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Prognostic Value of Plasminogen Activators and their Inhibitors in Colorectal Cancer

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Colorectal tumorigenesis is associated with remarkable changes in the plasminogen activation system at the tissue level. The sequence of normal mucosa–adenomatous polyp–adenocarcinoma–metastasis is accompanied by an increase in the urokinase-type of plasminogen activator, the urokinase receptor and the inhibitors type-1 and type-2, with a concurrent decrease in the tissue-type plasminogen activator. Overall survival analysis of colorectal cancer patients, with a follow-up of more than 5 years, revealed that several of these components, in both the carcinomas and their corresponding normal mucosa, are of prognostic value independent of major clinicopathological parameters. Therefore, the plasminogen activation cascade not only contributes to the invasive and metastatic growth of colorectal tumours, but might also have a clinical impact with respect to adjuvant and intervention therapy.

Key words: carcinomas, colorectal cancer, normal mucosa, plasminogen activators, plasminogen activator inhibitors, prognosis, urokinase receptor

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COLORECTAL TUMORIGENESIS

PATIENTS WITH a malignancy of the digestive tract have a relatively poor prognosis. Therefore, clinical and basic studies which could provide useful prognostic markers are of great value. Gastrointestinal carcinogenesis is a multistep process in which environmental factors and genetic abnormalities play an important pathogenetic role. These malignancies usually start with a local increase of proliferation resulting in premalignant lesions, such as adenomas of the colorectum. Endoscopical screening for early detection and/or removal of these premalignant lesions is the most effective and widely used preventive strategy for colorectal cancer. However, in many cases, a premalignancy is not clinically manifest and patients present themselves at a later stage with a carcinoma. One of the most important prognostic factors in gastrointestinal cancer is still the histological stage of the tumour at diagnosis. All tumour staging systems are based on invasion within the intestinal wall, lymph node involvement, and the presence of distant metastasis. This sequence in the process of malignant derailment involves several steps in which proteolytic activity has been shown to play an important role. Both behaviour and aggressiveness of tumour cells are probably reflected by the extent of this proteolytic activity. In this respect, the plasminogen activation system has received much attention, and has been shown to play a major role in the invasion and metastasis of several human malignancies [1–3].

PLASMINOGEN ACTIVATION SYSTEM IN COLORECTAL NEOPLASIA

Plasminogen activators are enzymes which catalyse the conversion of plasminogen into plasmin. Plasminogen is commonly present in human tissues and it is the zymogen, the inactive proform, of plasmin. Plasmin, a serine proteinase, is a potent degrader of various proteins. The lytic capacity of plasmin is best known from thrombolysis, the dissolution of blood thrombi in the circulation. Apart from the degradation of fibrin in blood clots, plasmin is also able to break down proteins that are found in tissues, e.g. laminin, fibronectin, and proteoglycans (Figure 1). These proteins are especially found in basement membranes and interstitial stroma, two structures forming the extracellular matrix, an important constituent of tissue architecture. Activation of plasminogen in tissues is shown to be an important mechanism for cells that are migrating through the extracellular matrix. The formation of blood vessels, so-called angiogenesis, is a process in which the extracellular matrix is temporarily degraded by plasmin to make way for the evolving

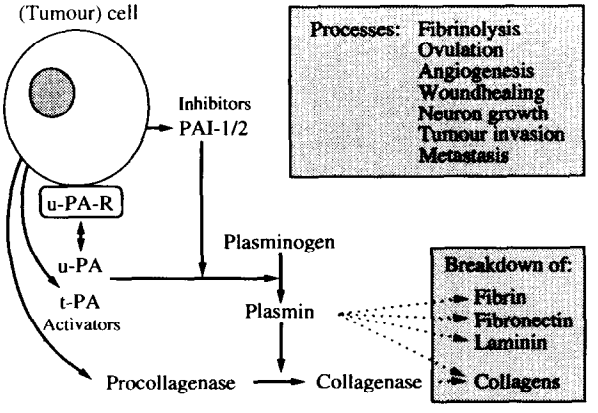

Figure 1. Contribution of the plasminogen activation cascade to the breakdown of extracellular matrix components in diverse pathophysiological processes.

