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PROSPECTS

Tumor Suppressive Maspin and Epithelial Homeostasis

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Abstract Maspin is a 42-kDa novel serine protease inhibitor (serpin) with multifaceted tumor suppressive activities. To date, the consensus that maspin expression predicts a better prognosis still largely holds for breast, prostate, colon, and oral squamous cancers. Interestingly, however, more detailed analyses revealed a biphasic expression pattern of maspin in early steps of tumorigenesity and re-expression of maspin in dormant cancer metastatic revertants. These data suggest a sensitivity of maspin expression to changes of epithelial microenvironments, and a role of maspin in epithelial homeostasis. Experimental evidence consistently showed that maspin suppresses tumor growth, invasion and metastasis, induces tumor redifferentiation, and enhances tumor cell sensitivity to apoptosis. Maspin protein isolated from biological sources is a monomer, which is present as a secreted, a cytoplasmic, a nuclear, as well as a cell surface-associated protein. Nuclear maspin is associated with better prognoses of cancer. It is further noted that extracellular maspin is sufficient to block tumor induced extracellular matrix degradation, tumor cell motility and invasion, whereas intracellular maspin is responsible for the increased cellular sensitivity to apoptosis. Despite these exciting developments, the mechanistic studies of maspin have proven challenging primarily due to the lack of a prototype molecular model. Although the maspin sequence has overall homologies with other members in the serpin superfamily, it does not behave like a typical serpin, that is, non-inhibitory toward active serine proteases in solution. This novel feature is in line with the X-ray crystallographic evidence. Several recent studies dedicated to finding the maspin partners support a paradigm shift. The current review is intended to summarize these recent findings and discuss a new perspective of maspin in epithelial homeostasis. J. Cell. Biochem. 97: 651–660, 2006.

Key words: maspin; serine protease inhibitor; tumor suppressor; homeostasis

MASPIN EXPRESSION AND SUBCELLULAR LOCALIZATION IN TUMOR PROGRESSION

The maspin cDNA encodes a 42 kDa protein (376 amino acids) with the overall sequence homologies with serine protease inhibitors, or serpins, and an Arginine residue at its reactive site loop (RSL) p_1 site [Zou et al., 1994]. The protein sequence of maspin is highly conserved among human, mouse and rat [Umekita et al., 1997; Zhang et al., 1997a]. The human *maspin* gene has been mapped to a cluster of serpins at chromosome 18q21.3-q23 [Sager et al., 1994]. Mouse maspin, mMaspin, is found to have a similar tissue expression pattern as human maspin [Zhang et al., 1997a]. Maspin protein isolated from biological sources is a monomer, which is present as a secreted, cytoplasmic, nuclear, as well as a cell surface-associated protein [Pemberton et al., 1997; Shao et al., 1998; Katz and Taichman, 1999; McGowen et al., 2000; Chim et al., 2005; Lonardo et al., 2005]. The epithelial-specific expression of maspin in placenta during embryogenesis [Chim et al., 2005] and in normal somatic tissues is controlled by the methylation mechanism [Futscher et al., 2002].

The clinical relevance of maspin in human cancers is extensively investigated since its discovery in 1994. In breast, maspin is highly expressed in normal epithelial cells, especially in myoepithelial cells, downregulated in invasive and metastatic breast carcinoma cells [Zou et al., 1994; Lele et al., 2000]. In oral squamous carcinoma, maspin expression correlates with better prognoses [Xia et al., 2000]. In prostate cancer, loss of maspin expression correlates with higher tumor stages and increasing histological

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dedifferentiation [Pierson et al., 2002]. In contrast, prostate cancer patients who retained maspin expression had a significantly longer recurrence-free survival [Machtens et al., 2001]. The expression of maspin in both breast and prostate epithelial cells may be directly activated by tumor suppressor p53 [Zou et al., 2000], or inactivated by hormone [Zhang et al., 1997b]. Consistently, nude mice induced maspin expression in LNCaP xenograft tumors [Zou et al., 2002]. Zou et al., subsequently reported that when patients were treated with neoadjuvant androgen ablation therapy before radical prostatectomy, maspin expression was significantly higher [Zou et al., 2002].

