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The Natural Tumor Suppressor Protein Maspin and Potential Application in Non Small Cell Lung Cancer

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Abstract

The grim prognosis of lung cancer, that has an overall 10–15% survival at 5 years, remains in the US the leading cause of cancer mortality, providing a compelling rationale for studying the molecular basis of this malignancy. Surmising the common, general association with smoking, lung cancers differ at the microscopic, anatomical, epidemiological and clinical level and harbor complex genetic and epigenetic alterations. Currently, lung cancer is divided into small cell lung carcinoma (SCLC) and non small cell lung carcinoma (NSCLC) for the purpose of clinical management. NSCLC constitutes 80–85% of lung cancers and is further divided into histological subtypes such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, etc.

The ultimate goal for lung cancer research is to develop a strategy to block the tumor progression and improve the prognosis of lung cancer. This goal can realistically be achieved only when the biological complexity of this disease is taken into account. To this end, identification and understanding of molecular markers that are mechanistically involved in tumor progression are needed. Our recent studies suggest histological subtype-dependent distinct correlations between the expression and/or subcellular localization of tumor suppressive maspin with the progression and prognosis of NSCLC. Maspin is an epithelial specific member of the serine protease inhibitor (serpin) superfamily but recently identified as an endogenous inhibitor of histone deacetylase 1 (HDAC1). This novel biochemical activity coincides with a consensus emerged recently from the evidence that nuclear maspin confers better differentiated epithelial phenotypes, decreased tumor angiogenesis, increased tumor sensitivity to drug-induced apoptosis, and a more favorable prognosis. In the current review, we discuss the evidence that maspin may be a marker that stratifies the progression and prognosis of different subtypes of NSCLC.

Keywords

lung cancer; molecular marker; prognosis; histological subtypes; histone deacetylase 1; natural HDAC inhibitor; subcellular localization; differential expression; drug discovery

THE COMPLEXITY OF LUNG CANCER

Lung cancer is a leading cause of cancer morbidity and mortality worldwide, with an alarming increase in developing countries, largely as a result of tobacco smoking. In the US,

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death from lung cancer accounts for approximately 25% of all cancer deaths and exceeds the second, third, fourth and fifth most common causes of cancer death combined. Lung cancer can be generally divided into two types: small cell lung cancer (SCLC) and non small cell lung cancer (NSCLC). SCLC, of neuroendocrine origin, is highly aggressive and has a high potential for metastases. NSCLC constitutes 80–85% of lung cancers and can be further divided into several histological subtypes that differ clinically, epidemiologically and at the molecular level. Three major NSCLC subtypes are adenocarcinoma, squamous cell carcinoma and large cell carcinoma. Adenocarcinoma is the most frequently diagnosed NSCLC, representing 35–40% of all lung cancers and originates more commonly from epithelial cells at the terminal bronchioles/alveoli in the periphery of the lung. Adenocarcinoma is thought to progress through the morphological sequence atypical adenomatous hyperplasia→bronchiolo-alveolar carcinoma growth pattern→invasive carcinoma. Squamous cell carcinoma accounts for 25–30% of all lung cancers. The classic manifestation is a cavitary lesion in a proximal bronchus, and is characterized histologically by keratinization and/or intercellular bridges. Squamous cell carcinoma is thought to progress through the steps of normal bronchial mucosa→squamous metaplasia→low grade dysplasia→high grade dysplasia→invasive carcinoma. Large cell carcinoma, typically manifesting as a large peripheral mass on chest radiograph, accounts for 10–15% of lung cancers. Histologically, large cell carcinoma has sheets of highly atypical cells with focal necrosis, with no evidence of keratinization or gland formation.