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endothelium. Since the beginning of this century, many studies reported that the activation of plasmin is also involved in malignant growth of various types of carcinomas. Malignancy is the ability of a tumour to cross anatomical barriers that separate neoplastic cells from the normal host tissue. The degrading capacity of plasmin is considered to play a key role in these proteolytic events, and it enables the tumour cells to invade the surrounding normal tissue (Figure 2).

The two most potent activators of plasminogen are called plasminogen activators, namely tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). Earlier studies in homogenates of tissues from the large bowel and rectum showed that there is more u-PA present in carcinomas than in the surrounding normal tissue [4, 5]. This rise in the level of u-PA was accompanied by an increase in activity of this enzyme. Remarkably, the levels of t-PA were found to be decreased in carcinoma tissue. Levels of plasminogen activators in premalignant stages of colorectal cancer, adenomatous polyps, were shown to be intermediate between normal tissue and carcinomas. Subsequently, the regulation of u-PA was studied in normal colorectal mucosa, in premalignant adenomatous polyps, in primary carcinomas, and in liver metastases.

Isolation of RNA and northern blotting revealed that the increase in amount of u-PA protein in colorectal neoplasia was also seen at the mRNA level, suggesting that the u-PA which is present in adenomas and carcinomas is synthesised in the tumours and does not come from other parts of the body [6]. In conclusion, the enhancement of u-PA antigen levels in colorectal neoplasia is regulated at the transcriptional level. This study did not definitely show which cells were responsible for u-PA synthesis, although immunohistochemistry showed that u-PA protein appeared to be localised primarily in the neoplastic epithelial cells of adenomas and carcinomas from the colorectum [7].

The amount of u-PA does not necessarily have to be correlated with the quantity of u-PA activity. Apart from the presence of an inactive pro-form of u-PA (pro-u-PA), u-PA as well as t-PA can be inactivated by the formation of complexes with two specific plasminogen activator inhibitors, PAI-1 and PAI-2.

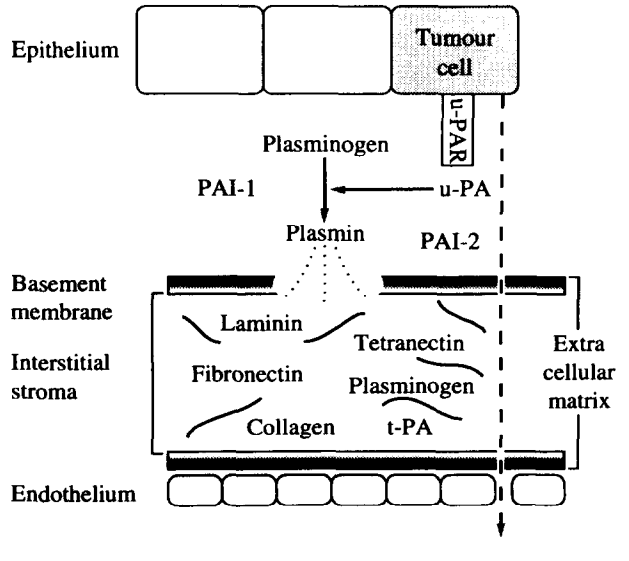

Figure 2. Schematic presentation of tumour cell-associated plasminogen activation in invasive growth through basement membranes and stroma, with some of the important components involved.

Specific ELISAs for these inhibitors revealed that the levels of both PAI-1 and PAI-2 are enhanced in adenomas and are especially high in carcinomas of the colorectum [8]. Northern blotting also showed enhanced PAI-1 mRNA levels in colorectal neoplasia. In contrast, mRNA coding for PAI-2 was only occasionally found [6]. Remarkably, the increased amount of inhibitors was correlated with a decrease in t-PA activity, but not with enhanced u-PA activity. Apparently, at least part of the u-PA escapes physiological control by the inibitors. Although homogenates of liver metastases also showed enhanced concentrations of u-PA, they did not contain detectable plasminogen activator activity. This absence of activity was most probably caused by extremely high levels of inhibitor PAI-1 [9]. These findings suggest that regulation of the activity of u-PA in colorectal neoplasms is a delicate balance between the amount of activator and inhibitors, although other regulating factors are involved.