While the loss of maspin expression is often detected at the step of tumor invasion, using both radical prostatectomy and prostate autopsy specimens, we studied the expression of maspin in early steps of tumor development. We found that in normal prostate, the basal epithelial cells uniformly express maspin at a high level, mostly in the nuclei. In contrast, secretory prostate epithelial cells express little or no maspin. Intraepithelial neoplasm (PIN) (especially high grade PIN) and low grade prostate carcinoma (LGPC) expressed a high level of maspin as compared to the secretory epithelial cells, and with mixed nuclear/cytoplasmic positivities. In LGPC, maspin was more associated with the cell membrane towards the lumen (Fig. 1). High grade prostate carcinoma (HGPC) express maspin at a significantly reduced level [Pierson et al., 2002]. Overall, the expression of maspin in PC and PC-associated PIN lesions is significantly decreased when the Gleason's grade increases from 6 (or lower) to 7 (and above). Clinically, this division marks a transition towards poor prognosis [Sakr and Partin, 2001].

Specific subcellular localizations of maspin seems to be associated with distinct tumor progression pathways. For example, in invasive breast cancer, a nuclear maspin signal was associated with estrogen receptor (ER) and progesterone receptor (PR) positivities, but not to S-phase fraction or ploidy. In contrast, cytoplasmic staining was related to ER and PR negativity, high S-phase fraction and aneuploidy [Mohsin et al., 2003]. In invasive ovarian cancers, maspin signals were more likely to have predominantly cytoplasmic staining compared with benign and low-malignant-potential tumors, which featured with nuclear maspin signal [Sood et al., 2002]. In lung cancer, a nuclear, opposed to a combined nuclear and cytoplasmic localization, has been associated with increased survival in human malignancies including non small cell lung cancer (NSCLC) [Heighway et al., 2002; Smith et al., 2003]. Immunohistochemistry revealed maspin expression to be virtually universal in NSCLC.

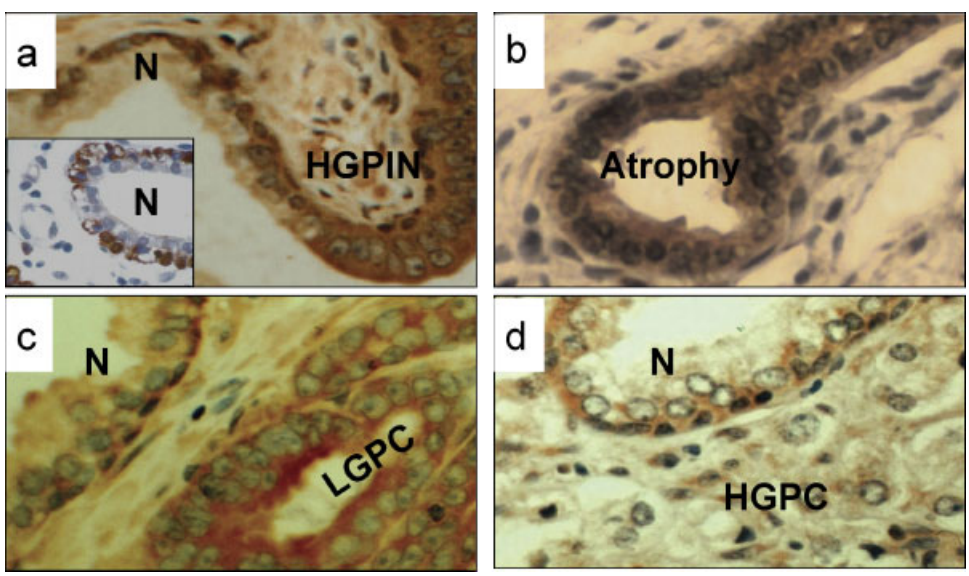

Fig. 1. Maspin immunoreactivity (brown color) in (**a**) benign prostate epithelium with high grade PIN (inset: normal human breast); (**b**) atrophic prostate epithelia; (**c**) low grade prostate carcinoma (LGPC); (**d**) high grade prostate carcinoma (HGPC). ×200. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Interestingly, squamous cell carcinoma of the lung showed almost exclusively a combined nuclear and cytosolic maspin stain. In contrast, in adenocarcinoma of the lung, nuclear maspin (but not combined nuclear-cytoplasmic maspin) significantly correlated with low histological grade, lower proliferative rate, absence of invasion, and negative p53 stain. Thus, the nuclear localization of maspin may stratify subtypes of cancer with favorable clinical–pathological features [Lonardo et al., 2005]. Furthermore, since the eventual loss of maspin is associated with more aggressive phenotypes (reviewed in Sheng [2004]), the seemingly paradoxical increase of cytoplasmic maspin in early steps of tumor progression may signal a suboptimal suppressive effect in the transition from non-invasive to invasive diseases.

