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Clinical significance of the plasminogen activator system in relation to grade of tumor and treatment response in colorectal carcinoma patients

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Urokinase (uPA) plays an essential role in the activation of plasminogen to plasmin, and together with its receptor (uPAR), tissue activator (tPA) and urokinase inhibitors (PAI 1, PAI 2, PAI 3 and protease nexin) forms the plasminogen activator system (PAS), a component of metastatic cascade importantly contributing to the invasive growth and angiogenesis of malignant tumours. In our project we examined the expression of uPA, uPAR, PAI 1 and PAI 2 in tumor tissue and we also studied the plasma levels of PAI 1 before and after the initiation of therapy in patients with colorectal carcinoma in relationship to grade of tumor and the treatment response. In our prospective evaluation we included 80 patients treated for adenocarcinoma of the colon and rectum. Analysis of collected data revealed statistically significant evidence of a relationship between the level of PAI 1 in plasma before treatment and grade of the tumor, which increases with tumor grade (p=0.025). We demonstrated that there exists a statistically significant relationship between the expression of PAI 2 (p<0.001) and uPAR (p=0.031) and grade of tumor. We also confirmed a statistically significant relationship between soluble levels of PAI 1 before treatment and therapeutic response (p=0.021). In our group of patients the expression of uPA, uPAR, PAI 1 and 2 in tumor tissue in relation to response to treatment was also assessed. Our results suggest that the greater expression of these parameters in tumor tissue is linked to a worse response to therapy. In conclusion, PAS factors help as a prognostic indicators and could also act as a predictive factor in colorectal carcinoma.

Key words: colorectal cancer, plasminogen activator system, grade, treatment response.

Colorectal cancer is one of the most common site of cancer in the Czech Republic, representing about 15% of all oncological diseases. It is the most common site of cancer of the digestive tract. In 2007 its incidence reached 91.3 per 100 000 in men and 60.2 per 100 000 in women [1]. Cancer of the colon and rectum is therefore becoming not only a medical but also a social problem. The main form of treatment is surgical therapy, in combination with chemotherapy and radiotherapy (in the case of cancer of the rectum) according to stage of the disease. To date, there has yet to be identified an independent factor, by which one can clearly assess the patient's prognosis and help in the indication of treatment, both chemotherapy and radiotherapy. When evaluating the patient's prognosis, we are still dependent on a number of parameters with rather limited prognostic value.

Fibrinolysis is a process, which leads to the degradation of fibrin to fibrin monomers. Fibrinolysis helps to regulate hemostasis and prevents the creation of an inappropriately large thrombus, which could reduce blood flow in the bloodstream. The main enzyme involved in fibrinolysis is plasmin, which is produced by the liver in inactive form as plasminogen, its half-life is about 2 days, but depending on the activation of the fibrinolytic system, may be significantly shorter [2]. Tissue plasminogen activator (tPA) and urokinase (uPA) are agents converting plasminogen into active plasmin.

Urokinase (uPA) is a serine protease that plays an essential role in the activation of plasminogen to plasmin, which belongs to the serine proteases and also participates in the activation of matrixmetaloproteinases, latent elastases, growth factors and

cytokines, involved in the degradation of elements of extracellular matrix, such as fibrin and laminin. uPA is synthesized from prourokinases proenzymes (pro-uPA or sc-uPA). Plasmin together with its receptor (uPAR), tissue activator (tPA), which is synthesized from proenzymes pro-tPA or sc-tPA) and urokinase inhibitors (PAI 1, PAI 2, and PAI 3 – which is identical to the protein C inhibitor and protease nexin) forms the plasminogen activator system (PAS) [2], which is part of the metastatic cascade and significantly contributes to invasive growth and angiogenesis of malignant tumors [3]. In contrast to tPA that is fundamental in fibrinolysis, uPA plays an essential role in tissue degradation as part of physiological and pathological processes. Plasminogen activator inhibitor 1, which comprises 60% of all plasminogen activator inhibitors, inhibits both uPA and tPA. It is of interest that, contrary to colorectal carcinomas that show low tPA and high uPA activity, the tissue of a healthy colon demonstrates low uPA and high tPA expression [4].

uPAR is a membrane glycoprotein, receptor for uPA. The binding of uPA to uPAR leads to the activation of signal transduction pathways and consequent stimulation of cell proliferation, modulation of adhesion and facilitation of cell migration [5].

