Invasion and metastasis are hallmarks of tumor progression and depend on the ability of tumor cells both to degrade and to migrate through connective tissue barriers. These processes require complex interactions between tumor cells and the surrounding extracellular matrix. These interactions are modified by proteolytic enzymes and their receptors. The urokinase plasminogen activator system is well known to play an important role in cancer invasion and metastasis. It contains the urokinase type plasminogen activator (uPA), the urokinase receptor (uPAR) and specific plasminogen activator inhibitors, PAI-1 and PAI-2. The receptor for uPA, uPAR may reside on the surface of tumor cells. uPA which is secreted by normal and tumor cells binds with high affinity and specificity to uPAR. The uPA-uPAR complex focuses proteolytic activity to the tumor cell surface by converting the plasma protein plasminogen into the serine protease plasmin. Plasmin degrades components of the extracellular matrix in the vicinity of the tumor cells thus facilitating tumor cell invasion and metastasis. PAI-1 may be needed for the optimal function of the uPA system in these processes, by being necessary for the dynamic state of the system, by regulating cell adhesion and for selective protection of vitronectin against proteolysis. PAI-1 may counteract migration and invasion. uPA, uPAR and PAI-1 can be used as strong independent markers of prognosis in many types of cancers – high levels of these proteins are associated with both shorter disease-free interval and shorter overall survival of the subjects with cancer.

Key words: cancer, metastasis, plasminogen activation system, urokinase, urokinase receptor, plasminogen activator inhibitor-1 (PAI-1)

INTRODUCTION

The processes of cancer invasion and metastasis require the remodelling of the extracellular matrix (ECM) (see [10] for review). Remodelling of the ECM may consist of its degradation by means of excision and deletion of ECM components, regeneration of the ECM components and their spatial and temporal reorganization. Several enzymes are known to be integrated...
components of the ECM, others are secreted and may be associated with the cell membrane. The proteolytic function of the enzymes are counterbalanced and regulated by appropriate protease inhibitors. These proteases and their inhibitors actively participate in determining the complex structure and biochemical characteristics of the ECM, which lead to the expression of specific forms of biochemical behavior. In order to establish metastatic deposits, the adhesive ability of cancer cells must be regulated. In the initial stage of dissemination, the release of cells from the primary tumors requires a decrease in intracellular adhesion. The cells that are released have to acquire the ability to adhere to, and penetrate the barriers presented in the form of the ECM, host tissue stroma and the vascular endothelium. Remodelling of the ECM [75, 76] provides the adaptability of the cell to enter into these interactions with the ECM and other cellular elements. This process is assisted by the proteolytic enzymes synthesized by the tumor cells.

Fig. 1. Tumor invasion and metastasis of solid malignant tumors (reproduced with permission from Schmitt et al., 1997)

Tumor invasion and metastasis of solid malignant tumors involves invasion and extravasation of tumor cells into and out of vessels and into the ECM (Fig. 1). Three steps control tumor spreading: attachment to and interaction of tumor cells with components of the basement membrane and the ECM, local proteolysis and tumor cell migration [73]. Four different classes of proteases are known to be correlated with local proteolysis: matrix metalloproteases including collagenases, gelatinases and stromelysins [46, 47, 53, 83], cysteine proteases including cathepsin B and L [5, 51, 77, 78, 79], the aspartyl protease cathepsin D [52, 69, 71] and serine proteases
including components of the plasminogen activation system [8, 19, 48-50].
The suggestion that the components of the urokinase plasminogen activation
system may play an important role in the processes of cancer invasion and
metastasis goes back several decades [18].

**UROKINASE PATHWAY OF PLASMINOGEN ACTIVATION**

The main components of the urokinase plasminogen activation system
are displayed in Fig. 2.