The itinerant subcellular compartmentalization combined with its novel biphasic expression profile in early tumor development also suggests that maspin has versatile biological functions under different pathophysiological influences. It is noted that a high level of maspin in both nuclear and cytoplasmic compartments was found in all atrophic secretory prostate epithelial cells examined (Fig. 1). Atrophy is considered as a stressed state of prostate epithelia and has been linked to chronic inflammation [De Marzo et al., 1999, 2004; Nelson et al., 2004]. Not surprisingly, in vitro promoter activity study showed that, in addition to p53 [Zou et al., 2000], a list of stress-related signals including DNA-damaging agents, cytotoxic drugs [Zou et al., 2000], peroxisome proliferator-activated receptor-gamma [Mueller et al., 1998], noxide [Khalkhali-Ellis and Hendrix, 2003] and manganese superoxide dismutase (MnSOD) [Li et al., 1998] activates maspin expression. Consistently, several stress signals that induce maspin expression also induced more differentiated phenotypes [Li et al., 1998; Mueller et al., 1998; Zou et al., 2000; Khalkhali-Ellis and Hendrix, 2003]. Interestingly, Barsky and colleagues reviewed 200 cases of metastatic human breast cancer and found 21% of these cases showed features of reversion to a ductal carcinoma in situ (DCIS) growth pattern. These "revertants" can be easily distinguished for the expression of maspin [Barsky et al., 1997]. This data raises the possibility that maspin re-expression may contribute to metastasis dormancy in vivo. Taken together, a new theme emerged from the clinical correlative studies suggests that maspin is a responder to changes in epithelial microenvironment in favor of differentiation.

COMPARTMENTALIZED MASPIN FUNCTIONS IN TUMOR SUPPRESSION

Maspin has been consistently shown to suppress the aggressive tumor phenotypes, inhibiting invasion and motility in vitro and inhibiting tumor growth and metastasis in experimental animal models (reviewed in Sheng [2004]) including the SCID-Hu model for human prostate cancer bone metastasis [Cher et al., 2003]. Consistent with the clinical correlative studies, experimental evidence further supports that the biological functions of maspin are compartmentalized.

A Novel Extracellular Mode of Maspin Action and Pericellular Proteolysis

An anti-invasive effect was observed with both endogenous maspin (re-expression or overexpression) and exogenously added purified maspin protein, and can be partially reversed by a maspin-neutralizing antibody made against the maspin RSL peptide (Abs4A). Consistently, the maspin effect on cell motility and invasion seems to be localized at the interface of cell and extracellular matrix (ECM) [Sheng et al., 1996]. Although maspin does not directly inhibit an active serine protease, in light of the following considerations, it remains a possibility that extracellular maspin plays an important role to block pericellular proteolysis.

A current consensus suggests that cell motility and invasion require both the dynamic formation of new adhesion and the detachment from matured (or established) cell–matrix interaction [Zamir and Geiger, 2001]. In fact, mature focal adhesion contacts (FAC) have been shown to retard cell detachment and limit cell migration [Schlaepfer and Mitra, 2004]. On the other hand, both cell adhesion and detachment may be associated with, and further propelled by, ECM remodeling [Friedl and Wolf, 2003]. In vitro studies have demonstrated that maspin enhances cell adhesion to ECM protein fibronectin (FN) with increased FAC [Seftor et al., 1998; Odero-Marah et al., 2003]. Since increased cell adhesion dynamics in the absence of retraction control may lead to a net increase of tumor cell migration and invasion, the maspin

effect on cell adhesion to FN alone may not fully explain the inhibitory effect of maspin on cell motility and invasion. In a recent study, we found that maspin strengthened established FAC and inhibited cell detachment [Yin et al., 2005a].

Exogenously added maspin protein was sufficient to inhibit cell detachment, motility, and invasion, indicating the pre-existence of the maspin-responsive pathway. Previously, we showed that maspin inhibits the activity of cell surface-associated urokinase type plasminogen activator (uPA) [McGowen et al., 2000; Biliran and Sheng, 2001]. Since extracellular maspin is efficiently internalized [Biliran and Sheng, 2001; Odero-Marah et al., 2003], it is possible that internalized maspin may initiate an inside-out signaling mechanism that subsequently changes the FAC dynamics. The identification of several intracellular molecules as candidate maspin partners [Bailey et al., 2005; Yin et al., 2005b] may be of value for future research in this direction. In the meantime, it is not unreasonable to assume that the endocytosis of maspin must start with some kind of molecular interactions on the cell surface. To this end, the uPA/uPAR (uPA receptor) complex is the only cell surface-associated target of maspin implicated thus far. Although we cannot rule out the possibility that maspin may interact with other cell surface-associated molecules, the evidence that disruption of uPA and uPAR interaction prevents maspin binding to the cell surface further suggests that the cell surface-associated uPA/uPAR complex may be the primary extracellular target of maspin [Yin et al., 2005a].

