Maspin—A Novel Protease Inhibitor with Tumor-suppressing Activity in Breast Cancer

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Maspin (mammary serpin) is a novel serine protease inhibitor related to the serpin family with a tumor-suppressing function in breast cancer. Maspin was originally identified from normal mammary epithelium by subtractive hybridization and might function as a class II tumor-suppressor gene. Maspin's decreased expression with increased level of malignancy and its loss in metastatic cells is regulated at the transcriptional level. Cytosin methylation and heterochromatinization in the promoter region might account for this down-regulation of maspin. Transfection of tumor cells with maspin cDNA inhibits invasion and motility and decreases tumor growth and metastatic ability in nude mice. Maspin interacts with the p53 tumor-suppressor pathway and function as an inhibitor of angiogenesis in vitro and in vivo. The progressive loss of expression of maspin during tumor progression makes this new protein an interesting diagnostic and prognostic marker. The re-expression of maspin by pharmacological intervention potentially offers a promising approach as a therapeutic option in breast cancer therapy.

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Proteases and protease inhibitors are known to play important roles in tumor invasion and metastasis. Proteases degrade the extra cellular matrix (ECM), whereas protease inhibitors function to prevent this process. Two classes of proteases have been studied extensively in breast and other cancers: serine proteases (e.g. plasminogen activators) and their inhibitors, PAI-1 and PAI-2, and metallo proteases (e.g. collagenases) and their inhibitors, the tissue inhibitors of metallo-proteases (1–3). The most extensively investigated serpins are PAI-1 and PAI-2. Both have been shown to inhibit extracellular matrix degradation in vitro (4).

High tumor levels of urokinase type plasminogen activator (uPA) and PAI-1 are poor prognostic factors for disease-free and overall survival in breast cancer (1, 5). In contrast, high PAI-2 expression correlate with a favorable prognostic marker in breast cancer (6, 7).

IDENTIFICATION AND LOCATION

As a member of the serpin super family, maspin (mammary serpin) is a novel protease inhibitor related to other inhibitors such as plasminogen activator inhibitor (PAI-1 and PAI-2) and α-1-antitrypsin, as well as non-inhibitor serine proteins such as ovalbumin (3, 8).

The maspin gene was originally isolated from normal mammary epithelium by subtractive hybridization on the basis of its expression at the m-RNA level (9). The subtractive hybridization provides a positive selection system based on recovery of c-DNAs of genes expressed differentially or exclusively in normal cells (10).

Maspin's c-DNA consists of 2584 nucleotides encoding for a 42-kDa peptide. The maspin gene has been localized to chromosome 18q21.3-q23 within the same region as the plasminogen activator inhibitor –2 gene (PAI-2), the tumor-suppressor genes DCC (11) and SCCA 1 + 2 (12), and the BCL-2 gene, which is commonly rearranged in non-Hodgkins lymphoma (13, 14).

Maspin m-RNA and protein were found in normal mammary epithelial cells, but were down-regulated in mammary carcinoma cells, as shown by Northern, Southern, and Western blot analyses as well as by immunohistochemical staining (9).

TUMOR-SUPPRESSOR ACTIVITY

Several lines of evidence support the role of maspin as a tumor-suppressor gene.

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1. Functional studies in nude mice show that mammary carcinoma (MDA-MB 435) transfectants expressing maspin are inhibited in tumor growth and metastasis (15).
2. Invasion assays demonstrate that endogenous and exogenous maspin inhibits invasion through the basement membrane matrix in culture (8).
3. Motility assays showing that cell motility can be blocked by endogenous or exogenous maspin (15). Inhibition of invasion and cell motility can be reversed with anti-maspin antibodies (15). In addition, maspin shows sequence similarity to other serpins with protease inhibitor activity (9).

Several studies done by Pemberton, Sheng, Hendrix and Sager support the hypothesis that maspin functions at the level of invasion and metastasis by blocking tumor cell migration and proliferation (8, 15–18).