Urokinase plasminogen activator (uPA) is a serine protease, which is
released from cells as a single polypeptide-chain, virtually inactive proenzyme
(pro-uPA) [61]. pro-uPA is proteolytically converted into a two chain,
active form of uPA, which in turn activates plasminogen into plasmin.
pro-uPA itself is efficiently activated by plasmin, leading to amplification
of the overall reaction. However, the mechanism of the initial activation
of pro-uPA under physiological conditions is not yet clarified. Pro-uPA
and uPA can bind to a specific cell surface receptor, uPAR [19, 70, 85]
and concomitant binding of pro-uPA to uPAR and of plasminogen to as
yet unidentified cell surface binding sites strongly enhances plasmin generation
[19, 27]. uPA activity is regulated by two inhibitors, plasminogen activator
inhibitors type 1 (PAI-1) and type 2 (PAI-2) [2]. The complexes uPA-inhibitor
are internalized in a process dependent on both uPAR and the a-macrog-
lobulin receptor [17, 57].
In model systems, uPA and uPAR are required for invasion and metastasis [15, 42, 44]. The receptor for uPA, uPAR resides on the surface of tumor cells (Fig. 3). uPA which is secreted by normal and tumor cells binds with high affinity and specificity to uPAR. The uPA-uPAR complex focusses proteolytic activity to the tumor cell surface by converting the plasma protein plasminogen into the serine protease plasmin. Plasmin degrades components of the ECM (tumor stroma) in the vicinity of the tumor cells thus facilitating tumor cell invasion and metastasis [73].

**UROKINASE-TYPE PLASMINOGEN ACTIVATOR**

The protein and the gene

Urokinase-type plasminogen activator, uPA, is a serine protease of molecular weight of approximately 50 000. The molecule consists of 2 disulfide bridge-linked polypeptide chains, a C-terminal serine proteinase domain, and a N-terminal chain which contains a kringle domain and a growth factor-like domain. uPA converts the inactive zymogen plasminogen into plasmin that has tripsine-like activity. Plasmin is involved in degradation of extracellular matrix proteins such as fibronectin, type IV collagen,
laminin and fibrin [18, 48] and activates latent collagenase to potentiate their lytic activity [74, 85]. uPA is produced and released by normal cells like pneumocytes, fibroblasts, keratinocytes or phagotic cells and from tumor cells as a single-chain zymogen (pro-uPA) with very low or without any activity. Pro-uPA is converted to active-high molecular-weight two chains form of uPA (HMW) [19] by cleavage of the peptide bond. This reaction may be catalyzed by plasmin [18] and other molecules, like kallikrein and blood coagulation factor XIIa [18] thermolysin, nerve growth factor-γ [20], cathepsin B [41], cathepsin L [18], prostate specific antigen [33], T-cell associated serine proteinase [5], human mast cell tryptase [81] or tripsine-like proteinases isolated from human ovarian tumor [54]. Pro-uPA may be converted to inactive HMW uPA by thrombin and elastase from granulocytes [74]. HMW uPA can be further degraded by plasmin and other proteinases such as unknown proteinase [63] existing in the urine or matrix metalloproteinase Pump I [34]. These reactions produce low-molecular-weight two-chain form of uPA (LMW) and amino-terminal-fragment containing kringle domain and the growth factor-like domain [73]. Concentration of uPA in the blood is about 20 pM. Most of it is complexed with its type 1 inhibitor, the rest is in the pro-uPA form [3]. Pro-uPA and uPA can be bound, through its growth factor domain, to a specific cell surface receptor uPAR with the same affinity. Activation of pro-uPA can occur on binding to uPAR. The binding strongly accelerates activation of pro-uPA and increases the enzymatic activity of uPA itself [27]. Conversion of pro-uPA into uPA is inhibited by specific plasminogen activator inhibitor type 1 and 2 or protein C and nexin proteinase (PN-1) but reactions with these factors are much slower [81]. uPA has mitogenic activity for some cell lines [52]. Urokinase with plasmin are involved in proteolytic activation of growth factors: hepatocyte growth factor/scatter factor or basic fibroblast growth factor but these are devoid of proteolytic activity [3].

The human uPA gene is located on the 10-th chromosome, organized in 11 exons and is 6.4 kb long. At the promoter region there are repeated ALU-type sequences, the enhancer and negative-regulation regions. A fragment which shows promoter activity contains the hexanucleotide sequence GGCGGG that is repeated three times between the CAAT-box and the TATA-box and may play important role for promoter activity [65]. Two polymorphisms of the uPA gene are known: more frequent one near the beginning of exon 8 and less frequent resulted in the replacement of leucine residue by proline in the kringle domain [14].
uPA and cancer

