Commentary

Maspin is a tumour suppressor that inhibits breast cancer tumour metastasis in vivo

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Abstract

Maspin is a member of the serpin family of serine proteases and functions as a tumour suppressor. A study using a new syngeneic mouse model for breast cancer suggests that maspin can inhibit metastasis *in vivo*.

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Introduction

Proteases that remodel the extracellular matrix (ECM) play an important role in the progression of neoplasia [1]. Excessive protease activity can lead to major changes within the microenvironment of tumour tissue to promote cell migration, and it thereby contributes to metastasis. Moreover, subtle changes in the levels and activities of proteases can expose cryptic sites in ECM molecules that alter integrin usage, and release matrix-bound growth factors, which both potentiate proliferation and survival of tumour cells, and induce angiogenesis [2-4]. Thus, several of the hallmarks of tumour progression occur as a result of alteration in protease activity within the extracellular environment of a nascent tumour [5]. Direct evidence that inappropriate expression of both matrix metalloproteinases (MMPs) and serine proteases, the two main classes of ECM-degrading proteases, is involved in tumour progression comes from mis-expression studies in genetically altered mice [6-8].

It is little wonder, then, that nature has devised means of keeping ECM-degrading proteases under tight control. Tissue inhibitors of MMPs suppress the activity of MMPs, whereas serpins are a class of serine protease inhibitor. The expression of these protease inhibitors is closely regulated in developmental morphogenetic processes. For example, tissue inhibitors of MMPs control ECM remodelling during mammary gland development, suppressing excess MMP activity and therefore preventing matrix remodelling from occurring prematurely in postlactational involution [9,10]. However, there can be disastrous consequences if the expression of matrix proteinase suppressing enzymes is mis-regulated. Just as over-expression of MMPs and serine proteases can contribute to carcinogenesis, so can down-regulation of their inhibitors. Levels of one such inhibitor of serine proteases, namely maspin, are frequently reduced or even absent in invasive cancer [11].

Maspin: a serine protease inhibitor

Maspin was identified by subtractive hybridization of cDNAs from normal versus tumourigenic breast cells [11]. This 42-kDa protein has significant homology to serpin and contains a carboxyl terminal reactive serpin loop domain, which is essential for its antiprotease activity. Several features of its expression and function, which are discussed below, indicate that maspin is a tumour suppressor.

First, maspin is strongly down-regulated in some cancers. Its levels inversely correlate with the stage of malignancy during breast cancer progression [12,13]. Moreover, maspin levels are also reduced in prostate and oral squamous carcinoma, and in mouse models of mammary tumourigenesis [14–16]. Two mechanisms for its altered expression in cancer have thus far been identified. The gene that encodes maspin is silenced in some tumours through hypermethylation at CpG islands [17]. Moreover, maspin is under the control of p53 and may therefore not be expressed in tumour cells with abnormal p53 function [18].

Second, recombinant maspin blocks the invasion of several tumour cell lines in Matrigel™ culture assays [19], indicating that it has a migration suppression function. One possible mechanism for this is by reducing the cell surface proteolytic activities required for breaking and making cell-matrix adhesions during migration [20]. Tissue plasminogen activator, localized to the cell surface, is implicated in migration of tumour cells [21], whereas membrane type 1 MMP stimulates migration of MCF7 cells [22]. An alternative possibility is that maspin, through an unknown mechanism, prevents invasion by increasing the strength of integrin mediated adhesion to the ECM. Recombinant maspin elevates the cell surface levels of $\alpha_5\beta_1$ integrin in MDA-MB-435 cells, thereby reducing their motility on fibronectin [23]. As with many cell regulatory factors, maspin may be presented to the cell surface by matrix molecules themselves, because it binds collagen types I and III [24].

The migration suppression function of maspin for cancer cells is potent, with a median effective dose of 0.2–0.3 µmol/l, and requires the reactive serpin loop [25]. Importantly, maspin also has an equally effective but quite different function, because it blocks endothelial cell migration in culture and neovascularization *in vivo*, independently of the reactive serpin loop [25]. Also implicated in tumour angiogenesis are MMPs [26], and analogous antiangiogenic activity is provided by a novel membrane anchored MMP inhibitor, RECK [27]. Thus, a third tumour suppressor activity of maspin is to inhibit angiogenesis, a role that now appears to be extended to other classes of protease inhibitor [28].

