Urokinase-type plasminogen activator: a potent marker of metastatic potential in human cancers

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Abstract

Urokinase-type plasminogen activator (uPA) is a serine protease that is causally involved in cancer progression, especially invasion and metastasis. Multiple studies have shown that breast cancer patients whose primary cancer contains high levels of uPA have a significantly worse outcome than patients with low levels. As a prognostic marker for breast cancer the information supplied by uPA is both independent of traditionally used factors and significant in the important subgroup of axillary-node patients. Paradoxically, high levels of plasminogen activator inhibitor-1 (PAI-1), an endogenous inhibitor of uPA, also predict for aggressive disease. Recently, the prognostic impact of both uPA and PAI-1 in axillary node-negative breast cancer was confirmed using two different Level 1 Evidence studies, i.e. in both a randomized prospective trial and a pooled analysis. Therefore, uPA and PAI-1 appear to have fulfilled all the criteria for the routine assessment of prognosis in newly diagnosed breast cancer patients.

Introduction

Metastasis, or the spread of malignant cells from a primary tumour to a distant site, is the main cause of death in patients with cancer. Metastasis is...
multistep event involving degradation or remodeling of the extracellular matrix (ECM), local invasion, angiogenesis (growth of new blood vessels), intravasation, survival of malignant cells in the circulation, extravasation and, finally, growth at a secondary site (reviewed in [1]). A key molecule causally involved in a number of these processes is the serine protease, urokinase-type plasminogen activator (uPA).

**uPA: structure and function**

The human uPA gene is located on chromosome 10q (10q22) [2] and encodes a 53 kDa protein. The protein is initially synthesized as a catalytically inactive single chain peptide. Conversion into the active form can be brought about, at least in vitro, by a number of proteases such as plasmin, cathepsin B and cathepsin L (reviewed in [3]). The active form of uPA consists of a two-chain molecule in which the N-terminal A-chain is linked to the B-chain by a single disulphide bond. The A-chain (amino acids 1–158) contains a growth-factor-like domain (amino acids 1–49) while the B-chain contains the catalytic site [3].

uPA can be regarded as a multifunctional protein that is involved in both proteolysis and signal transduction. As a protease, its best known reaction is catalysis of conversion of the zymogen plasminogen into active plasmin. Plasmin, in contrast with uPA, has multiple substrates. It can promote degradation of diverse ECM substrates such as fibrin, fibronectin and laminin [3]. It can activate the precursor forms of certain matrix metalloproteases (MMPs) such as MMP-3, MMP-9, MMP-12 and MMP-13 [4]. The formation of the active MMPs allows further degradation of the ECM, especially interstitial and type IV collagen. Plasmin can also activate or release specific growth factors such as fibroblast growth factor 2, vascular endothelial growth factor and transforming growth factor β [5]. These pleitrophic growth factors have the potential to enhance tumour progression by stimulating angiogenesis and enhancing both cell proliferation and migration.

uPA-catalysed proteolysis occurs in vivo while the protease is attached to a membrane-anchored receptor known as the uPA receptor (uPAR). uPAR, which is a member of the Ly-6 family of molecules, is a 55–60 kDa glycoprotein [6]. It consists of three homologous domains and is bound to the cell membrane by a glycosylphosphatidylinositol moiety. The primary binding between uPA and uPAR involves the growth factor domain of uPA (amino acids 12–32) and the N-terminal end, or domain 1, of the receptor. Other regions of ligand and receptor may also participate in binding, for example amino acids 136–142 of uPA [7] and domains 2 and 3 of uPAR [8].

Binding of uPA to its receptor has two main consequences. First, it leads to both enhanced and focused proteolysis. Secondly, ligand–receptor interaction results in signal transduction, including activation of mitogen-activated protein kinase, extracellular signal-regulated kinases 1 and 2 and other signalling pathways [8]. Proteins shown to be induced following uPA signalling include Fos and Jun [8].

**Endogenous inhibitors of uPA**

uPA activity can be neutralized in vivo by two inhibitors known as plasminogen activator inhibitors 1 and 2 (PAI-1 and PAI-2), which belong to the serpin family of protease inhibitors (reviewed in [9,10]). Both form equimolar complexes with uPA that are at least partially SDS-resistant. PAI-1 is thought to be the primary endogenous inhibitor of uPA as it reacts more rapidly with the protease than PAI-2.

Like uPA, both PAI-1 and PAI-2 are also multifunctional proteins. Both serpins were recently found to inhibit apoptosis [11,12]. In addition, PAI-1 has been shown to modulate both cell adhesion and migration [13]. These latter actions of PAI-1 are independent of its protease-inhibitory role.

**Role of uPA in cancer metastasis**

The evidence implicating uPA in cancer invasion and metastasis in model systems is substantial (reviewed in [1,3]). This evidence is summarized here. (i) Positive correlations are found between levels of uPA in both cell lines and animal tumours and metastatic potential. (ii) Inhibition of uPA activity (e.g. by inhibitors or antibodies) or uPA expression (e.g. by antisense oligonucleotides) suppresses metastasis in model systems. (iii) Transfection of cell lines with uPA cDNA enhances the metastatic phenotype. (iv) Preventing uPA from binding to uPAR decreases metastasis in model systems. (v) Tumours in uPA-deficient mice undergo less progression than in control wild-type mice.