Pemberton demonstrated that maspin is present in the epithelium of many human organs (such as prostate, thymus, testis, small intestine and colon) and particularly in the myoepithelia of the breast where it functions both intra- and extracellularly (19).

TRANSCRIPTIONAL REGULATION AND FUNCTION

We have shown that the differential expression of maspin in normal and carcinoma-derived mammary epithelial cells is regulated at the transcriptional level. The loss of maspin expression during tumor progression results, at least in part, from the absence of transactivation through the Ets– and AP1–DNA binding sites within the maspin promotor region (20, 21). Aberrant cytosine methylation patterns and chromatin condensation in the promoter region of the maspin gene have been recently discussed to account for the silencing of maspin gene expression (22).

At present the molecular and biological mechanisms of maspin's function is unknown. Maspin is located in the cell membrane and extracellular matrix and does not behave as a classical inhibitory serpin against any known target proteases, including trypsin, chymotrypsin, elastase, plasmin, thrombin and plasminogen activators (17, 18, 23). Thus it remains to be determined whether maspin functions as a substrate and not as an inhibitor of serine proteases ('non-inhibitor serpin') or acts at the cell membrane and requires a reactive site loop ('inhibitory serpin') (8, 11, 17, 19).

We cloned and sequenced a homologous mouse maspin (mMaspin) (24) to take advantage of the mouse model system, such as hormonal regulation of maspin in the mouse mammary glands, the production of transgenic animals to study the effects of overexpression of maspin on mammary gland development, and knock out mice to study the loss of function of maspin. mMaspin shows 89% homology with human maspin at the amino acid level and demonstrates expression in normal mouse mammary epithelial cells and down-regulation in mouse breast tumor cell lines, like its human homolog (24).

Maspin gene expression in tumor suppression can be induced by gamma linolenic acid (GLA) (25) and by over-expressing manganese-containing superoxide dismutase (MnSOD) (26). Therefore the reduction of tumor cell motility induced by GLA and other essential fatty acids and the inhibitory effect on cell invasion induced by MnSOD may be related to the up-regulation of the tumor-suppressor gene maspin (25, 26).

Recently published data from Seftor et al. indicate that recombinant maspin (rMaspin) reduced the invasive phenotype of mammary carcinoma cells (MDA-MB 435) by altering their integrin profile (particularly $\alpha 5$). The treated breast cancer cells convert to a more benign, less invasive epithelial phenotype (27). Sheng et al. found that single-chain tissue plasminogen activator (sctPA) specifically interacts with the maspin reactive site loop peptide and forms a stable complex with recombinant maspin. These interactions between maspin and sctPA in vitro suggest a mechanism by which maspin regulates plasminogen activation by sctPA bound to the epithelial cell surface (28). These data support the potential diagnostic and therapeutic relevance of maspin in the management of breast cancer.

Only a very few studies have been done with surgical specimens. An exception is the work done by Zou et al. who demonstrated the progressive loss of expression of maspin during progression of primary tumor cells from ductal carcinoma in situ to invasive carcinoma using immunohistochemical staining (9). Luppi et al. developed a RT-PCR assay of a maspin transcript to identify mammary carcinoma cells in peripheral blood samples and bone marrow samples of patients with breast cancer (29). Sabbatini et al. from the same group confirmed recently, in a clinical study of 30 patients, that reverse transcription-polymerase chain reaction (RT-PCR) for maspin mRNA is a sensitive assay for the detection of circulating neoplastic mammary cells in patients with breast cancer (30).

Although at present the molecular and biological mechanisms of maspin's function(s) remain unknown there is new evidence that maspin interacts with the p53 tumor-suppressor pathway and might function as an inhibitor of angiogenesis.