PAI 1 is a single-chained glycoprotein predominantly found in vascular smooth muscle, in megakaryocytes, endothelium, granulocytes, monocytes, macrophages and tumor cells. It is a serpin, present in plasma and α-granules of platelets and has the ability to bind to vitronectin [6,7,8]. Complex vitronectin PAI 1 has a longer elimination half-life and inhibits the migration of smooth muscle tissue cells by blocking the bond of $\alpha_v\beta_3$integrin to vitronectin [9,10]. Apart from $\alpha_v\beta_3$integrin, it also binds to other groups of integrins, including $\alpha_v\beta_1$, $\alpha_v\beta_5$, $\alpha IIb\beta_3$ and $\alpha_8\beta_1$. The interaction of plasminogen activator inhibitor 1 with the uPA/uPAR complex leads to internalization of the triplet, which stimulates cell proliferation, whereas the PAI 2/uPA/uPAR complex is not internalized, but it is processed on the cell surfaces [11]. PAI 1 is the most effective inhibitor of uPA and tPA.

PAI 2 is also known as placental plasminogen activator inhibitor, which exists in two forms: the intracellular nonglycosyled and extracellular glycosylated form. PAI 2 is found in the trophoblastic epithelium and is synthesized by leukocytes. Its plasma level is elevated in pregnant women. Until recently, according to some authors, PAI 2 played a role in the modulation of coagulation during pregnancy. Further investigations of its function suggest a significant role in the process of malignant tumor growth.

Therefore, the effects of PAI 1 and PAI 2 on tumor cells are a reflection of their distinct physiological biological functions. By binding to the uPA/uPAR complex thus PAI 1 stimulates growth as well as redistribution of uPAR on cell surfaces; this redistribution provides tumor tissue invasiveness. On the other hand, PAI 2 should be a true PAS inhibitor, not just because of inhibition of urokinase activity but also because it inhibits tumor cell migration by means of uPAR blockade [12].

The precise mechanism of local growth and metastasizing of malignant tumors is not yet well known. It is, nevertheless, clear that a number of factors contribute to this process, particularly proteolytic enzymes that cause erosion of extracellular matrix. What is generally accepted with regards to colorectal carcinoma is Morson's theory, which claims that the majority of colorectal carcinomas develop from adenomatous polyps [13]. However, the biochemical background to this process has not been explored sufficiently yet. The first step in the malignant transformation of a benign tumor involves the destruction of the basement membrane, by proteolytic enzymes alone, which facilitates both, local invasiveness of the tumor and its metastatic dissemination, being the basic processes of malignant tumor growth. This sequence of transformation from normal mucosa – adenomatous polyps – adenocarcinoma – metastasis is associated with increasing expression of uPA, uPAR and PAI 1 as well as PAI 2 with concomitant decrease in tPA expression [14,15].

It can be said that plasminogen-bound molecules play an essential role in tumor invasion and process of metastasis. Activation of uPA is regulated by the receptor for urokinases (uPAR). Binding uPA to the uPA receptor accelerates the activation of uPA from inactive proenzymes pro-uPA, whose conversion is catalyzed by plasmin, coagulation factor XIIa, and cathepsin B and L. Interaction of uPAR with the extracellular matrix is mediated by vitronectin [16], this interaction is amplified by the binding of uPA and attenuated by PAI 1. The complex of uPA/uPAR has a high affinity to vitronectin.

Vitronectin is bound to fibrin, contributes to the migration of tumor cells in a fibrin´s matrix, local tumor invasion and distant metastasis. The basic process of invasive growth of tumors is the degradation of basement membrane and extracellular matrix proteins, allowing migration of tumor cells. uPAR is linked to the cell membrane by glycosylphosphatidylinositol. The binding of uPA to uPAR results in activation of the protein tyrosine kinase [17], protein kinase C [18] and MAP kinase [19]. At the same time, the direct signaling pathway via Jak/STAT cascade utilizing signaling transduction of Scr-like protein tyrosine kinase has also been described [20]. uPAR expression is regulated by many growth factors, e.g. EGF, FGF-2 and HGF. Extracellular interaction between uPA, uPAR, $\alpha_3\beta_1$integrin and fibronectin initiates an intracellular signaling cascade mediated by epidermal growth factor [21]. The uPAR/uPA/PAI1 system is also involved in VEGF-induced angiogenesis [22].