It is important for the invading carcinoma that the degradation of the matrix surrounding the migrating cells is restricted to certain areas, and that the proteolytic activity is specifically localised. For the urokinase-type plasminogen activator, a specific receptor is known, which localises the proteolytic activity. We evaluated the presence of u-PA receptors in homogenates of normal colorectal mucosa, adenomatous polyps, carcinomas, and liver metastases. While the levels of free u-PA receptor were enhanced in carcinomas and metastases, the adenomas did not show higher amounts of receptor than adjacent normal mucosa. The protein measurements of u-PA receptors in colorectal neoplasia were confirmed by northern blotting of u-PA receptor mRNA [6]. These data suggest that the presence of the receptor for u-PA could be an important factor in the regulation of localised proteolytic activity in colorectal malignancies.

PROGNOSTIC IMPACT

Recently, the altered levels of components of the plasminogen activation cascade in the tissues have been shown to be related to the prognosis of patients with breast cancer [3, 10–13]. We evaluated several parameters of the tissular plasminogen activation cascade for their prognostic value for the overall survival of patients with colorectal cancer, in comparison with major clinicopathological parameters. High levels of u-PA and PAI-2 antigen, a high u-PA(carcinoma)/t-PA(normal mucosa) antigen ratio, and a high u-PA receptor level in colorectal carcinomatous tissue were found to be significantly associated with a poor overall survival. Moreover, we found a low t-PA level, both antigen and activity, in normal mucosa of patients with a colorectal carcinoma to be significantly associated with a poor overall survival as well [14]. Multivariate analyses revealed that all these parameters, except for the u-PA antigen, retained prognostic value independent of major clinicopathological parameters, e.g. localisation, differentiation, diameter of the tumours, gender of the patients, etc. From these latter parameters, only the age of the patient and the Dukes' stage of the tumour were found to be of strong prognostic impact. Thus, these studies clearly show that several components of the plasminogen activation system are of additional prognostic value to the overall survival of colorectal cancer patients.

Most experimental and clinical studies claimed that u-PA is the most important component of the plasminogen activation system for tumour cell invasion, taking into account the fact that the activity of u-PA is highly dependent on the cell surface receptor for u-PA. Similar to our findings in colorectal cancer, however, u-PA was not found to be a single independent

prognostic factor for breast cancer [12, 13]. Therefore, u-PA seems to be an important factor for tumour invasion, but does not appear to be a solitary prognostic marker by itself, although a recent immunohistochemical study in Dukes' B colorectal cancer identified u-PA as a single independent prognostic parameter [15].

PAI-2, the major inhibitor of u-PA, was found to be an independent prognostic marker in colorectal cancer. Several *in vitro* studies revealed that PAI-2 has an inhibitory effect on invasion. Therefore, high PAI-2 levels were expected to be associated with less invasion and a better survival. In breast cancer, high levels of PAI-2 were indeed found to be associated with a good survival and therefore presumed to be protective [12]. Our findings, however, indicate exactly the opposite in colorectal cancer, i.e. high PAI-2 levels are associated with a poor overall survival. It is important to emphasise that the prognosis of colorectal cancer is largely determined by the depth of tumour invasion and the presence of local and/or distant metastasis. These are two different, although related, processes. Based on our findings, it could be possible that PAI-2 is more related to the metastatic potential of a colorectal tumour than to its invasiveness. Nevertheless, PAI-2 is an important parameter in the prognosis of patients with colorectal cancer.