A long standing question is how maspin binds to uPAR-associated uPA if it has no affinity for active uPA and does not act as a proteolytic inhibitor of soluble active uPA. It is worth noting that uPAR initially recruits pro-uPA. The pro-uPA then gets proteolytically cleaved, presumably by an adjacent plasmin, and becomes active uPA. At a steady state, while some cell surface uPAR may be occupied by active uPA, other uPAR molecules may be either unoccupied or occupied by pro-uPA. It has been shown that pro-uPA has a low intrinsic reactivity to activate plasminogen [Behrendt et al., 2003]. This intrinsic proteolytic activity is postulated to help maintain the reciprocal uPA-plasminogen activation loop [Behrendt et al., 2003]. It is not clear how pro-uPA is enzymatically controlled. To this end, our kinetic studies revealed a novel preference of maspin for pro-uPA via non-covalent interactions [Yin et al., 2005a].

Targeting the cell surface-associated uPA/uPAR complex may be particularly effective to block tumor-mediated ECM remodeling, since plasmin derived from plasmiongen activation can directly degrade non-fibrillar ECM proteins and activate other types of proteases such as matrix metalloproteinases (MMPs) [Mueller, 1996]. Plasminogen activator inhibitor type 2 (PAI-2), a tumor suppressive homolog of maspin that also triggers the internalization of cell surface-associated uPA/uPAR complex [Tsatas et al., 1997], has been shown to counteract the uPA-mediated cell detachment in vitro [Reinartz et al., 1996]. The inhibitory effect of maspin on cell surface-associated uPA activity semi-quantitatively correlates with its effect on cell motility and invasion [McGowen et al., 2000], tumor cell-mediated ECM degradation in vitro and tumor-mediated osteolysis in vivo [Cher et al., 2003]. As more detailed biochemical and biophysical studies are underway to further characterize the novel maspin/pro-uPA interaction, considering the evidence that both pro-uPA and active uPA can be internalized by the LRP mediated mechanism [Kounnas et al., 1993], maspin may prove to be an efficient quencher of the cell surface-associated uPA/uPAR complex by triggering the internalization even before pro-uPA becomes proteolytically activated.

It is important to note that the negative correlation of maspin expression with tumor progression [Sheng, 2004] is to be contrasted by the positive correlation of uPA and uPAR with tumor progression [Duffy, 2002]. This clinical observation may argue that the effect of maspin on uPA/uPAR complex may be biologically irrelevant but only of therapeutic significance. Alternatively, it is intriguing to speculate that during tumor progression, uPA and uPAR are upregulated as a result of maspin down-regulation. Cell surface-associated uPA/uPAR complex may lead to activation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) [Aguirre Ghiso et al., 1999; Nguyen et al., 2000], a consequence of which is the transactivation of AP-1 genes including uPA and uPAR [Hsu et al., 2000]. Thus, a positive feedback regulation of uPA and uPAR transcription by cell surface uPA/uPAR is implicated. If this is the case, the inhibition and/or depletion

of cell surface uPA/uPAR complex by maspin may further down-regulate the expression of both uPA and uPAR. Indeed, maspin was reported to suppress the expression of uPA and uPAR even when the tumor cells were challenged with hypoxic conditions [Amir et al., 2005].

Maspin is a secreted, a cytoplasmic, a nuclear, as well as a cell surface-associated protein. Missing in the correlative clinical data base is the profile of secreted and cell surface-associated maspin. To this end, an inhibitory effect of extracellular maspin on the uPA/uPAR system helps explain the experimental evidence that purified maspin and maspin stable transfection both effectively block tumor cell motility and invasion, tumor-mediated ECM degradation and angiogenesis, and induce tumor cell redifferentiation (reviewed in Sheng [2004]), despite the fact that in most of these cases, the bioengineered maspin was not predominantly nuclear. For example, although overexpressed maspin in cytosol is an independent marker for bad prognosis of ovarian cancer, transfection of invasive ovarian cancer cell lines with maspin cDNA inhibited tumor invasion in vitro [Sood et al., 2002]. Taken together, extracellular maspin may specifically regulate the cell surface-presentation of uPA/uPAR complex, inhibit the dynamic ECM remodeling, and consequently stabilize matured FAC-dependent cell–matrix interactions.