uPA is involved in the cell migration by plasmin generation on a cell surface by uPAR bound to uPA over ECM substrate. During this process uPA and its receptor are found at the leading cell edge. It was reported that inactive variants of uPA, bound to uPAR, may inhibit cell migration [3]. The cell migration may be stimulated by at least two mechanisms: a proteolytic one and non-proteolytic. Proteolytic mechanism may involve plasmin generation at focal adhesion sites, catalysed by uPAR bound uPA and may initiate ECM degradation. Second mechanism may stimulate cell migration by changes in the adhesion at the leading edge. Tumor cells secret large amounts of plasminogen activators and evoke increased cell migration and development of secondary tumor focus. Moreover, uPA/plasmin system may play a role of the mediator in tumor invasion and propagation by changes of cell surface and by hydrolysis of fibrin deposition around the tumors. uPA may break normal cell barriers and decrease cellular attachment to ECM by affecting ECM and cytoskeleton proteins. Proteinase secretion and tissue degradation are elements of the invasive and metastatic phenotype and correlate directly with tumor cells invasiveness. The level of uPA activity is significantly higher in the malignant tumors in comparison to normal tissues or in benign tumors of the same tissue [22, 23]. uPA was the first proteinase which was used as a prognostic marker in human malignancy. The results of studies have shown that patients with tumors displaying high level of uPA activity have shorter disease-free survival than patients with low level. The level of the uPA antigen appear to be one of the strongest prognostic markers in the multivariate analysis comprising node status, tumor size, estrogen receptor status and cathepsin D levels [24, 25, 74]. Increased level of uPA have been proposed as a prognostic marker in many cancers including cancers of breast [8, 39], lung [58, 59], bladder [34], stomach [55], colorectum [53], cervix [42], ovary [45], kidney [36], brain [7], prostate [1] and many soft tissue sarcomas [14]. Elevated level of uPA and PAI-1 may be prognostic marker for patients survival such as in the cancers of ovary like endometrial carcinoma [43], gastric [13], colorectal and dedifferentiated chondrosarcoma [33]. For other types of cancer level of uPA is independent prognostic factor in a case of breast, prostate and kidney cancer. In only a few studies of colon [67], breast [21], ovarian [72] and pancreatic carcinomas [30] uPA was shown to be uPAR-dependent prognostic marker.
PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1

The protein and the gene

Plasminogen activator inhibitor type 1, PAI-1, is a 50 kDa glycoprotein, which belongs to the serine protease inhibitor (Serpin) superfamily [81]. It contains 379 amino acids and is a fast-acting inhibitor of both tissue-type and urokinase-type plasminogen activators. The deduced amino acid sequence of PAI-1 reveals a signal peptide of 23 amino acid residues and a mature protein containing 379 amino acid residues with three potential sites of N-linked glycosylation [9]. PAI-1 is produced by platelets, endothelial cells, granulosa cells, hepatocytes and smooth muscle cells [9]. Insulin and triglyceride rich lipoproteins stimulate PAI-1 production by cultured endothelial cells or hepatocytes [40]. Insulin resistance syndrome, which predisposes to diabetes and ischemic heart disease, may be a major regulator of PAI-1 expression [40]. There are two distinct pools of PAI-1: one in the α-granules of blood platelets and the other in plasma. PAI-1 is rather unstable but the formation of complex with vitronectin stabilizes PAI-1 activity [68]. The expression of PAI-1 is enhanced in several situations. Elevated level of PAI-1 was observed after major surgery, trauma and myocardial infarction. Its activity is correlated with body mass index (BMI) and serum triglyceride levels [87]. Plasma PAI-1 level is positively correlated with plasma insulin level. Insulin and triglyceride rich lipoproteins stimulate PAI-1 production by cultured endothelial cells or hepatocytes [40]. Its biosynthesis is regulated by a number of hormones, cytokines, glucocorticoids, thrombin [20]. PAI-1 activity in plasma is also induced by agents such as interleukin-1 (IL-1), tumor necrosis factor (TNF), transforming growth factor (TGF). The level of antigens against PAI-1 of hepatocytes was stimulated by cytokines (IL-6, IL-1, TGFβ) [20]. It is likely, that cytokines can effect in regulation of the PAI-1 gene transcription.