The adoption of improved imaging techniques has made possible the detection of smaller and earlier stage lung cancers. However, since 1970, the 5-year survival rate has only increased from 7% to 15%. Currently, the strategy of lung cancer treatment is based on tumor stage as well as on the tumor type as either SCLC or NSCLC. SCLC tends to develop distant metastases early in the course of the disease, and are highly chemosensitive, therefore surgical resection is rarely considered in the management of the disease. First-line chemotherapy is the combination of platinum and etoposide. Although most patients are initially responsive, more than 90% of them develop resistance and eventually succumb to the disease. Treatment of NSCLC is based on its resectability. If NSCLC can be surgically removed, the cancer may be cured by surgery alone or by surgery followed by chemotherapy. If the tumor cannot be surgically removed, combination chemotherapy and radiation can be curative. For patients with metastatic NSCLC at presentation, chemotherapy remains the only therapeutic option and almost all the patients eventually succumb to their disease. It has been noted that the efficacy of pemetrexed (a folate antimetabolite) differs in patients with adenocarcinoma and squamous cell carcinoma [1]. In addition, high response rates have been observed with EGFR tyrosine kinase inhibitors (EGFR-TKIs) but this is limited to a histologically distinct subgroup of adenocarcinoma that have EGFR mutations [2]. Genetic studies have revealed multiple histological subtype-specific changes. For example, p16 inactivation is frequent and Rb mutations are infrequent in NSCLC, whereas the opposite is true in SCLC. In addition, both Rb and p53 are most commonly affected in SCLC. HER2 overexpression/mutation, EGFR mutations, and KRAS mutations occur almost only in adenocarcinomas, where they tend to be mutually exclusive [3,4]. These data demonstrate the necessity and possibility of personalized therapeutic strategies, taking into account the complex etiology, histology and genetics/epigenetics of different subtypes of this disease.

To develop personalized therapeutic strategies of NSCLC, we need to better understand how lung cancer progression and prognosis can be stratified in addition to by histological subtypes. Specific molecular markers, in combination with other diagnostic tests (bronchoscopy, chest radiography, tissue biopsy, cytological tests of sputum, MRI, CT, and ultrasound), may be the key. Lung cancer cells may express carcinoembryonic antigen (CEA), NSE (neuron specific enolase), SCC (squamous cell carcinoma antigen-1 and -2),

CYFRA21-1 (fragment of cytokeratin 19), TPA (tissue polypeptide antigen), or CA125 [5–9]. It is noted that many of these markers do not have a uniform marker value across different NSCLC subtypes. For example, CEA is significantly higher in adenocarcinoma than in large cell carcinoma, whereas NSE is low in adenocarcinoma but high in large cell carcinoma. Furthermore, these markers are not known to be functionally critical for lung cancer progression, and are not lung cancer-specific, thus have not been proven useful for either stratifying the progression or selecting treatment. Here we review the evidence that supports the clinical utility of maspin [10] as a marker for stratifying the prognosis of different subtypes of lung cancer and, possibly, predicting the effectiveness of lung cancer therapy.

THE EXPRESSION LEVEL AND/OR SUBCELLULAR LOCALIZATION OF MASPIN AS A MARKER FOR STRATIFYING THE PROGNOSIS OF DIFFERENT SUBTYPES OF LUNG CANCER

Although maspin expression has been detected in several types of cells, it is expressed predominantly by epithelial cells [11]. Since the discovery of the maspin gene [12], hundreds of published studies with human specimens indicate that maspin expression predicts a better prognosis for several types of carcinomas including breast, prostate, colon, and oral squamous cell carcinoma (for review, see [13]). The differential expression of maspin in tumor progression seems to be multifaceted and further dependent on the specific tumor types or subtypes.

Adenocarcinoma, in different tissues, seems to share a common differential maspin expression pattern in tumor progression [14,15]. For example, in normal breast and prostate epithelial tissues, maspin protein was localized in the nucleus [12,16]. In preneo-plastic or early stage of carcinogenesis, in the prostate, maspin expression is transiently up-regulated and the maspin protein is detected both in the nucleus and in the cytoplasm. As tumor further progresses to less differentiated states, maspin expression level is down-regulated or lost [16]. In lung cancer, nuclear localization, opposed to a combined nuclear and cytoplasmic localization of maspin, segregates with increased overall survival in early stage lung adenocarcinoma.

Since squamous cell carcinoma also originates from normal lung epithelial cells, i.e., bronchial ciliated epithelial cells, it remains a possibility that the switch from an exclusive nuclear maspin localization pattern to a combined nuclear and cytosolic pattern occurs prior to the step of carcinoma *in situ*. To this end, four other studies published on the differential expression of maspin in head and neck squamous cell carcinoma (oral squamous cell carcinoma and lyaryngeal squamous cell carcinoma), revealed a correlation of nuclear maspin with better differentiated phenotypes [17]. Interestingly, our earlier study of oral squamous cell carcinoma indicated a positive correlation of the maspin protein level with better cancer prognosis [18]. These data suggest that loss of maspin expression may still occur at a late stage of lung squamous cell carcinoma progression. To this end, Takanami *et al* recently showed that high levels of maspin expression predict favorable prognosis of lung squamous cell carcinoma [19]. It is important to understand the biological significance of the differential expression and/or subcellular localization of maspin as lung squamous cell carcinoma undergoes the full continuum of progression from normal epithelia to metastatic lesion.