Intracellular Maspin Is Implicated in Cellular Stress Responses

Accumulated evidence suggests that the in vivo inhibitory effect of maspin on tumor growth is, at least in part, due to increased apoptosis [Zhang et al., 2000a; Shi et al., 2002, 2003]. As compared to maspin transfected MDA-435 and DU145 cells, normal breast or normal prostate epithelial cells that express a high level of maspin exhibited a strong resistance to TRAIL or staurosporine (STS)-induced apoptosis as judged by the specific $116 \rightarrow 85$ kDa PARP cleavage (Fig. 2). Later, we found that maspin expression sensitized tumor cells to a series of apoptotic stimuli, ranging from death ligands (TRAIL and TNF-α) to brefeldin-induced endoplasmic reticulum stress [Jiang et al., 2002; Liu et al., 2004a]. We also showed that maspin also sensitized DU145 cells to apoptosis induced by doxazosin, an α1-adrenoceptor antagonist in clinical trials for prostate cancer [Tahmatzopoulos et al., 2005].

We screened both pro- and anti-apoptotic factors to identify the maspin effectors, and found that maspin expression led to elevated expression of pro-apoptotic Bax [Liu et al., 2004a]. Although the elevated Bax expression did not result in spontaneous apoptosis, it is associated with a slightly increased translocation from cytoplasm to mitochondria. When induced by apoptotic stimuli, the translocation of Bax was almost driven to completion. TRAIL

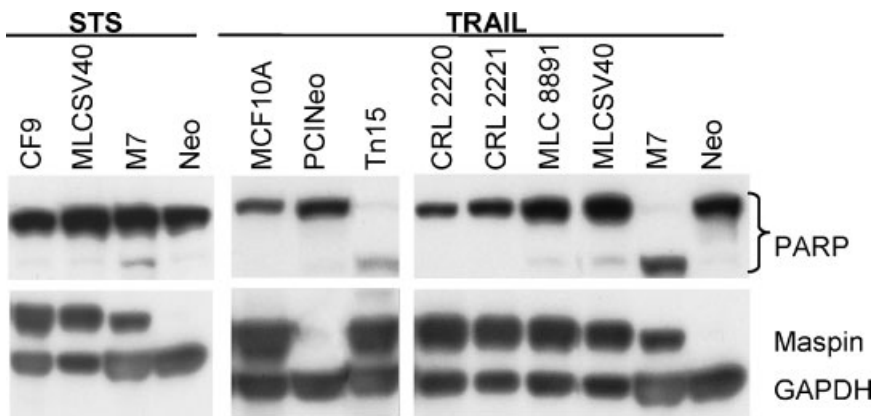

Fig. 2. Western blotting of PARP, maspin and GAPDH in cells treated with either STS (0.5 mM, 3 h) or TRAIL (50 ng/ml, 1 h). CF9, MLC8891 and MLCSV40 are immortalized normal prostate epithelial cells (from Dr. Rhim, NIH). CRL2220 and CRL2221 are immortalized non-tumorigenic human prostate epithelial cells (ATCC). MCF10A is a naturally immortalized normal breast epithelial cell line. PCIneo and Tn15 were derived from mock and maspin transfection of MDA-MB-435 cells, respectively. Neo and M7 were derived from mock and maspin transfection of DU145 cells, respectively.

(or TNF-α) treatment of maspin transfected cells led to a more dramatic increase of cytochrome-*c* release, caspase-9 and caspase-3 activities, PARP cleavage, and nuclear DNA fragmentation, which can be partially abolished by specific caspase-9 inhibitor. Consistently, Bax knockdown by siRNA blocked the sensitizing effect of maspin on TRAIL-induced apoptosis. We also demonstrated that intracellular maspin, but not secreted or exogenously added rMaspin sensitizes drug-induced tumor apoptosis [Jiang et al., 2002]. While the extracellular maspin depends on its intact RSL to inhibit cell surface-associated uPA/uPAR, cell motility and tumor invasion, intracellular maspin needs both the N-terminal and the C-terminal domains to sensitize cellular apoptotic response. Two maspin-PAI-1 swapping chimeras lost the ability to sensitize breast cancer cells to STS-induced apoptosis [Jiang et al., 2002]. This is the first evidence linking intracellular maspin to a biological function. Furthermore, our data further supports the notion that intracellular maspin may act in a mechanism distinct from that for extracellular maspin.