Some authors believe that PAI 1 or its deficiency interferes in signaling pathways such as PI3K/Akt and JAK / STAT and it is included in the processes of maintaining the integrity of the endothelial cells and thereby regulation of cell death. PAI 1 affects apoptosis by reducing cell adhesion (anoikis) and the functioning of intracellular signaling pathways. This allows its ability to inhibit the generation of plasmin, inhibition of caspase 3 and inhibition of cell adhesion mediated by vitronectin. It is the direct inhibition of caspase 3 by PAI 1, which can change intracellular cell signaling from induction of apoptosis to induction of proliferation [23,24].

Patients and methods.

Patients. Prospective evaluation involved a total of 80 patients treated for adenocarcinoma of the colon or rectum at the Department of Clinical Oncology, University Hospital Brno, comprising of 55 men and 25 women, aged 39-80 years with a median of 62.5 years. The group consisted of 25 (31.3%) patients of TNM classification stage I, 19 (23.8%) stage II, 17 (21.3%) stage III patients and 19 (23.8%) stage IV patients. Sixteen patients died during the evaluation period, the death of 3 patients were not associated with the primary disease, 13 patients died due to the progression or relapse of the colorectal cancer. Of the total of 80 patients, there were 36 (45%) with cancer of colon and 44 patients (55%) were diagnosed with adenocarcinoma of the rectum. The main patient characteristics are summarized in Tab. 1.

Table 1. Description of patients

Age (mean, median (min – max))	62.5, 62.5 (39.0 – 80.0)
Men – N (%)	55 (68.8%)
Follow-up median (min – max) – months	18.5 (3.0 – 42.0)
PAI 1 tumor – positive – N (%)	33 (41.3%)
PAI 2 tumor – positive – N (%)	44 (55.0%)
uPA tumor – positive – N (%)	29 (36.3%)
uPAR tumor – positive – N (%)	40 (50.0%)
Stage (TNM classification)	
1 – N (%)	25 (31.3%)
2 – N (%)	19 (23.8%)
3 – N (%)	17 (21.3%)
4 – N (%)	19 (23.8%)
Stage (Dukes classification)	
A – N (%)	25 (31.3%)
B – N (%)	19 (23.8%)
C – N (%)	17 (21.3%)
Grade	
1 – N (%)	25 (31.3%)
2 – N (%)	39 (48.8%)
3 – N (%)	14 (17.5%)
4 – N (%)	1 (1.3%)
unknown – N (%)	1 (1.3%)

For the project the tumor tissue of patients was used after resection for colorectal cancer (primary tumor or metastases) conducted in the Surgical Clinic of the University Hospital Brno. Patients were included in the project after giving their informed consent. Participation in the study was represented only by a sampling of tumor tissue, which was not carried beyond the standard treatment and diagnostic procedures and collection of peripheral blood at the same time, also as part of standard tests. Patients, in addition to the above characteristics (uPA, uPAR, PAI 1 and PAI 2 in tumor tissue, PAI 1 in peripheral blood before treatment and 6-8 weeks after initiation of therapy {for stages I and II after surgery, for stage III after the initiation of adjuvant chemotherapy, for stage IV after start of palliative chemotherapy}) were assessed for age, gender, type and grade of tumor, TNM classification, Dukes classification, response to treatment (by RECIST criteria [25], overall survival, the application of adjuvant chemotherapy, type of adjuvant or palliative chemotherapy or antithrombotic therapy.

As the adjuvant chemotherapy, the 5-day protocol FUFA Mayo was administered. In the scope of palliative treatment only the effect the first line chemotherapy based on irinotecan or oxaliplatin, possibly in combination with bevacizumab was evaluated. During the monitoring, 2 patients diagnosed with coronary artery disease were treated with acetyl salicylic acid and 1 patient diagnosed with deep-vein thrombosis of the leg was treated with low molecular weight heparin, and none of these patients were treated with warfarin or other oral anticoagulation therapies.