In addition to the evidence for distinct patterns of maspin differential expression in histological subtypes of NSCLC, a critical consideration in favor of maspin as a marker for potential personalized lung cancer management is that maspin may directly regulate tumor

progression. Earlier, we have shown that maspin curtails aggressive tumor phenotypes, inhibiting invasion and motility *in vitro* and inhibiting tumor growth and metastasis in experimental animal models [13,20,21]. Maspin was sufficient to induce prostate carcinoma cell redifferentiation *in vivo* [22]. The better differentiated phenotype of maspin-expressing tumor cells is also associated with increased sensitivity to apoptosis *in vitro* [23–25]. In preclinical biological studies, we showed that maspin re-expressed by both breast and prostate cancer cells sensitized cellular response to a broad spectrum of apoptosis stimuli [25] including TRAIL, TNF-α, endoplasmic reticulum stress inducer, and staurosporine (STS). It is important to note that the pro-apoptotic effect of maspin was tumor-specific. In addition, intracellular maspin, but not extracellular maspin, seems responsible for increased tumor cell sensitivity to drug-induced apoptosis [23]. A study with laryngeal carcinoma tissue specimens, nuclear maspin expression correlates with M30, a marker for epithelial cells at an early apoptotic stage [26].

Depending on the state of epithelial differentiation, maspin protein isolated from biological sources may be a 42 kDa monomer which is present as a secreted, a cytoplasmic, a nuclear, as well as a cell surface-associated protein [20]. While extracellular maspin seems to play a critical role in blocking the initiation step of the plasminogen activation cascade [22,27,28], intracellular maspin is specifically associated with a proapoptotic effect in tumor cells [ref]. Following the observation that nuclear maspin, but not the nuclear combined with cytoplasmic maspin, correlated with better survival of lung adenocarcinoma, we examined the correlation of maspin subcellular localization with tumor proliferation index [14]. Strictly nuclear maspin localization correlated with significantly lower proliferation index Ki67 in lung adenocarcinoma. Furthermore, maspin nuclear localization inversely correlated with VEGF-A. As compared to other factors implicated in tumor prognosis such as histological grade, p53, Ki67, VEGF-A, and tumor size, nuclear maspin was the most significant indicator for better survival. The inverse correlation of maspin with VEGF-A in lung adenocarcinoma is in line with our earlier evidence that maspin blocks the oxidative-stress induced VEGF-A production in tumor cells [29], and blocks tumor angiogenesis in xenograft tumor models [22,30].

BEAS-2B is a non-transformed immortalized lung epithelial cells line that can be transformed by cigarette smoke condensate (CSC) [31–33]. BEAS-2B cells express maspin predominantly in the nucleus. To further clarify whether the translocation of maspin from the nucleus to the cytoplasm represents snap shots of tumor heterogeneity or a carcinogenesis-associated biological process, we examined the expression and subcellular distribution of maspin in BEAS-2B [15] and its CSC-transformed derivative. We have shown that CSC-transformed BEAS-2B clonal cell lines were significantly more invasive [15]. These CSC-transformed cells also expressed less maspin as compared to the parental BEAS-2B cells. Upon subcellular fractionation, nuclear maspin was found to be disproportionally further decreased in the CSC-transformed cells. This observation was consistent with the evidence from the resected lung adenocarcinomas studies by IHC.

A UNIQUE THERAPEUTIC OPPORTUNITY WITH MASPIN

Our recent data revealed that association of nuclear maspin with better differentiated epithelial phenotype is, at least in part, due to an inhibitory effect of maspin on histone deacetylase 1 (HDAC1) [24]. The identification of HDAC1 as a maspin-associated protein first came from a yeast two-hybrid screening [24]. This association is consistent with the nuclear localization of maspin. Indeed, the prostate tumor cell line PC3 that expresses maspin at a moderate level was used to show that maspin and HDAC1 interaction occurred in the nucleus [24]. Upon further structural analyses, the specific interaction between maspin and HDAC1 could be explained. HDAC1 catalyzes the removal of the ε-acetyl group from

the side chain of a lysine in the protein substrate. The catalytic domain of HDAC1 contains a peptide recognition pocket and a catalytic triad (shown by the dashed line highlight in Fig. 1). The overall catalytic domain of HDAC1 is similar to that of a protease such as serine protease or metalloproteinase [34,35]. The x-ray crystal structure of maspin [36,37] suggests that maspin can use its reactive center loop (RCL) as pseudo substrate domain to interact with the catalytic domain of HDAC1. An arginine located at the center of maspin RCL could interact with catalytic triad of HDAC1, mimicking the substrate acetylated lysine and having both structural and electrostatic similarity. The molecular interaction of maspin with HDAC1 was confirmed in an array of normal and maspin-expressing tumorous prostate epithelial cell lines [24]. In paired human prostate tissues, maspin and HDAC1 levels were inversely correlated. Furthermore, more maspin-HDAC1 association was detected in the normal tissue of each pair.

