

BIOLOGY OF METASTASIS WITH FOCUS ON PROTEASES

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4.1 Introduction

Cancer invasion and metastases are multistep events involving local invasion of the extracellular matrix, angiogenesis, invasion of the blood vessels, survival of malignant cells in the vascular system, extravasation and establishment of the secondary growth. During most of these steps, natural barriers have to be degraded. The breakdown of these barriers is catalyzed by different proteolytic enzymes released from the invading tumour. Different proteolytic enzymes, produced by tumour cells or by the surrounding stroma, were found to be involved in the proteolysis of the extracellular matrix that allows for cell migration as well as in the release of the stored angiogenic molecules that allows for neovascularization and growth of secondary deposits. The most well studied and recognized proteolytic enzymes involved in cancer invasion and metastasis are serine protease urokinase plasminogen activator (uPA) and its inhibitors plasminogen activator inhibitor-1 (PAI-1) and PAI-2, cysteine protease: cathepsins B, L, H and their inhibitors, stefins and matrix metalloproteinases (MMPs) and their tissue inhibitors. The levels and the activity of these enzymes in the tumour tissue of various malignancies was found to be related to the potential of local growth as well as distant spread of the tumour. Consistent with their role in cancer growth and spread, these factors have been shown to be prognosticators of the faith of disease in a variety of malignancies. In addition some of these factors were also found to have a predictive value for response to different treatment strategies in breast cancer.

4.2 Serine protease uPA and its inhibitors PAI-1 and PAI-2

Serine protease uPA is a protease with multiple activities. Its best known action is as a catalysis for the conversion of the inactive plasminogen to plasmin, a broad-spectrum protease which degrades most substrates in the extracellular matrix. In addition, uPA activates different growth factors which play an important role in tumour growth and angiogenesis, such as vascular endothelial growth factor (VEGF) and human growth factor (HGF). uPA inhibitors, PAI-1 and PAI-2, were found to be multifunctional

proteins involved in tumour remodelling. While PAI-2 acts as a true inhibitor, PAI-1 was found to be an actor necessary for optimal adhesion, and migration of tumour cells and its high levels were paradoxically positively correlated with aggressiveness and bad prognosis in different cancers.

Serine protease uPA and its inhibitors were found to have a prognostic value in a variety of cancers. Out of different cancers studied to date, the strongest and most consistent evidence of a prognostic role exists with uPA and PAI-1 in breast cancer. In this malignancy the independent prognostic value of uPA and PAI-1 was almost uniformly confirmed in numerous individual studies as well as in a meta-analysis, including 18 data sets of more than 8,000 patients. Clinically, even more relevant and important are the recent findings that uPA and/or PAI-1 levels in primary tumour may predict for a response to adjuvant systemic therapy in breast cancer. According to our data obtained on the collective of 460 operable breast cancer patients, high levels of uPA and PAI-1 in primary tumour may predict a better response to hormone therapy. On the basis of currently available evidence, serine protease uPA and its inhibitor PAI-1 are certainly the markers that help us to identify better the collective of breast cancer patients that benefit from adjuvant systemic therapy and may also be the markers that will improve treatment decision in each individual breast cancer patient in the future, which is of utmost importance.

Increased uPA and PAI-1 activity was found in a wide variety of human cancers and was often an independent prognostic factor for survival. In different studies, uPA and PAI-1 have been shown to be a prognostic indicator in colorectal, gastric, urinary bladder, ovarian, pancreatic cancer and others. The prognostic role of PAI-2 is not so widely confirmed. High levels of PAI-2 were found to be associated with bad prognosis in breast cancer; however the data are not as uniform as with uPA and PAI-1.

The convincing clinical data indicate a key role of uPA and PAI-1 in the spread of cancer. Inhibition of cancer progression might be possible by their inhibitors or down-regulators. A new generation of uPA inhibitors with proven activity in vitro are under development and they are planned to enter the clinical trials soon.

4.3 Cysteine proteases and their inhibitors

The cysteine proteases (CP) cathepsins B, H and L participate in the degradation of ECM and basement membrane and are also involved in the formation of new blood vessels. Up-regulation of cysteine cathepsins has been demonstrated in many human tumours, including breast, lung, brain, gastrointestinal, head and neck cancer, and melanoma. In addition the imbalance between cathepsins and their endogenous inhibitors named cystatins may facilitate tumour invasion and metastasis. According to the data available the levels of cathepsins and cystatins in tumour tissue as well as in the extracellular fluids can provide additional clinical

information to predict overall survival in variety of malignancies. However, the data on the prognostic value of cathepsins and their inhibitors in cancer are not uniform. This may be partially due to differences in methodology and materials taken for assessment. There are major differences in the levels of cathepsins determined by immunological or enzymatic test and concentration of cathepsins in serum is much lower in comparison to tissues. Hence the future way in cathepsins evaluation seems to be in the standardization and unification of experimental models to make data verification easier.

4.4 Matrix metalloproteinase inhibitors

Matrix metalloproteinases (MMPs) are a family of zinc-dependant enzymes involved in ECM proteolysis, activation and deactivation of growth factors and in angiogenesis. They are classified on the basis of their domain structure and substrate specificity into a number of groups: collagenases, gelatinases, stromalysins, matrilysins and a number of MMPs that do not fall into these groups. Matrix metalloproteinases are up-regulated in most human tumours and invasive malignant tumours express higher levels of MMPs. Most of the MMPs are produced by surrounding stroma and not by tumour cells. Although the expression of MMPs in malignancies has been studied widely, the specific role of distinct MMPs in various cancer types and their eventual prognostic value has to be assumed.

Naturally occurring tissue inhibitors of MMPs activity (TIMPs) are present in ECM. The balance between TIMPs and MMPs correlates with tumour-genesis and studies have shown that TIMP-1 expression actually correlates with bad prognosis. In a large study conducted into the large collective of rectal cancer patients, the plasma levels of TIMP-1 were found to be an independent prognostic factor for survival in these patients. According to this study data the TIMP-1 levels could be used to select rectal cancer patients who are at high risk of relapse and are candidates for adjuvant chemotherapy.

Synthetic MMPs inhibitors have been evaluated in clinical trials. Clinical trials using marimastat and some other compounds alone or in combination with cytotoxic agents in various solid tumours have been mostly disappointing. Except for marimastat in gastric cancer no clinical efficacy was found and the toxicity, especially musculoskeletal one, was substantial. However, based on the new knowledge and better understanding of different MMPs, the new approaches in clinical use of MMPs are to be tested.

4.5 Conclusion

Nowadays it is quite clear that numerous proteolytic enzymes play a major role in cancer invasion and metastasis. Their levels in tumour tissue could already serve as a surrogate marker for prediction of the faith of disease and survival in various malignancies. It is even more important that we already have some data indicating that at least some of these markers could predict a better response to standard systemic therapies of cancer, such as chemotherapy and hormonal therapy. Such a prediction helps us to identify the collective of patients that would benefit most from the particular systemic therapy. In addition these markers may serve as targets for novel biological therapies of cancer, so called targeted

therapies, which are expected to further improve the curability of cancer.

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