Material sampling and processing. The immunohistochemical proof of the urokinase-type plasminogen activator, its receptor and plasminogen activator inhibitor 1 and 2 is based on the immunohistochemical reaction in native tumor tissue. The tissue fixed in 4% formaldehyde solution (i.e. 10% formalin solution) was processed in a standard fashion into formalin-fixed, paraffin-embedded blocks. The blocks were cut into 4 micrometer thick sections that were subsequently deparaffinized. Endogenous peroxidases were inactivated (using 3% solution of hydrogen peroxide in methanol) and the tissue epitopes were demasked using heat induction at 98°C for 20 minutes in citrate buffer at pH 6.0. This was followed by inactivation of non-specific bonds using 1% Normal Rabbit Serum. Primary goat (anti-goat) polyclonal antibodies PAI-1-Santa Cruz, (C-20):sc-6642, PAI-2-Santa Cruz, (N18): sc-6647, uPA-Santa Cruz (C-20):sc-6830, uPAR-Santa Cruz (N-19):sc-9793 were then applied, all 1:50 diluted. Detection was performed using Goat Vectastain ABCkit (Vector) and DAB (diaminobenzidine) visualization for 5 minutes. The sections were stained with Gills Haematoxylin, dehydrated, mounted and examined in light microscope. Two independent and fully qualified doctors with the aid of the morphometric programme LUCIA executed the evaluation of the results of the immunohistochemical reactions.

Peripheral blood for plasma PAI 1 analysis was sampled pre-surgery or, in TNM stage IV patients, before the initiation of tumor therapy and, subsequently, 6-8 weeks later to avoid of effects of prolonged post–surgery antithrombotic prophylaxis. The evaluation of functional activity of PAI 1 was performed using the photometric microplate method on the ELISA reader Expert Plus (ASYS Hitech) with Spectrolyse/(pLPAI-1) set by Biopool (A Trinity Biotech Company). Plasma samples were prepared from blood samples collected into 1:10 diluted 0.109M sodium citrate and centrifuged for 15 minutes at 2500g in a cooled centrifuge (15°C) and stored at -70°C. The reference range of 0-10.0 AU/ml was used to assess PAI 1 plasma levels.

Clinical evaluation. All patients were monitored as part of their routine follow-up; patients in complete remission were, after the post-surgery sampling, evaluated at 3-monthly intervals using imaging methods. Chemotherapy patients were

Table 2. Relationship between plasma level of PAI 1 before therapy and grade of tumour

PAI 1 before therapy (AU/ml)	Grade[1]		
	1 [a]	2 [a]	3+4 [b]
N	25	39	15
Median	5.2	6.6	13.0
Min – max	0.0 – 15.0	0.0 – 36.2	2.4 – 42.2
Percentil 5 – 95%	0.0 – 14.9	0.0 – 26.0	2.4 – 42.2

[1] Tested with Kruskal Wallis test (p=**0.025**).
[a, b] – homogenous groups, without significant difference

evaluated at monthly intervals, using imaging methods every 3 months. Suspicion of progressive disease was verified by CT abdomen/thorax or chest X-ray. Treatment response was assessed according to RECIST criteria [25]

Ethical aspects. The study patients were enrolled after signing an informed consent form that was approved by the Ethical Committee of the University Hospital Brno.

Statistical analysis. Descriptive statistics (N, median, minimum and maximum, 5 and 95% percentile) were calculated for continuous variables and absolute and relative numbers of patients in each category for categorical variables. To determine the relationship between levels of PAI 1 before and PAI 1 after treatment and tumor grade and clinical response, the Kruskal-Wallis test was used. To identify homogeneous subgroups we used the post hoc test "Multiple Comparisons of Mean Ranks for All Groups". Contingency tables were tested with a Chi-square test and Fisher's exact test. The analysis was performed with the support of Statistica for Windows 8 and SPSS 17.

Results

We confirmed by statistical significance the relationship between the level of PAI 1 in plasma before treatment and grade of the tumor, which increases with tumor grade, there were also higher pretreatment levels of PAI 1 (p = 0.025) (Tab. 2). This group of patients consists of patients with tumor grades 1 + 2 clearly different from the group of patients with tumor grades 3 + 4, which were tested together, with respect to the number of patients.

