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The urokinase-type plasminogen activator, its inhibitors and its receptor - the new prognostic factors in solid cancers

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The urokinase-type plasminogen activator (uPA), its inhibitors (PAI-1 and PAI-2) and its receptor (uPAR) play an important role in the degradation of the intercellular tissue, the process which affects the ability of cancer cells to invade to surrounding tissue and to metastasize. The results of clinical studies performed in the past few years point out a significant influence of uPA, PAI-1, PAI-2 and uPAR on the course of the disease and survival of patients with solid tumours, particularly breast cancer. Hopefully the categorization of patients according to the content of the serine proteases and its inhibitors in tumour tissues could provide a basis for more rational treatment planning and thus improving patients' survival.

Key words: neoplasms; prognosis; urokinase; plasminogen inactivators; serine proteinases

Introduction

The projection of the progression of the disease and hence the prognosis for each individual patient is of extreme importance in clinical oncology. The treatment strategy of solid tumours is nowadays based on the established prognostic factors, such as the patho-histological type, the size of the tumour, and the stage of the disease, but the survival statistics of individual groups of patients with the same values of these prognostic factors is still diversified. Therefore, the main objective of the research work is to find those tumour characteristics which would enable us to discern less biologically aggressive tumours from the more aggressive ones. In recent years, the methods of molecular biology have helped to identify a number of new factors associated with the differentiation and proliferation of cancer cells, such as: Ki67, cyclin D1, the proportion of cells in S phase of the cell cycle, various growth factors and oncogenes. Although these factors have been proven to influence the differentiation and proliferation of cancer cells, their value as prognostic factors is still questionable. It was found that the ability of cancer cells to invade the surrounding tissue and form distant metastases rather than the differentiation and proliferation of cancer cells is of vital importance for the prognosis of a particular patients. Therefore, an increasing number of investigations have been dedicated to the study of factors which define tumor invasiveness.

The research *in vitro* showed that the invasion of tumour cells in the neighboring tissue and the formation of metastases depend significantly on the content of proteases in the tumor tissue.[1,2,3] There are four recognized classes of proteases: cisteine proteases, (cathepsin B, H and L), aspartyl proteases (cathepsin D), metalloproteases (gelatinases, collagenases, stromelysins) and serine proteases (plasminogen activators and the product of their activation - plasmin). Metalloproteases play an important role in the invasion and metastatic growth of tumours in animals, but their significance for tumours in humans is still not totally clear.[4] Cysteine proteases and the cathepsin C have a proteolytic effect on the intercellular tissue and they are especially important for the activation of the urokinase-type plasminogen activator.[4,5,6]

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The research work carried out over the last few years revealed a most important prognostic relevance of serine proteases.[3] There are two types of serine proteases - plasminogen activators: the tissue plasminogen activator (tPA) and the urokinase-type plasminogen activator (uPA). The tPA is mostly present in the blood, where it participates in the intra vascular thrombolysis.[1,3] The uPA is found mostly in the tumour tissue, where it plays, together with its inhibitors and receptor, a most important part in the activation and regulation of the tumour associated proteolysis in man.[3]

Malignant cells, as well as macrophages and fibroblasts, present in tumours produce an inactive proenzyme (pro-uPA), which is bound to a specific receptor on the cellular membrane - to the uPAR. Through the activation of the cathepsin B and L, the cathepsin D and the plasmin transform the pro-uPA into the active form uPA. The uPA triggers off a cascade-like transformation of plasminogen into the active proteolytic enzyme plasmin, which disintegrates intercellular proteins (fibrin, fibronectin, proteoglycane and laminin) and which indirectly induces basement membranes degradation by the procollagenase IV activation (Figure 1).[1,3,5,6] At the same time, the proteolytic activity of the uPA produced by malignant cells and macrophages induces the formation of angiogenic factors and hence the neo-vascularization both in the primary tumours margins and in metastases. Apart from the presence of proteolytic enzymes, neovascularisation is the most important factor for the local growth and metastatic potential of tumours.[5]

Proteolysis is also strongly affected by the inhibitors of the plasminogen activator. The most relevant of the four known types are the plasminogen activator inhibitor type 1 (PAI-1) and the plasminogen activator inhibitor type 2 (PAI-2). The capacity of the PAI-2 as a pure plasminogen activator inhibitor is clear and straightforward. Contrary to what is known about the PAI-2, clinical research results display a stronger metastatic potential of tumours with high PAI-1 levels.[7,8,9] That may result from the PAI-1 protecting malignant cells against self-destruction or, another possible explanation, from the PAI-1 functioning as a biochemical marker of angiogenesis.[9] The activation of the uPA is possible only after its binding to the receptor. The uPAR presence in the membranes of tumour cells is much more pronounced than in normal tissues.[6] In some instances the presence of the uPAR in breast tissue was detected in the tumour itself but not in the normal tissue of the breast.[10] The blockade of the uPAR with monoclonal antibodies was found to prevent the invasion of tumour cells through the basement membrane.

