited in number, could support the notion that progression from inflammatory response to hyperplasia and ultimately neoplasia is heralded by changes in maspin and perhaps could have some usefulness in diagnosis.

Prospective Usefulness of Maspin in Diagnosis

The potential use of maspin alone or in combination with mammaglobin B in detecting breast cancer has been investigated recently (45). Nested reverse transcription-PCR analysis of peripheral blood samples from healthy donors and previously untreated patients indicated the presence of maspin in 24%, whereas mammaglobin B was present only in 7% of patients. The presence of maspin correlated with cell proliferation of the primary tumor, whereas mammaglobin B positivity correlated with pathologic stage, and the presence of either marker was significantly related to nodal status. Thus, indicating the two markers in association could represent a potentially useful noninvasive tool to detect breast cancer.

A recent study has indicated differential DNA methylation of the *maspin* gene between the placenta and maternal blood cells (24), which could potentially be exploited to develop further markers for noninvasive prenatal assessment. Examination of paired placental tissues and maternal blood cells from pregnant women identified hypomethylation of maspin promoter in placental tissues compared with its densely methylated status in maternal blood cells. The unmethylated maspin sequences were detected in maternal plasma in all three trimesters of pregnancy but were cleared within 24 h after delivery. In addition, in preeclamptic pregnancies, the maternal plasma concentration of unmethylated maspin was elevated 5.7 times compared with non-preeclamptic pregnancies. Hypomethylated maspin DNA is the first universal marker for fetal DNA in maternal plasma, thus allowing the measurement of fetal DNA concentrations in pregnancy-associated disorders, irrespective of fetal gender and genetic polymorphisms.

Potential Therapeutic Use of Maspin

The established antitumorigenic/antimetastatic property of maspin in cancer has prompted investigation of its usefulness as a therapeutic agent. Animal studies using targeted delivery of maspin by liposome/DNA and/or adenoviral constructs to tumor and/or tumor vasculature (32, 46) have supported a viable approach in cancer treatment. However, the safety problems associated with the viral vectors, the structure-activity relationships of liposome/DNA complexes, and the variables affecting their interaction with serum components are still the subject of intensive studies, and their use in human trials need careful scrutiny. The usefulness of recombinant maspin in targeted therapy is questionable, as proteins undergo rapid clearance from the body (proteolysis, renal ultrafiltration, and liver clearance). The development of nanotechnology has provided a valuable option for targeted delivery of genes, drugs, and proteins. For gene delivery, both organic and inorganic polymeric nanoparticles have been extensively explored, and cationic polymeric nanoparticles exhibit strong gene binding and high transfection potential with low toxicity (47); however, for cancer gene therapy, the efficiency of transfection has to be improved considerably. Polymeric nanoparticles also hold promise for peptide and protein delivery and have been successfully used for delivery of small peptides (i.e., insulin and calcitonin) in animal models (47). Polymeric nanoparticles could be specifically designed for *in vivo* delivery of maspin (or its active fragments). The polymeric nanoparticles surface chemistry has to overcome the biological barriers for maspin uptake and prolong blood circulation, and the choice of polymer and fabrication process has to impart a high loading efficiency and maintain the bioactivity of maspin during the manufacturing processes. Undoubtedly, the challenges are numerous, but the prospects for improved therapeutic approaches for this debilitating disease could be immense.

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Table 1
Tissue expression of maspin, promoter methylation status, and changes in expression resulting from neoplastic alteration