In several other types of human malignancies, the functional role and the prognostic value of the plasminogen activation system has also been evaluated. For instance, in carcinomas of the stomach, lung, urinary bladder, breast, and in melanomas, one or more components were found to be of prognostic significance, which illustrates clearly the general clinical relevance of the plasminogen activation system in human cancer [10–13, 16–20]. The majority of these prognostic studies have been performed in breast cancer. Several of these studies showed that, based on the level of PAI-1 in the tumour, subgroups of high risk patients can be identified when they have already been classified according to lymph node involvement, and hormonal and menopausal status [13]. Several hypotheses have been forwarded to explain that high levels of plasminogen activator inhibitor are associated with a poor prognosis and short survival. It has been proposed that PAI-1 is produced for self protection of the normal tissue against the tumour-derived u-PA mediated tissue destruction, indicated by the fact that high levels of PAI-1 are correlated with high levels of u-PA. PAI-1 has also been proposed to participate in tumour associated neo-angiogenesis, and to be necessary for re-implantation of circulating tumour cells. However, in our studies, we did not find an association of PAI-1 with the overall survival of colorectal cancer patients [14]. The origin of this divergence between breast and colorectal cancer in the association of PAI-1 with survival is unclear.

As mentioned above, a high u-PA receptor level was also found to be associated with a poor overall survival of colorectal cancer patients [21] (Figure 3). A similar observation was recently made by Pedersen and colleagues [19] in squamous cell lung cancer. These findings illustrate once more that the u-PA receptor is an important component of the plasminogen activation system with respect to tumour invasion. Immunohistochemical as well as *in situ* hybridisation studies in colorectal and breast cancer revealed a strong staining of the u-PA receptor at the invasive front of the tumours [22, 23]. These observations indicate that the receptor is important in concentrating the u-PA activity to the invasive part of the tumour, which might facilitate tumour cell migration into the surrounding normal tissue. Recently, the u-PA receptor was also detected in ascites and in plasma of ovarian cancer patients [24]. Taking into account the

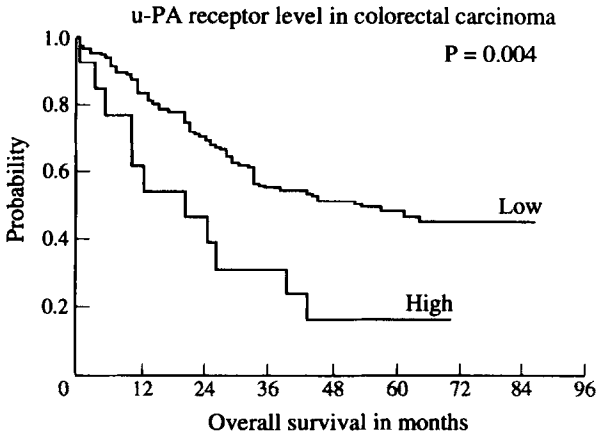

Figure 3. Overall survival curves based on a u-PA receptor cut-off level of 4.1 ng/mg protein (Cox's hazard ratio 2.6, $P=0.004$).

pivotal role of the u-PA receptor in peritumoral proteolysis, further studies in gastrointestinal cancer patients are indicated to determine the value of the circulating (soluble) u-PA receptor in plasma and urine as a marker for the size or malignant behaviour of the tumour. Moreover, administration of recombinant u-PA receptor, to bind u-PA, or antibodies and antagonists which block the receptor could be new approaches in anticancer therapy [25].

Most interesting was our general finding that a low t-PA level of activity as well as antigen, in normal mucosa of patients with colorectal cancer was associated with a poor overall survival [14]. The digestive tract is in continuous contact with environmental factors present in the food and stools. This is thought to be one of the causes of the increased proliferation of epithelial cells in normal mucosa of patients with colorectal cancer and of patients with a high risk of developing colorectal cancer. This abnormal cell proliferation in the normal mucosa of patients with colorectal cancer is also of prognostic value, and, therefore, supports and increases the acceptability of our finding that the t-PA level in normal mucosa is related to cancer prognosis. t-PA is mainly produced by endothelial cells, and the tissue levels are most likely dependent on the vascularisation of the tissue. Within and surrounding the tumour, inflammatory reactions and neovascularisation are frequently found, which probably serve as defence reactions of the host against the tumour. Increased vascularisation might be accompanied by high levels of t-PA and a decreased ability of tumour cells to invade through adjacent structures. This hypothesis, together with our previously reported finding that there is a gradual decrease of t-PA during the adenoma–carcinoma sequence [4, 5, 7, 8], could explain why a low t-PA level in normal mucosa of colorectal cancer patients is associated with a poor overall survival. Moreover, a high t-PA activity was indicative of a relatively good prognosis. These latter observations are of clinical interest because they might help to discriminate patients with a good prognosis from those with a poor prognosis.