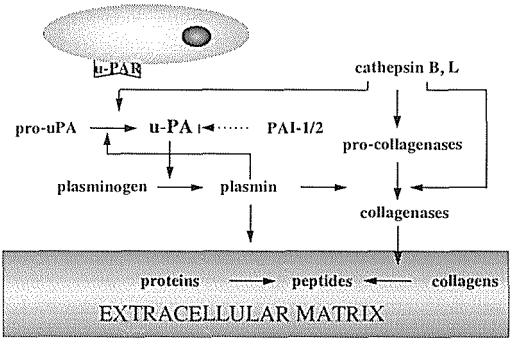

Figure 1. Scheme of the u-PA catalyzed plasminogen-plasmin cascade. Dashed line indicate inhibition. (u-PA - the urokinase type plasminogen activator, PAI-1/2 - plasminogen activator inhibitor type 1/2, u-PAR - specific receptor for u-PA).

The evaluation of the content of the uPA, PAI-1, PAI-2 and uPAR in tumours can be biochemical and immuno-histochemical. The biochemical method has been applied more frequently in the research performed so far. It involves the extraction of the urokinase system components with special puffers and detergents, and their evaluation with monoclonal antibodies. The referential values depend on the use and on the type of detergent. The best extraction can be obtained with the addition of the triton detergent.[12] The comparison of the values of the uPA content in the tissue of breast carcinoma obtained with biochemical and immuno-histological methods showed good correlation.[13]

The prognostic significance of uPA, PAI-1, PAI-2 and uPAR for different types of cancer

Breast cancer

By far the greatest number of studies on the prognostic significance of the uPA system components have been made for breast cancer, the most frequent type of cancer in women, the incidence of which has been on the increase.[14] The primary treatment of breast cancer is devised with respect to the established prognostic factors, such as the size of the tumour, the histological type and the gradus of the tumour, the axillary lymph nodes involvement and the presence of the hormone receptors in the

tumour.[15] Additional prognostic factors are being intensely investigated and the serine proteases being among the most promising ones.[16]

A number of studies proved that the levels of the uPA, PAI-1, PAI-2 and uPAR in the breast carcinoma tissue are considerably higher than in benign tumours and in normal breast tissues.[1,3,17,18] The content of the uPA can be 11 times,[13] the content of its inhibitor PAI-1 74 times and the content its inhibitor PAI-2 29 times higher in malignant that in benign breast tumours.[19] The level of the tPA is the same in malignant and in benign breast tumours.[3,20]

The uPA content in the tumour tissue does not correlate with other components of the uPA system and with common prognostic factors,[13,19] The PAI-1 correlates inversely with hormone receptors.[8] The PAI-2 correlates inversely with the size of the tumour and positive with the age of patients.[21] The uPAR correlates inversely with the estrogen receptors.[22]

Most of the clinical research has identified both the uPA and the PAI-1 as independent prognostic factors for breast cancer. Higher values of both uPA and PAI-1 in primary tumour are not only associated with higher risk of recurrence, but also significantly influence the overall survival rates. The question of a prognostic value of uPA and its inhibitor in prognostically different sub-groups of patients remains unsolved.[7,9,13,17,23,24,25] While they have both proved to be independent prognostic factors in the subgroup of patients with positive lymph nodes,[7,8,23] the uPA has not been established as an independent prognostic factor in the group of patients with negative axillary lymph nodes.[23] Only one study with small number of patients enrolled reports results supporting the uPA as an independent prognostic factor in this group of patients as well.[6] While a study involving over 600 patients revealed only the PAI-1 as an independent prognostic factor in patients with negative axillary lymph nodes.[7]

The prognostic value improves with the combination of individual system components. In patients with a high level of uPA content in primary tumours, the high level of PAI-2 is an independent prognostic factor while those with low PAI-2 levels have poor prognosis,[21] which is to be expected in the light of the function of the PAI-2 as a pure uPA inhibitor.