Tissue	Cell type	Promoter status	Subcellular site	Neoplastic alteration	Ref.
Breast	Epithelial	Hypomethylated	Cytoplasmic	Methylation/↓	(3, 11)
Breast	Adipocytes	Hypomethylated*	Cytoplasmic	Methylation/↓*	Khalkhali-Ellis [‡]
Breast	Endothelial	Hypomethylated*	Cytoplasmic	Methylation/↓*	Khalkhali-Ellis [‡]
Prostate	Epithelial	Hypomethylated	Cytoplasmic	Methylation/↓	(3)
Skin	Keratinocytes	Hypomethylated	Cytoplasmic	Methylation/↓	(15, 48)
Skin	Melanocytes	Hypermethylated	Cytoplasmic?	Hypomethylation/↑	(49)
Gall Bladder	Epithelial	Methylated?	ND [‡]	Hypomethylation/↑	(44)
Biliary tract		Hypermethylated	ND [‡]	Hypomethylation/↑	(50)
Thyroid	Epithelial	Methylated	ND [‡]	Hypomethylation/↑	(13, 51)
Pancreas	Epithelial	Methylated	Nuclear?	Hypomethylation/↑	(52, 53)
Ovary	Epithelial	Methylated	Nuclear?	Hypomethylation/↑	(11)
Lung	Epithelial [§]	Methylated		Hypomethylation/↓	(54)
Larynx			Cytoplasmic	↑	(55)
Bladder		Methylated	ND [‡]	Hypomethylation/↑	(23)
Stomach	Epithelial		ND [‡]	↑	(29)
Colon	Epithelial?	Hypomethylated	Cytoplasmic//	Hypermethylation/↑	(14)
Hair follicle		Hypomethylated*	Cytoplasmic	Methylation/↓*	(15), Khalkhali-Ellis [‡]
Sebaceous gland		Hypomethylated*	Cytoplasmic	Methylation/↓*	(15), Khalkhali-Ellis [‡]
Cornea	Endothelial				(29)
Cornea	Stromal		Cytoplasmic/nuclear		(29)
Cornea	Epithelial				

NOTE: In addition to the tissues listed in this table, maspin has been detected in small and large intestine, testes, thymus, vagina (25), and placenta (56), and in the absence of further information, they have not been included in the table.

* The promoter status of maspin in these tissues is not determined, the author is proposing that their promoter status is hypomethylated, and neoplastic transformation will be associated with methylation, resulting in decreased maspin expression.

[‡] Unpublished observation.

‡Lack of maspin expression.

§ Cell type specific expression, positive maspin expression in basal cells of the bronchial surface epithelium and myoepithelium of the gland and negative expression in alveolar spaces.

//Nuclear staining was observed in poorly differentiated tumors.

Table 2

Maspin-interacting proteins and their putative functions

Identified partner	Tissue	Function	Ref.
Collagen I		Matrix adhesion	(36)*
Collagen III		Matrix adhesion	(36)*
Transketolase (U55017)		Glucose metabolism	(36)
Metallothionein (BC008408)		Copper homeostasis	(36)
Human elongation factor (BC018641)		Protein synthesis	(36)
IRF-6	Breast	Cell differentiation, immune response	(33)*, Z. Khalkhali-Ellis [†]
Prostate cancer antigen 1	Breast		C.M. Bailey [‡]
β-Catenin	Breast	Transcription factor	Z. Khalkhali-Ellis ^{*†}
β-Actin	Breast	Scaffolding	Z. Khalkhali-Ellis ^{*†}
EGR1	Breast	Transcription factor	C.M. Bailey [‡]
Glutathione peroxidase (GPX1)	Breast	H ₂ O ₂ scavenger	C.M. Bailey [‡]
Pyruvate carboxylase	Breast	Anaplerotic enzyme	C.M. Bailey [‡]
GST	Prostate	Oxidative stress	(34)*
HDAC1	Prostate	AR regulation	(34)
Hsp70	Prostate	Heat-inducible molecular chaperone	(34)
Hsp90	Prostate	Heat-inducible molecular chaperone	(34)
uPA/UPAR	Prostate/breast	Pericellular proteolysis	(26)*
β ₁ integrin	Breast	Cell attachment	(57)*

Abbreviations: IRF-6, IFN regulatory factor 6; EGR1, Early growth response protein 1; GST, glutathione S-transferase; HDAC1, histone deacetylase 1; Hsp70, heat shock protein 70; Hsp90, heat shock protein 90; uPA, urokinase-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor.

* The interaction of the protein with maspin is confirmed.

[†] Unpublished observation.

[‡] Personal communication.