We have tested a possibility that maspin may directly inhibit HDAC1. Purified recombinant protein not only binds HDAC1, but also inhibits its activity *in vitro*. Down-regulation of maspin led to increased activity of HDAC1 in PC3 cells [24]. Conversely, reintroduction of maspin to prostate cancer cell line DU145 that lost ability to express detectable endogenous maspin resulted in increased histone acetylation and increased expression of HDAC1 target genes Bax and p21. This data helped to explain our earlier finding that the pro-apoptotic effect of maspin was mediated, at least in part, by increased Bax expression and function [25]. Further, as predicted by the structural considerations, the interaction between maspin and HDAC1 depends on maspin RCL. Together, these findings allow one to postulate a discovery of the first endogenous inhibitor of HDAC1.

An unexpected finding that maspin inhibits the most abundant HDAC in mammalian cells, HDAC1, in fact represents a major breakthrough that helps to explain why maspin-expressing epithelial cells are less proliferative *in vivo* [38,39], more sensitive to induced apoptosis [23,25,40] and maintain better differentiated phenotypes [20,22,41]. Briefly, the homeostasis of gene expression is, at least in part, controlled by the acetylation/deacetylation of chromatin. When the acetyl groups are removed by the action of housekeeping HDACs, chromatin will be packed into a closed structure, disallowing the access of transcriptional factors, thus repressing gene expression [42,43]. It has been estimated that approximately 7% of total expressed genes in mammalian cells may be affected by HDAC1 [44]. Many of these targets (such as p21, p27, Bax, and cytokeratin 18) are involved in apoptotic response to adverse tissue microenvironment or growth inhibition. Several synthetic HDAC inhibitors show clinical activities with objective tumor regression in clinical trials [45–47]. Considering that malignant tumors acquire survival and drug resistance through multiple dysregulated networks of signaling events, the HDAC-targeted therapies have a conceivable advantage since chromosomal modulation by HDAC inhibition can lower the overall threshold of apoptotic sensitivity. In contrast, other therapeutic agents such as gefitinib (which inhibits the tyrosine kinase activity of EGFR [48,49] are designed to target one specific molecule or pathway. Maspin is the only endogenous HDAC inhibitor identified thus far. Either maspin or HDAC1 knockout in mouse leads to embryonic lethality [38,50], suggesting high biological significance of a fine balance between HDAC1 and maspin. It is of particular interest to note that maspin expression in tumor cell lines can be up-regulated by synthetic HDAC inhibitors [24]. It is likely that this positive feedback between the maspin effect on HDAC1 and maspin expression be a self-propelling mechanism to sustain effective HDAC1 inhibition.

Since maspin and synthetic HDAC inhibitors share similar biochemical and biological activities, it is intriguing to speculate that drug-resistant cancer cells that express low levels of maspin may become sensitized by synthetic HDAC inhibitors to the therapeutic agents. It is also possible that maspin combined with a synthetic HDAC1 inhibitor that has a broad

spectrum of specificity may exert synergistic anti-tumor activity. In general, pharmacological HDAC inhibitors have a standard, modular construction with structural similarities to the HDAC acetyl-lysine substrate (Fig. 2) (also see reviews [51,52]). The natural HDAC inhibitor, in the example of maspin, however, may have multiple contacts with HDAC at and near its catalytic site, as suggested by Fig. 1. It is known that under physiological and pathological conditions, HDAC-mediated gene transcription repression is dependent on the relative level of different HDAC isoforms and make-up of each HDAC protein complexes [53–55]. Furthermore, different HDAC isoforms in different classes may have distinct substrate specificities. While further structural-functional analysis is needed to address whether maspin only inhibits HDAC1 in specific molecular contexts, drug development to target the biochemcial and biological activities of specific HDAC isoforms may benefit from a better understanding of the mode of molecular interaction between HDAC1 and its natural inhibitor maspin.