In search of the intracellular partners of maspin, we constructed a full-length maspin bait for yeast two-hybrid screening (using both human prostate epithelial cDNA library and a HeLa cDNA library) and identified GST (μ and ω isoforms), Hsp90 and HDAC1 as candidate maspin interactors [Yin et al., 2005b]. We confirmed these interactions in human prostate tissues. Consistent with clinical data [Pierson et al., 2002], noninvasive prostate tumor sample a comparable level of maspin as the matching normal tissues, while invasive prostate tumor expressed significantly less maspin as compared to their matching normal samples. Interestingly, significantly greater amounts of maspin, Hsp90, and HDAC1, were pulled down from normal tissues as compared to the tumor tissues.

The maspin/GST interaction was initially characterized [Yin et al., 2005b]. Endogenous maspin correlates with increased cellular GST activity, even though purified maspin does not affect the activity of GST in vitro. The basal levels of ROS in maspin transfected tumor cells were significantly lower than that in the transfection control cells. In contrast, siRNA knockdown of maspin in prostate cancer cells PC3 increased the basal ROS level. Oxidative stress, that is, treatment of with H_2O_2 (or PMA) but not with TRAIL, further increases the maspin/GST interaction in DU145 cells, and significantly attenuated H_2O_2-induced ROS generation and VEGF expression. This evidence is further supported by the evidence that maspin transfected tumor cells produced less VEGF than the transfection control cells when treated with doxazosin [Tahmatzopoulos et al., 2005]. Interestingly, a single point mutation at the RSL p_1 position of maspin (Mas^{R340A}) greatly reduced the affinity for GST. Consistently, treatment with purified wild type maspin, but not Mas^{R340A}, significantly increased cellular GST activity.

In a complementary yeast two-hybrid expedition, Bailey et al., reported a specific interaction between maspin and interferon regulatory factor 6 (IRF6) [Bailey et al., 2005]. IRF6, a member of the IRF family, is expressed in normal mammary epithelial cells, but downregulated in invasive breast cancer cells [Bailey et al., 2005]. IRF6 expression in mammary epithelial cells correlates with cell morphological changes that are associated with epithelial-to-mesenchymal transition and an increase of N-cadherin. The interaction between maspin and IRF6 appears to be regulated by IRF6 phosphorylation, and may negatively regulate the IRF6 activity [Bailey et al., 2005]. The exact biological activity of IRF6 remains to be elucidated. In the meantime, many other IRF family members have been shown to regulate interferon and interferon-inducible genes. Interestingly, IRF1 is thought to regulate the growth and differentiation of keratinocytes in Psoriasis, a chronic inflammatory skin disease in which the cells are subjected to physico-chemical and immunological stress [McKenzie and Sabin, 2003]. The activation of IRF3, on the other hand, is thought to represent a cellular detection pathway that recognizes viral nucleocapsid structure, as a part of the innate immune response to infection [Servant et al., 2003].

Intracellular maspin is functionally associated with differentiated epithelial phenotypes and epigenetics as well as cellular sensitivity to apoptosis. Considering the complex correlation of maspin expression with different stages of tumor progression, its itinerant subcellular localization, and its unique meta-stable structural features, it is likely that maspin regulates cellular responses to changes in

epithelial microenvironment by its versatile interactions with various stress-responsive proteins.

A PARADIGM SHIFT AND ITS IMPLICATIONS

The mechanistic studies of maspin have proven challenging in the absence of a prototype molecular model. Earlier attempts to test whether maspin acts as a "classical serpin" to inhibit an active serine protease led to the identification of fibrin-activated tissue type plasminogen activator (tPA) [Sheng et al., 1998] and cell surface-associated uPA [McGowen et al., 2000; Biliran and Sheng, 2001] as candidate extracellular targets. While additional evidence suggests that maspin actually binds to the zymogen form of the protease target [Yin et al., 2005a], the "classical serpin" model has become increasingly insufficient to explain the multifaceted biological functions of maspin. For example, intracellular maspin specifically sensitizes tumor cells, but not normal cells, to induced apoptosis. This apoptosis-sensitizing effect of maspin not only depends on its reactive site loop, but also the N-terminal domain. Furthermore, systemic maspin knockout is lethal at embryogenesis [Gao et al., 2004]. This dramatic finding suggests a uniquely important function of maspin in development, which can not be compensated by other serpins. Based on the recent X-ray crystallographic analyses, maspin does seem to have retained certain structural flexibilities to undergo limited conformational changes. This meta-stable conformation without the ability to engage in a strong partnership may confer versatile chaperon functions in different subcellular compartments. In light of the recent advances in maspin research, a new paradigm has emerged that maspin may act as a "chaperone" to restores normal cellular response to environmental stress signals. Although at the present time, the temporal and spatial regulation of maspin interaction with each of the implicated stress-response proteins is not clear, based on the existing literature, the biological implications of these interactions may include the followings.