The uPAR has not proved to be such a strong prognostic factor as the uPA and the PAI-1 are. However, patients with a higher level of the uPAR in the breast tumour have significantly shorter relapse-free and overall survival[19] and the uPAR has proved to be an independent prognostic factor in the sub-group of post-menopausal patients with positive axillary lymph nodes.[22]

The components of the uPA system are not only prognostic but also predictive factors in breast cancer patients. In the study of 274 patients Foeckens *et al.* observed that the response to hormonal therapy with tamoxifen was better in patients with low levels of uPA, PAI-1 and uPAR and a high level of PAI-2 in tumour tissue.[26]

Ovarian cancer

The established prognostic factors for the ovarian cancer are the performance status, the stage of the disease, the histological type of cancer, the age of the patient, the tumour gradus, the size of the residual tumour after surgery and the presence or absence of ascites.[27]

The prognostic significance of the uPA system has been investigated for the ovarian cancer, too, but the studies are few and the number of patients involved is small.

The levels of the uPA, PAI-1 and uPAR were found to be significantly higher in the malignant tissue than in the benign tumours of ovaries,[28,4] which does not apply to the PAI-2.[4] The uPA was found to positively correlate with the number of metastatically involved lymph nodes, less differentiated tumours and excessive production of ascites.[28]

A study of 51 patients with advanced ovarian carcinoma (FIGOIII) revealed that the patients with lower levels of uPA and PAI-1 in the tumour had better prognosis.[28] In addition to the size of the residual tumour, the uPA and the PAI-1 levels proved to be the most reliable independent prognostic factors for patients with advanced ovarian carcinoma. The uPA and the PAI-1 levels were especially significant in the group of patients with no residual tumour.[28] These are the patients with relatively good prognoses, but their 5-year survival rate is still around 55%.[29] The evaluation of the components of the uPA system offers the possibility to find those with less favorable prognoses among these patients, and to adjust their treatment accordingly.[28]

A high content of the PAI-2 in the ascites of the patients with ovarian carcinoma proved to be an independent prognostic factor of the unfavorable outcome of the disease,[30] which is a surprising re-

sult in the light of the capacity of the PAI-2 as a pure uPA inhibitor. The level of the PAI-2 in the tumour was not measured in this study.[30]

Cervical cancer

The strongest prognostic factors of cervical cancer is the stage of the disease. Other prognostic factors are the size of the tumour, the amount of the cervical stroma involved, the involvement of lymphatic vessels and the presence of metastases in lymph nodes.[31]

There are few data in literature on the prognostic relevance of the levels of the uPA system components for cervical cancer. A clinical study involving 62 patients revealed a correlation between high levels of the uPA and/or PAI-1 in the tumour and the number of involved lymph nodes. High levels of the uPA significantly correlated with relapse free survival, and high levels of the PAI-1 with early relapses and shorter survival.[32]

The independent prognostic values of the uPA system components for the outcome of cervical cancer has not been studied yet.[32]

Lung cancer

The prognostic relevance of the uPA system components has been examined in three histological types of pulmonary carcinoma: adenocarcinoma, squamous cell carcinoma and large cell carcinoma.[33,34]

A clinical study involving 106 patients with pulmonary adenocarcinoma showed that the level of the PAI-1 did not correlate with other prognostic factors, such as the age and sex of the patient, the stage of the disease, the size of the tumour, the number of the involed lymph nodes and the surgical radicality. However, the uPA levels were statistically significantly higher in old patients.[33] The uPA and PAI-1 values did not correlate with each other.[33] High levels of the PAI-1 in the tumour tissue of patients with pulmonary carcinoma significantly correlated with poor survival, which was not the case for the uPA.[33] The multi-variant analysis showed that the PAI-1 is an independent prognostic factor for the survival of patients in stages I-III of the disease. The PAI-1 proved to be an independent prognostic factor also for patients in a prognostically favorable stage I of the disease. This fact suggests a possible selection of patients qualifying for adjuvant treatment after the surgical removal of pulmonary carcinoma.[33]