CONCLUDING REMARKS

Our data, albeit not all obtained with lung cancer specimens or lung cancer models, consistently support a role of maspin as a novel epithelial specific endogenous HDAC1 inhibitor that may both mark the better differentiated lung cancer phenotype and confer tumor cell apoptotic sensitivity. A unique and exciting direction for further research emerged from these studies is how maspin may be used to stratify different subtypes of NSCLC to predict the prognosis. On the other hand, tumor cells that no longer express maspin may become sensitive to drug-induced apoptosis if maspin expression can be restored. Since small molecular weight HDAC inhibitors and maspin shared the pro-apoptotic property, one may speculate further that pharmacological HDAC inhibitors may sensitize tumor cells that have lost maspin expression to drug-induced apoptosis.

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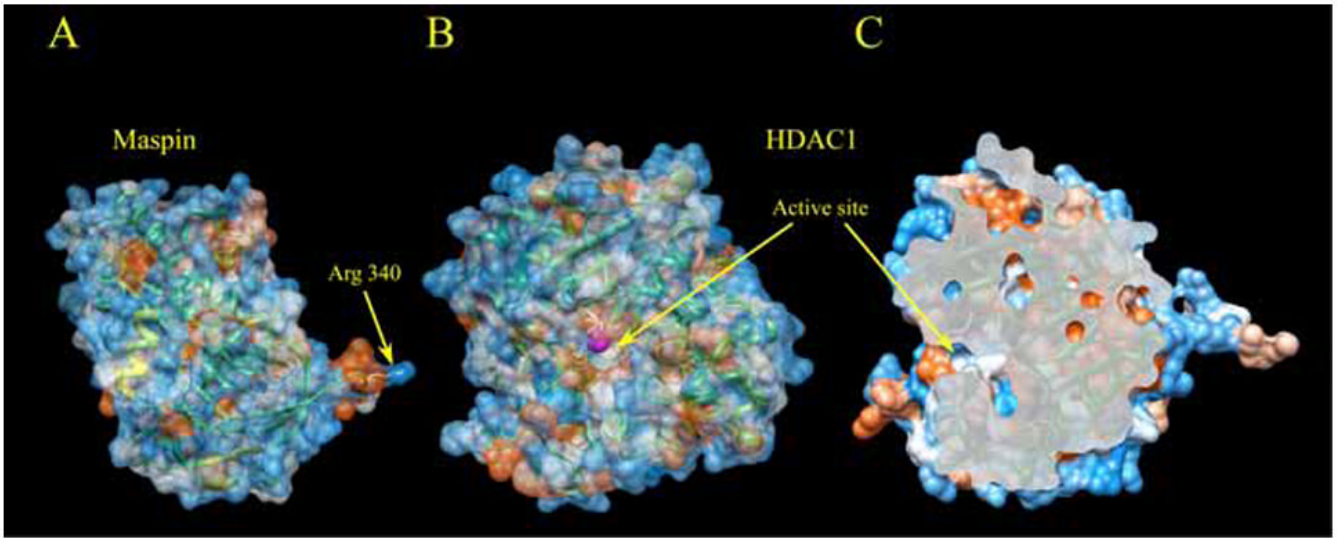
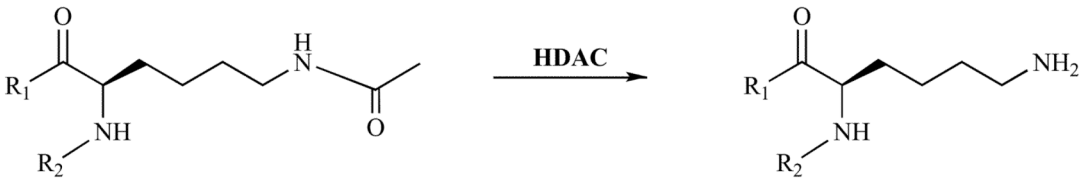


Fig. 1. The Possible Mode of Molecular Interaction of Maspin and HDAC1

Surface representations of maspin and HDAC1, colored according to electrostatic potential. **A.** Crystal structure of maspin. Arg-340 is shown in stick representation. **B.** Model of HDAC1 structure produced basing on crystal structure of HDAC8. Catalytic triad residues are shown in stick representation and Zn^{2+} ion as magenta sphere. **C.** Slice of the molecular surface of HDAC1 active site shows the feasibility for maspin RSL to fit into the catalytic domain of HDAC1. View is rotated approximately 90° about the vertical axis of **B**.



Substrate as a Template for
HDAC Synthetic Inhibitors

Fig. 2.
The Substrate Acetylated Lysine as A Template for Synthetic HDAC inhibitors.