Originally, uPA was thought to promote metastasis by degrading the ECM, thereby permitting local invasion and ultimately the formation
of distant metastases. However, it is now clear that uPA has additional actions allowing it to play a role in cancer cell dissemination [3]. These activities include its ability to enhance angiogenesis, and stimulate both cell proliferation and migration. For formation of a clinically significant metastasis, these actions of uPA are necessary in both the primary tumour and at the metastatic site.

**uPA as a marker of metastatic potential in human cancers**

For optimum management of patients with newly diagnosed cancer, an ability to predict the tumour's likely propensity to metastasize is important. For example, a patient with a tumour likely to disseminate should be given additional or adjuvant treatment (e.g. chemotherapy, radiotherapy or hormone therapy in addition to surgery). On the other hand, those patients with an indolent malignancy could be spared the side effects and costs of this additional therapy. Since metastasis is the principal cause of mortality in patients with cancer and since uPA is a critical mediator of the process, uPA is a good candidate for investigation as a marker for predicting the likely formation of metastasis. Furthermore, as mentioned above, positive correlations are found between uPA levels and metastatic ability of both cell lines and animal cancers.

In 1988, Duffy et al. [14] first reported that breast cancer patients with high levels of uPA activity in their primary cancer developed metastasis more rapidly than those with low activity levels. These original findings have now been confirmed by at least twenty independent groups, world-wide (reviewed in [15,16]). As a predictor of outcome for breast cancer patients, uPA is one of the most potent biological markers described to date. For example, the clinical information provided by uPA is: (i) independent of or additional to that provided by the traditional prognostic factors for this disease, such as tumour size, tumour grade or axillary node status; (ii) stronger than that of other biological factors such as oestrogen receptor, progesterone receptor, HER-2 or p53; (iii) independent of the cut-off point used for separating patients with low and high levels of uPA, i.e. whether the median, tertile, or quartile value is used [15], uPA is also predictive when used as continuous variable [15]; (iv) prognostic in axillary node-negative patients, the subgroup of patients with breast cancer for which new prognostic factors are most urgently required [15].

The ability of uPA to predict the formation of metastasis in human cancers is not confined to breast cancer. High levels of the protease are also correlated with adverse outcome in patients with cancer of the stomach, colorectal region, bladder, ovary, endometrium and brain [15,16]. uPA levels may, therefore, correlate with a propensity to metastasize and, thus, with prognosis in many different types of malignancy.

**PAI-I as a marker of metastatic potential in human cancers**

Intuitively, it might be expected that high levels of an inhibitor of uPA in cancer tissue would correlate with a low probability of metastasis and thus with good outcome. Paradoxically, high levels of PAI-1 in different types of cancer are also strongly associated with aggressive disease and poor outcome [3,16]. Indeed in breast cancer, PAI-1 provides prognostic information that is additional to that of both uPA and the traditionally used factors.

The reason that high levels of PAI-1 correlate with adverse prognosis is not clear. There are several possible explanations. (i) A critical concentration of PAI-1 may be necessary to prevent excessive degradation of the ECM by uPA during cancer invasion. Excessive breakdown of the matrix could leave insufficient substrate on which cancer cells could migrate. (ii) PAI-1 is necessary for angiogenesis [17] which in turn is essential for malignant cells to gain access to the circulation. (iii) PAI-1 can modulate both cell adhesion and migration [13] and as result may accelerate the metastatic process. (iv) PAI-1 can inhibit apoptosis [11]. In order for malignant cells to complete the metastatic process, they must survive and evade apoptosis. Resistance to apoptosis might therefore be expected to increase the likelihood of forming a distant metastasis.

**Use of uPA and PAI-I in a clinical setting**

Prior to entering routine clinical use, a new marker must be rigorously evaluated with respect to both analytical and clinical performance. A number of commercially available ELISA kits have been subject to technical evaluation [18] and one of these (American Diagnostica, Greenwich, CT, U.S.A.) was further studied in an External Quality Assurance (EQA) trial [19]. A commercially available ELISA kit for PAI-1 (American Diagnostica) has also been evaluated in an EQA programme [19].
For clinical validation of a new marker, a Level 1 Evidence study must be performed [20]. A Level 1 Evidence study can either be a large randomized prospective trial in which evaluation of the marker is the primary objective of the study or a meta-analysis/pooled analysis of small-scale prospective or retrospective trials. Recently, the prognostic impact of uPA/PAI-1 in breast cancer patients was confirmed using both these types of Level 1 Evidence studies [21,22].

**Conclusion**

uPA and PAI-1 are among the very few tumour markers whose clinical value has been validated using Level 1 Evidence data, and to the author’s knowledge are the only markers to have been validated using two different types of such studies. Since assays for these markers have also been shown to perform satisfactorily in EQA trials, both should now be ready for routine clinical use.

In the clinic, the main application of these markers is likely to be in selecting node-negative breast cancer patients who do not need or are unlikely to benefit from adjuvant chemotherapy, i.e. patients with low levels of uPA and/or PAI-1. uPA and PAI-1 may also be of value in deciding which oestrogen receptor-positive breast cancer patients should receive combined chemotherapy and endocrine therapy instead of endocrine therapy alone, i.e. patients with low levels of both proteins could receive just the endocrine therapy whereas if either or both markers are elevated, combined therapy could be considered.

**References**


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