PAI-1 gene is localized to 7q21.3-q22 ad its 12.3 kb DNA contains nine exons and eight introns [9]. Exons range from 84 to 1823 bp, introns from 119 to 1764 bp in length. 12 Alu elements and 5 repeats of a long polyurine element are present in PAI-1 gene. 8 polymorphisms of PAI-1 are currently known: two G/A substitutions at positions -844 and +9785, 4G/5G insertion/deletion at position -675, two polymorphism in the 3' untranslated region - substitution T/G at position +11 053 and a 9-nucleotide insertion/deletion located between nucleotides +11 320 and +11 345, two (CA)n repeat polymorphisms, one in the promoter and one in intron 4, and HindIII restriction fragment length polymorphism
Several polymorphic sites in the PAI-1 gene can be associated with circulating levels of the inhibitor, including a 3' *Hind*III restriction site and an intronic dinucleotide (CA) repeat [12]. It has also been shown that the polymorphism 4G/5G found in the promoter region of the PAI-1 gene is of functional importance in regulating PAI-1 gene expression [35]. This polymorphism can be associated with elevated level of the protein. PAI-1 may be an important component in the regulation of a variety of both physiological and pathological processes.

**The role in cancer invasion and metastasis**

Considerable evidences support the view that elevated plasma PAI-1 activity is often observed in individuals with angina pectoris, deep vein thrombosis, diabetes and in survivors of myocardial infarction [13, 87]. The increase of plasma PAI-1 activity is associated with early recurrence of myocardial infarction [87]. PAI-1 may play an important role in the development of atherotrombotic diseases. Patients with other thrombotic disorders such as deep vein thrombosis and stroke have higher PAI-1 levels than controls. Increased level of PAI-1 is commonly found in patients with coronary heart disease [28]. Moreover, PAI-1 may have functions other than intravascular control of plasminogen activators activity. Epidemiological studies have revealed that plasma PAI-1 activity is positively correlated with body mass index and serum triglyceride levels [87].

PAI-1 is involved in tumor invasion and metastasis. Moreover, invasion and metastasis of solid tumors require the concerted action of various proteolytic enzyme systems, which degrade the basement membranes [84]. As mentioned above uPA may play a key role among the proteases and the activity of uPA during metastasis is regulated by PAI-1 that inhibits uPA through the formation of a covalent inhibitor-enzyme complex. PAI-1 is an inhibitor of both free and receptor bound uPA [54]. The role of PAI-1 in tumor biology has not been defined clearly. Several studies have suggested that PAI-1 protects the tumor tissue against the proteolytic degradation that the tumor imposes upon the surrounding normal tissue [54]. PAI-1 may bind to the cell surface associated uPAR/uPA complex forming an enzymatically inactive trimeric receptor-protease-inhibitor complex which is internalized by the tumor cell [73]. In addition, PAI-1 may be involved in the modulation of uPAR binding to ECM components and interfere with cell attachment to the matrix. PAI-1 has strong effect on the malignant phenotype of tumor cells [74]. High level of PAI-1 in tumors
predicts poor prognosis for the patient. Increased levels PAI-1 may be a cause for the development or the progression of cancer [82]. High level of PAI-1 is observed in a variety of malignancies including cancer of the breast, cervix uteri, ovary, stomach, colon, lung, brain, kidney [73]. Cancer patients may be defined as low- or high-risk patients depending on the amount of PAI-1 in primary tumor [74]. Recently, it has been proposed that PAI-1 is a potentially important prognostic factor in breast carcinoma [74]. In tumor tissues from patients with cancer elevated levels of PAI-1 are observed when compared with normal tissues.

An insertion/deletion polymorphism in the promoter of the PAI-1 gene may have an influence on plasma PAI-1 level. We studied frequency of 4G/5G genotype in subjects with cancer. Blood was drawn from 53 patients (9 gastric cancers, 16 breast cancer, 9 melanomas, 12 colorectal cancers, 7 head and neck cancers) and 53 matched controls [80]. Our study showed that the genotype distribution differed significantly between these two groups. The 4G/5G genotype was observed more frequently in patients with cancer than in the controls. These results suggest that 4G/5G genotype may be associated with elevated PAI-1 level in cancer, what predicts poor prognosis. Further studies are needed to check whether the prevalence of the 4G/5G genotype may influence an individual's plasminogen activation system capacity and thereby contribute to the cancer risk profile.