Maspin is the only proapoptotic serpin amongst all serpins implicated in apoptosis regulation. The underlying molecular mechanism remains elusive. However, we speculate that maspin may regulate cellular apoptotic sensitivity via its interaction with Hsp90. Malignant tumors evolve to acquire solitary survival by dysregulating signaling mechanisms. The oncogenic signaling pathways are, therefore, potential therapeutic targets. However, as often noted, targeting one or two specific signaling pathways may be insufficient to elicit cytostatic/apoptotic effects, and may eventually lead to drug-resistance. Complex interactive networks of signaling pathways are involved in regulating cellular response to pro- and anti-survival stimuli. To this end, Hsp90, a cellular chaperone that is overexpressed in many types of cancer, may be a unique target for cancer therapy for it assists in proper folding of a variety of clients. The known Hsp90 clients include oncogenic or pro-survival proteins including c-Src, AKT, FAK, EGFR, MEK, c-Raf, HIF-1α [Beliakoff and Whitesell, 2004; Citri et al., 2004; Sreedhar et al., 2004; Zhang and Burrows, 2004]. The correct protein folding of the client proteins is protected by Hsp90 at the cost of ATP-hydrolysis, inhibition of which will release these client proteins to proteasome-mediated degradation. Hsp90 activity is regulated by its co-factors [Hostein et al., 2001; Isaacs et al., 2003]. Recently, synthetic 17-Allyamino-17-demethoxygeldanamycin (17-AAG) that locks Hsp90 in its ADP-bound form has been shown to either sensitize tumor cells to apoptosis or directly induce tumor apoptosis. Currently, 17-AAG is in clinical trials at several leading cancer centers including the Barbara Ann Karmonos Cancer Institute.

The homeostasis of gene expression is, at least in part, controlled by the acetylation/deacetylation of chromatin. Considering the evidence that maspin directly interacts with HDAC1, the most abundant HDAC, it is possible that maspin may regulate stress-responsive gene expression by negatively regulating the HDAC1 activity. When the charged acetyl groups are removed by the action of housekeeping histone deacetylases (HDACs), chromatin will be packed into a closed structure, disallowing the access of transcriptional factors, thus repressing gene expression [Barnes et al., 2005; Bhalla, 2005; Mai et al., 2005; Monneret, 2005]. Although the underlying mechanisms are far from clear, HDAC inhibitors in clinical trials, with some tolerable side effects, show clinical activity with objective tumor regression [Marks et al., 2001]. It is noted that HDAC1 seems to be specifically associated with p53-mediated stress response. PID/MTA2, a p53-interacting protein that induces p53

deacetylation by recruiting the HDAC1 complex, can attenuate p53 transcriptional activity [Gu et al., 2004]. Consistently, HDAC1 and its associated HDAC2 prevent the p21-dependent tumor cell apoptotic sensitivity [Zhu et al., 2004]. Conversely, DNA-damaging treatments increase p53-depedent p21 expression [Lagger et al., 2003] and the NF-κB function [Rocha et al., 2003] by simultaneously inducing the formation of a p53–Sp1 complex and the dissociation of HDAC1 from the C-terminus of Sp1. The deacetylated p53 can also be recruited by MDM2, leading to p53 degradation [Ito et al., 2002], and downregulation of Bax expression [Juan et al., 2000]. These data suggest that the biological activities of HDACs may be gene-specific. To this end, maspin sensitizes tumor cell to apoptosis through upregulated Bax expression [Liu et al., 2004a].