A study of the squamous cell cancer of lungs in 84 patients showed positive correlation between the uPAR and the PAI-1.[34] None of the uPA system components correlated with other prognostic factors. The uPA and the PAI-1 did not have any impact on the outcome of the disease, but the uPAR correlated significantly with survival.[34] Besides the size of the tumour, the uPAR was the only additional prognostic factor in squamous cell pulmonary cancer.[34]

In half a smaller group of patients affected by the large cell cancer a correlation was found between the uPAR and PAI-1, and the uPAR and uPA.[34] None of the components of the uPA system correlated with other prognostic factors.[34] None of the components of the uPA system had any impact on the outcome of large cell cancer.[34]

Gastric cancer

A number of studies of the prognostic relevance of the uPA system components in gastric cancer patients have been made. Here, too, the content of the uPA was found significantly higher in the carcinoma tissue than in the normal mucosa of the stomach.[35] The uPA significantly correlated with the established prognostic factors of gastric cancer. Higher levels of the uPA were found in more advanced stages of the disease, positive lymph nodes and less differentiated tumours.[35]

The uPA, the uPAR and the PAI-1 were found to inversely correlate with the length of survival. The PAI-2 did not show any impact on the outcome of the disease.[36] Two clinical studies of 203 [36] and 97 [37,38] patients with resectable gastric cancer identified only the PAI-1 as an independent prognostic factor of survival in all patients, [37,38,39] as well as in subgroups of patients in pathological stage pT1/2 and pN1/2.[36]

Besides the PAI-1, the uPA and the uPAR proved to be independent prognostic factors in the subgroup of patients with diffuse type of gastric carcinoma.[36] None of the three was confirmed as such in the subgroup of patients with intestinal type of gastric carcinoma.[36]

Colorectal cancer

In addition to the established prognostic factors of colorectal cancer, such as the stage of the disease and surgical radicallity,[39,40] other prognostic factors have been examined, and among them serine proteases are the most promising ones.

Compared with normal mucosa, colon tumours have higher levels of the uPA [41,42] and its inhibitors PAI-1 and PAI-2, [42,43] and lower levels of the tPA.[42]

In the clinical study of 92 patients with colorectal cancer, Ganesh *et al.* report a positive correlation between the uPA content in the tumour and survival of patients, whereas the PAI-1 did not show any prognostic relevance. [42]

However the PAI-2 content in tumour inversely correlated with survival,[42] which is surprising and contrary to the results obtained on breast cancer, where high levels of the PAI-2 in tumours represent a favorable prognostic factor. A high level of the uPAR in the tumour was associated with shorter survival of patients.[44]

Brain tumours

The established prognostic factors for brain tumours are the age and the performance status, the radicality of tumour resection and the histological type of the tumour.[45] Patients with high levels of the uPA in malignant brain tumours or in metastases of lung cancer, breast cancer, colon cancer or of malignant melanoma, have significantly poorer prognosis.[46]

Two clinical studies involving a small number of patients showed that the uPA as well as PAI-1 levels are significantly higher in malignant astrocytoma, especially in glioblastoma, than in the low-gradus glioma and normal brain tissue.[46,47] Neither of the two studies examined the correlation between serine proteases and the usual prognostic factors. Independent prognostic values of the uPA system components for the outcome of brain tumours have not been examined in studies made so far.

Bladder cancer

Nowadays the depth of invasion and tumour grade are considered to be the most relevant prognostic factors of invasive bladder cancer.[48]

According to the data available, the possible role of uPA as a prognostic factor in bladder cancer has been reported only by Hasui *et al.* They found higher levels of the uPA in cancer tissue than in normal urinary bladder tissue and its content increased with the depth of the invasion and the gradus of the tumour.[49] A higher level of the uPA in the tumour was significantly associated with the local relapse and growth of non-invasive tumours and with the survival of patients with invasive cancer.[50,51]

The independent prognostic value of the uPA was not determined due to the small number of patients involved in this studies.

Conclusion

The urokinase type plasminogen activator, its inhibitors and receptors have proved to be independent prognostic factors in breast cancer patients. In breast cancer, serine proteases may also predict the response to hormonal therapy. According to the results of the research performed so far, the uPA and its inhibitors content in tumour tissue also affects the prognosis of patients with many other solid cancers such as ovarian, lung, gastric, bladder cancer etc .

Hopefully on the basis the serine proteases, their inhibitors and receptor determination in tumour tissue it will be possible to differentiate between biologically more and less aggressive tumours in the future. This would enable to select the most suitable treatment for individual patients and thus improve the patients survival.

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