Maspin and tumour progression

Given all of these intriguing properties, it would be useful to know whether maspin really can affect the progression of tumours toward malignancy *in vivo*. Not many good orthotopic models for human metastatic breast cancer are available to study this. MCF7 breast cancer cells can grow and metastasize from an orthotopic site [29]. In a further model, MDA-MB-435 cells that stably express hepatocyte growth factor rapidly metastasize to the lung [30]. However, MDA-MB-435 has now been shown to be derived from a melanoma rather than a breast cancer, thus reducing the number of available models for studying

metastasis from human breast tumours [31]. By contrast, several mammary orthotopic metastatic syngeneic models have been described for both mice and rats [32–37]. A recent study [38] described an additional syngeneic tumour implantation model to investigate the role of maspin in tumour progression *in vivo*.

The new model involves transplantation of TM40D cells derived from the Balb/c mammary epithelial strain FSK-4 [39] into the orthotopic site of syngeneic hosts [38]. Primary tumours develop 3-4 weeks after inoculation with 5×10^5 cells and become large 2 weeks later. The tumours are aggressive, showing little encapsulation, and metastasize to the intestine and lung in approximately 75% of cases. The model appears to be valuable, and hopefully further studies that involve more mice and more detailed pathology of the tumours and metastases will eventually be forthcoming. For the purpose of the present commentary, however, the important result concerns the effect of maspin expression in the tumour model.

Two approaches were used in the study conducted by Shi et al. [38]. In the first approach, maspin was transfected into TM40D cells under the control of the strongly expressed elongation factor promoter and stable clones were isolated. In the second, cells infected with a retrovirus expressing maspin and green fluorescent protein (GFP) were selected by flow cytometry on the basis of GFP expression. Maspin significantly reduced the percentage of mice that developed tumours, increased the time taken for tumours to become established, and reduced their growth rate. The effect was more marked using the cells selected after viral infection. These results are impressive but not as dramatic as those from the subsequent metastasis study, in which metastasis was completely abrogated by maspin expression [38]. In each case the primary tumour was encapsulated with a fibrous sheath, suggesting a possible mechanism for inhibition of secondary tumour formation.

These are certainly interesting data and point to a potentially important role for maspin as a tumour suppressor for breast cancer *in vivo*. However, a significant number of questions remain that will hopefully be resolved by future studies. First, the data sets are very small (12 mice with TM40D implants and only three with maspin transduced cells in the metastasis study), and so statistical analysis is not really possible at this stage. Second, control experiments using cells transduced with catalytically altered maspin have not yet been done. Finally, the mechanism responsible for maspin mediated suppression of tumour growth has not been elucidated; is it due to maspin's function in blocking epithelial cell migration or angiogenesis, or both?

Intriguingly, maspin is normally produced by myoepithelial cells [40,41]. In the early stages of breast cancer progres-

sion, tumours are ensheathed by a layer of myoepithelial cells that have tumour suppressor activity [42], and are themselves subtended by a laminin-rich basement membrane [43,44]. The transition from ductal carcinoma in situ to malignant lesions results in loss of this myoepithelial cell layer and consequent disappearance of the basement membrane [42]. This myoepithelial cell and basement membrane loss is likely to have several implications for tumour progression. For example, cells that would normally depend on basement membrane for preventing apoptosis [45] are selected for their ability to survive in an inappropriate ECM environment by over-expression of integrin signalling enzymes, such as focal adhesion kinase [46], which is frequently up-regulated in breast cancer [47]. A further consequence of myoepithelial cell disappearance, based on the study using the new syngeneic breast cancer model [38], is that the reduction in maspin's function in vivo results in an increase in serine protease activity and thereby contributes to metastasis and/or the associated neovascularization to feed the growing tumour.

Conclusion

We are left with a tantalizing image of maspin as a potent tumour suppressor, both in culture and now *in vivo*. Hopefully, future studies on the structure of maspin and the molecular details of how it interacts with substrates may ultimately yield novel therapeutics that mimic its activities.

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