It is not easy to understand why the elevated tumor tissue content of PAI-1 indicates a poor prognosis for the cancer patients. PAI-1 may be involved in the modulation of uPAR binding to extracellular matrix components and interfere with cell attachment to the extracellular matrix. PAI-1 has strong effect on the malignant phenotype of the tumor cells.

In cancer, the increase of PAI-1 is associated with tumor progression and with shortened disease-free and/or overall survival in patients afflicted with malignant solid tumors. Strong correlation between elevated PAI-1 values in primary cancer tissues and the tumor invasion/metastasis capacity of cancer cells, PAI-1 has been selected as target for anticancer therapy.

**UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR**

The protein and the gene

The receptor for uPAR was first identified on monocytes and the monocytoid cell line U937 as a membrane protein that binds the amino-terminal fragment of uPA with high affinity \(5 \times 10^{-10}\) M [13, 56, 85].
uPAR consist of a single polypeptide chain and behaves as an amphphilic membrane protein [29, 56].

The specific cellular receptor for uPA, uPAR binds its ligands through a high affinity interaction with the N-terminal epidermal growth factor (EGF)-like domain of uPA [27]. uPAR is a glycolipid-anchored membrane glycoprotein encoded as a 335-residue polypeptide that is posttranslationally processed to approximately 283 residues by removal of the N-terminal signal sequence and a C-terminal sequence responsible for the addition of the glycosylphosphatidylinositol (GPI) moiety [64]. It is established that uPAR is composed of three domains that are thought to share a common structure and form a part of the Ly-6/uPAR superfamily of GPI-anchored proteins, characterized by a highly conserved spacing and disulfide bonding of cysteine residues. The solution structure of the Ly-6/uPAR family member CD59 was solved by nuclear magnetic resonance [30] and confirms a prediction based on a variety of considerations that these proteins share a common structural framework with the large family of structurally defined snake venom α-neurotoxins [62, 63]. Definitive proof of this as a model for uPAR awaits its direct structural determination, but the model has already been useful in tentatively identifying a hydrophobic binding site in uPAR [62, 63].

The unique multidomain structure of uPAR and its potential role in the binding of uPA and other putative uPAR ligands are of continuing interest, as this multidomain structure has proved to be essential for uPA binding function. It was originally considered that the N-terminal domain 1 of uPAR contained all the determinants necessary for uPA binding [4]. The direct involvement of this domain in ligand binding is also supported by other observations, including the recent identification of Tyr57 as part of the uPA binding site [63]. It has been shown in competitive binding studies with recombinant C-terminally truncated soluble uPAR (termed s-uPAR), however, that liberation of domain 1 reduces its uPA binding affinity by at least 1500-fold, from 0.1 nM to greater than 150 nM [62]. This suggests that it cannot be excluded that the other domains of uPAR directly contribute to the interaction with uPA.

The uPAR gene is composed of seven exons (101, 111, 144, 162, 135, 147 and 563 bp) separated by six introns (approximately 2.04, 2.62, 8.42, 0.906, 3.10 and 2.78 kb) [86]. Exons 1-7 encode 19, 37, 48, 54, 45, 49 and 83 amino acid residues, respectively. A CpG-rich islands and sequences related to the transcription factors AP-1, AP-2 and c-Jun are present, but no potential TATA or CAAT boxes were found in the proximal 5' region of the uPAR gene.
Tumor invasion and metastasis

As mentioned above, EMC degradation in cancer is a result of an interaction between the epithelial tumor cells and the infiltrating stromal cells, so the cellular localization of uPAR in cancer cells may give information on its potential role in the process of cancer invasion. In 49 from 60 samples derived from patients with invasive ductal breast carcinomas, uPAR reactivity was observed in macrophages located in close vicinity to the infiltrating epithelial cancer tissue [66]. In another study, the cellular localization of uPAR was studied in 59 invasive breast cancer samples, 12 normal breast tissue cases and 4 fibroadenomas [6]. By using an anti-uPAR polyclonal antibody it was found 49/59 invasive breast carcinoma to express uPAR immunoreactivity. Strong surface staining of tumor-associated macrophages was evident in most of the cases. Staining of tumor cells was observed in 21/59 cases while no staining was seen in normal breast tissue or in the fibroadenomas. The expression of uPAR in tumor-infiltrating macrophages in breast cancer is consistent with the pattern of uPAR mRNA expression in human colon adenocarcinomas [64].