The plasminogen activation system is a complex cascade in which activators, inhibitors and receptor(s) interact to form a balanced proteolytic activity. Analyses with combinations of some of these components could, therefore, be a better reflection of this proteolytic capacity and their prognostic impact. Our finding that the u-PA/t-PA antigen ratio in normal mucosa, for example, is strongly associated with a poor overall survival of

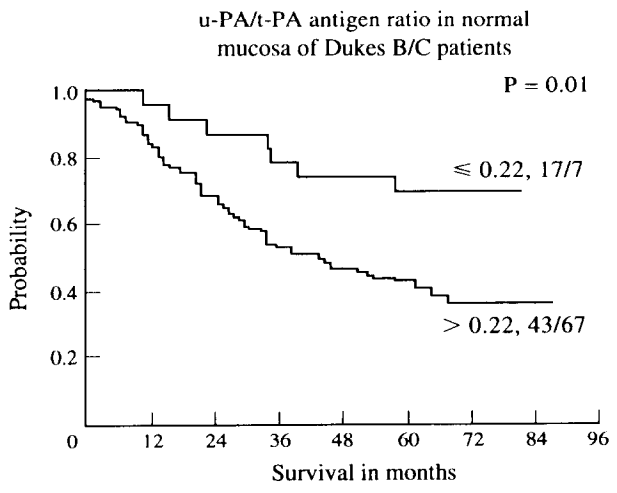

Figure 4. Overall survival curves according to high and low u-PA/t-PA antigen ratio in normal mucosa of patients with Dukes' stage B and C colorectal cancer. Values are the number of patients alive/dead at the end of follow-up (Cox's hazard ratio 2.8, $P=0.01$).

colorectal patients supports this impression (Figure 4). Moreover, in subgroups of colorectal cancer patients, combinations of some components of the plasminogen activation system were not only found to have a better prognostic value than the individual components, but also identified subgroups of patients with either a good or a poor prognosis [26]. Similar studies in breast cancer also showed combined plasminogen activation parameters to be of better prognostic value [11–13, 17].

Tumour cell invasion and dissemination is preceded by lysis of extracellular matrix. Tetranectin, as one of these matrix proteins, is abundantly present in tumoral extracellular matrix and is known to bind plasminogen. Therefore, tetranectin is thought to be actively involved in the plasminogen activation cascade, particularly in the local proteolytic activity of tumour tissues. We found that colonic neoplasia is associated with a change in the tissular distribution of tetranectin, from mucinous cells in the normal mucosa to the stroma of the carcinomas, and with a low plasma tetranectin level [27]. In several other human cancers, i.e. breast, pancreas, gastric, and ovarian cancer, significantly decreased plasma tetranectin levels have also been found. Moreover, the plasma tetranectin level was found to be of prognostic value in breast and ovarian cancer. An intense extracellular tetranectin staining within the tumours was also associated with shorter survival of ovarian cancer patients [28]. These findings suggest that the magnitude of the contribution of the plasminogen activation system to the tumour-associated proteolysis is also regulated by proteins of the extracellular matrix, such as tetranectin. Moreover, this kind of protein might reflect the invasiveness of the tumour and be of prognostic value, and, therefore, deserves further study.

CONCLUSIONS

In conclusion, several components of the plasminogen activation system, determined in both carcinomatous and normal tissue, are of clinical and particular prognostic value for the overall survival of patients with colorectal cancer. Combinations of plasminogen activation parameters have a better prognostic value than the separate parameters, and identify patients with colorectal cancer having either a poor or good overall survival. Based on these parameters, a further selection of patients for adjuvant therapy might be made to prevent overtreatment. Moreover, they could provide new criteria for screening, selection, follow-up, and treatment of high risk patients and may result in newly developed anti-invasive cancer therapies.

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