We also showed that maspin interaction with GST led to increased cellular capacity to block oxidative stress-induced ROS generation [Yin et al., 2005b]. This activity may be particularly important for protecting cells from genotoxic oxidative stress. The uncontrollable tumor growth, at both primary and metastatic sites, poses metabolic stress and exhausts local oxygen supply (hypoxia), leading to insufficient electron transfer in the mitochondrial respiratory chain reaction and elevated electrophilic metabolic intermediates. In cellular response to reoxygenate these intermediates, reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and superoxides, are accumulated. ROS can directly cause cell injury by damaging DNA, proteins, and lipids [Cook et al., 2004; Pervaiz and Clement, 2004; Shi et al., 2004]. In addition, ROS can inactivate prolyl hydroxylase (PHD). Inactivated PHD releases hypoxia-induced factor-1α (HIF-1α) from von Hippel-Lindau protein (VHL) in an E3 ubiquitin ligase complex, thus protecting HIF-1α from proteasome-mediated degradation. HIF-1α directly activates the transcription of vascular endothelial growth factor (VEGF-A) [Dachs and Tozer, 2000], a key tumor angiogenic factor. The detoxification of ROS is carried out by a redox circuitry composed of enzymes that directly convert peroxides and superoxides to oxides, and enzymes that catalyze glutathione (GSH)-based reduction [Perquin et al., 2000; Maulik, 2002]. Glutathione S-transferases (GST) and GSH peroxidases are major GSH-based reductases. Consistent with an earlier report that maspin is a potent inhibitor of tumor-induced angiogenesis in a subcutaneous xenograft animal model [Zhang et al., 2000b], we have recently shown that maspin expression leads to inhibition of tumor-induced angiogenesis in bone [Cher et al., 2003].

Tumor progression leads to and depends on ECM remodeling, which is mediated by a network of pericellular proteolytic enzymes [Tumber et al., 2003]. Tumor-induced ECM remodeling further facilitates tumor dissemination, angiogenesis, and the release of growth factors such as transforming growth factor-β (TGF-β). TGF-β is implicated as a driving force in tumor epithelial-mesenchymal transition (EMT), a manifestation of further dedifferentiation [Thiery, 2003]. Unfortunately, no drug has come out of the tremendous investment into developing specific inhibitors of ECM degrading enzymes. A major problem is that these synthetic inhibitors inhibit active proteases wherever they may be, thus may not be specific for the tumor microenvironment. On the other hand, these inhibitors may be sufficient to neutralize active enzymes but may not block the robust production and activation of these enzymes. In fact the pro-form of some proteases may have protease-independent activities. For example, plasminogen activator inhibitor type-1 (PAI-1) preferentially binds, inhibits and internalizes active uPA with insignificant effect at the step of pro-uPA activation [Durand et al., 2004]. PAI-1 is inefficient in inhibiting cell surface-associated uPA, in part, due to the pro-uPA/uPAR → plasminogen activation → active uPA/uPAR loop [Behrendt et al., 2003]. In addition, PAI-1 is upregulated together with uPA and uPAR in many types of cancer [Sheng, 2001; Sheng et al., 2002] and is shown to promote tumor invasion, angiogenesis, and metastasis [Sheng, 2001]. Our studies showed that epithelial-specific maspin inhibits tumor cell motility, inhibits cell surface-mediated uPA/uPAR activity, triggers rapid uPA/uPAR internalization, and inhibits tumor-mediated ECM degradation in vitro. Further biochemical evidence indicates that maspin binds specifically to pro-uPA [Yin et al., 2005a]. Thus, maspin may quench the uPA/uPAR function even before pro-uPA becomes activated.

Concluding Remarks

The homeostasis of a functional organ in our body has to be maintained at least in part by the reciprocal feedback control between the

functional cell types and their stromal environment. This reciprocal feedback relationship may become impaired due to aging or disease-related stresses. Tumor progression, for example, is accompanied by changes of its microenvironment at both cellular and molecular levels including decreased local oxygen concentration (hypoxia), increased extracellular matrix degradation, and proliferation and activation of stromal cells. In contrast to normal cells that respond to aberrant changes in the microenvironment by either a circuitry of defense mechanisms aimed at minimizing the damage or by undergoing cell death so that the source for increased instability is depleted, tumor cells may be propelled to acquire further instabilities at both genetic and epigenetic levels. While ample laboratory evidence suggests that tumor microenvironment may be specifically targeted in cancer treatment, an alternative approach to this strategy could be to block the receptive tumor response to the microenvironmental flares. To this end, maspin is a promising natural defense of epithelial homeostasis against the adverse effects of microenvironmental changes associated with tumor progression.

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