Zou et al. reported that p53 regulates the expression of maspin in breast and prostate cancer cell lines (31). They showed that p53 activates the maspin promoter by binding directly to the p53 consensus-binding site present in the maspin promoter. In addition, the authors reported that DNA-damaging agents and cytotoxic drugs induce endogenous maspin expression in cancer cells containing wild type p53, but not in cells containing mutant p53. Investigations to elucidate this potential interaction in vivo are ongoing.

Another interesting aspect opens a new discussion on maspin's function. Zhang et al. reported that maspin is capable of blocking angiogenesis both in vitro and in vivo, thereby reducing the density of tumor-associated microvessels and restraining tumor growth (32). Because angiogenesis is likely to play a crucial role in tumor development and progression (recently reviewed in ref. (33)), the dual mode of maspin-mediated tumor-suppressor activity may become of major interest.

These new aspects: interaction of maspin with the p53 pathway and its effect on angiogenesis, have been recently summarized by Hendrix and provide new mechanistic information about factors that regulate tumor cell metastasis (34).

EXPRESSION OF MASPIN BY RT-PCR AND IMMUNOHISTOCHEMISTRY

We recently published data demonstrating further evidence of decreased maspin expression in human breast cancer tissues: We used Northern blot analysis and RT-PCR to detect maspin mRNA expression in >50 primary breast tumors. Only 48% of primary breast tumors showed maspin expression. In contrast, maspin mRNA was detected in 94% of normal breast epithelial tissues from the same patients. We did not find a strong correlation between maspin expression and a number of established prognostic factors including age, tumor stage, lymph node status, tumor histology, estrogen/progesterone receptor status, p53 and c-erbB2 oncogene expression. However the down-regulation of maspin in half of the investigated human breast cancer tissues suggests that maspin may possess tumor-suppressor activity in vivo (35).

Own data of immunohistochemical staining of human breast tissue specimens show decreasing maspin expression with increasing malignancy. We have examined a large series of breast tissue specimens ($n = 234$) including normal breast glands ($n = 7$), fibrocystic changes ($n = 22$), ductal carcinoma in situ (DCIS, $n = 12$), infiltrating carcinomas ($n = 128$) and their lymph node metastases ($n = 65$) with use of a specific monoclonal antibody. The highest maspin expression was found in normal myoepithelial cells, which is congruent with the idea that myoepithelia build up a defense against stromal invasion by production and secretion of matrix proteins and proteinase inhibitors (36, 37). Accordingly, this reactivity was fully preserved in the intact myoepithelial layer enveloping carcinoma in situ. In epithelial cells, the highest maspin content was found in normal breast and fibrocystic change.

A significant stepwise reduction of maspin expression ($p < 0.0001$) occurred along with the progression from DCIS to invasive carcinomas to lymph node metastasis. Carcinomas that had metastasized to lymph nodes at the time of diagnosis exhibited significantly lower maspin levels than node negative tumors ($p < 0.01$), independently of tumor stage and grade.

Considering that tumor spread to lymph nodes is a critical step in the progression of breast cancer this result suggests that the decline in maspin expression may reflect the metastatic potential of breast carcinomas. This prognostic value has recently been demonstrated for oral squamous cell carcinoma, in which a high maspin content correlates with the absence of lymph node metastasis and improved overall survival (38). We are currently undertaking retrospective studies to verify this association in mammary carcinomas.

CONCLUSION

In summary, maspin is a novel serine protease inhibitor with tumor-suppressor activity in breast cancer. Maspin acts at the level of invasion and metastasis, interacts with the p53 tumor-suppressor pathway and functions as an inhibitor of angiogenesis. Although the mechanisms of maspin remain unknown it might function as a class II tumor-suppressor gene. These genes are not altered at the DNA level but affect the phenotype by modulation at the level of expression. Re-expression of class II tumor-suppressor genes such as maspin by pharmacological intervention offers a promising approach to influence the differentiation of tumor cells as a therapeutic option in cancer therapy (39, 40).

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