uPAR also appears to be a prognostic marker in certain tumors. In colorectal malignancies, high levels of uPAR have been reported to constitute an independent prognostic marker [31]. In breast cancer also, high uPAR levels are associated with both shorter disease-free interval and shorter overall survival [26, 32]. Furthermore, uPAR was shown to be an independent marker of disease outcome in squamous carcinoma of the lung but not in large-cell lung cancer [59]. Finally, it was demonstrated that expression of uPAR by disseminated carcinoma cells in bone marrow of patients with gastric cancer is a highly significant predictor of early disease relapse [34].

CONCLUDING REMARKS

The urokinase plasminogen activator system consist of pro-enzymes, active enzymes and binding protein that interact in a highly organized way with ECM proteins facilitating cancer invasion and metastasis.

uPA enhances in vitro cell migration and invasion, but there are suggestions that the function of the system does not rely on the unbrided enzyme activity of uPA. The dynamic state of the system at the cell surface allows spatial and temporal rearrangements of its components during these processes. Recent work has suggested the existence of non-proteolytic function of the system, consisting of intracellular signal-transduction cascades
being initiated by binding of uPA to uPAR, of uPAR as a vitronectin receptor and as a regulator of integrin function and of a function of PAI-1 as a regulator of binding of uPAR and of integrins to vitronectin. Some observations support the hypothesis that PAI-1 is needed for the optimal function of the uPA system in these processes, by being necessary for the dynamic state of the system, by regulating cell adhesion and for selective protection of vitronectin against proteolysis. PAI-1 may counteract migration and invasion.

The correlation between uPA and uPAR concentrations in human tumors and poor prognosis is in agreement with the basic idea of uPAR-bound uPA at the surface of cancer cells being necessary for cancer invasion and metastasis. uPA and uPAR engaged in tissue remodelling events in the tissues surrounding the cancer cells may also contribute to the levels of these components and poor patient prognosis. The role of PAI-1 as a strong prognostic marker in cancer may be related to a possible role of PAI-1 in cancer cell migration and invasion. Differential expression of uPA and uPAR, on one side, and of PAI-1, on the other, by different cell types and in different tissue areas may contribute to proteolysis being directional at the level of tissue areas. In this situation, PAI-1 would be playing a part in tissue remodelling events and stroma remodelling by protecting newly deposited ECM in the center of islands of cancer cells and in the normal tissue surrounding them.

Further studies are needed to elucidate the role of the urokinase plasminogen activator system in cancer invasion and metastasis. An exact identification of the processes in tumors in which various components of the uPA system are implicated is a challenge for the future research. The non-proteolytic interactions of the components of the system with ECM proteins should be taken into account as possible mechanism involved in tumor progression.

REFERENCES

Inwazyjność nowotworów i ich zdolność do przerzutowania są klinicznymi cechami progresji nowotworów i wymagają degradacji i migracji przez tkankę łączną przez komórki rakowe. Procesy te zależą od złożonego oddziaływania pomiędzy komórkami nowotworu i otaczającą macierzą zewntrzkomórkową. Oddziaływania te są modyfikowane przez enzymy proteolityczne i ich receptory. Urokinazowy układ aktywacji plazminogenu odgrywa ważną rolę w inwazyjności nowotworów i ich zdolności do przerzutowania. Układ ten zawiera urokinazowy aktywator plazminogenu (uPA), receptor urokinazowy (uPAR) i specyficzne inhibitory aktywatorów plazminogenu, PAI-1 i PAI-2. Receptor uPA może znajdować się na powierzchni komórek rakowych. uPA może być uwalniany zarówno przez komórki normalne jak i zmienione nowotworowo. Kompleks uPA-uPAR skupia aktywność proteolityczną na powierzchni komórek rakowych przez konwersję osocznego białka plazminogenu do proteazy serynowej plazminy. Plazmina degrduję macierz zewntrzkomórkową w sąsiedztwie komórek rakowych ułatwiając w ten sposób inwazję nowotworów. uPA, uPAR i PAI-1 mogą być stosowane jako markery prognoistyczne w wielu typach raka.