Tab. 3 shows that it is statistically significantly an increased expression to the increasing grade of tumor, but this was found only in the expression of PAI 2 in the tumor. It is interesting that PAI 1 expression is high in grades 3 + 4, meaning in the more aggressive types of tumors and uPA expression is low in grade 1, in the less aggressive types. We demonstrated that there is a statistically significant relationship between the expression of uPAR and grade of tumor, however individual grades of tumor could not be statistically significantly distinguished from others.

We confirmed a statistically significant relationship between soluble levels of plasminogen activator inhibitor 1 before treatment and therapeutic response (p = 0.021), in the context of increasing levels of plasminogen activator inhibitor 1 in patients with progression and low levels of PAI 1 in patients having complete remission after treatment (Tab. 4, 5). This is clearly related to the stage of the disease, where higher stages are accompanied by higher levels of soluble PAI 1 and early stages, such as Dukes A and B, where it is more likely to achieve complete remission, by lower levels of plasmatic PAI 1. The relationship between plasmatic PAI 1 levels after initiation of treatment and therapeutic response was also

Tab. 3. Relationship between expression of PAI 1, PAI 2, uPA, uPAR in tumorous tissue and grade of tumor.

	Grade			p[1] (negative vs. positive in all grades)
	1	2	3+4	
PAI 1 expression in tumor				
negative – N (%)	18 (39.1%)	23 (50%)	5 (10.9%)	
positive – N (%)	7 (21.2%)	16 (48.5%)	10 (30.3%)	
p (negative vs. positive in the same grade) [1]	0.143	1.000	**0.041**	0.054
PAI 2 2 expression in tumor				
negative – N (%)	19 (54.3%)	13 (37.1%)	3 (8.6%)	
positive – N (%)	6 (13.6%)	26 (59.1%)	12 (27.3%)	
p (negative vs. positive in the same grade)[1]	**p<0.001**	**0.047**	**0.043**	**p<0.001**
uPA expression in tumor				
negative – N (%)	20 (40%)	23 (46%)	7 (14%)	
positive – N (%)	5 (17.2%)	16 (55.2%)	8 (27.6%)	
p (negative vs. positive in the same grade)[1]	**0.048**	0.486	0.146	0.070
uPAR expression in tumor				
negative – N (%)	17 (43.6%)	18 (46.2%)	4 (10.3%)	
positive – N (%)	8 (20%)	21 (52.5%)	11 (27.5%)	
p (negative vs. positive in the same grade)[1]	0.053	0.655	0.083	**0.031**

[1] Tested with maximum likelihood Chi-square test (more than two categories) and Fisher's exact test

Tab. 4. Relationship between plasma level of PAI 1 before therapy and treatment response

PAI 1 before therapy (AU/ml)	Treatment response[1]			
	complete remission[a]	partial remission[ab]	stabilization[ab]	progression[b]
N	53	8	4	15
Median	5.9	13.5	4.8	13.0
Min – max	0.0 – 31.4	0.0 – 23.2	0.0 – 6.7	0.0 – 42.2
Percentil 5 – 95%	0.0 – 21.7	0.0 – 23.2	0.0 – 6.7	0.0 – 42.2

[1] Tested with Kruskal Wallis test (p=**0.021**).
[a, b] – homogenous groups, without significant difference

Tab. 5. Relationship between plasma level of PAI 1 after therapy and treatment response

PAI 1 after therapy (AU/ml)	Treatment response[1]			
	complete remission[a]	partial remission[ab]	stabilization[ab]	progression[b]
N	53	8	4	15
Median	2.5	3.6	7.0	9.4
Min – max	0.0 – 31.3	0.0 – 30.2	3.0 – 12.8	0.0 – 33.0
Percentil 5 – 95%	0.0 – 13.1	0.0 – 30.2	3.0 – 12.8	0.0 – 33.0

[1] Tested with Kruskal Wallis test (p=**0.004**).
[a, b] – homogenous groups, without significant difference

shown to be statistically significant, its value is reduced in relation with therapeutic response. Tab. 4 and 5 clearly show that the group of patients in partial remission and stabilization of the disease is homogeneous, in distinction to the group of patients in complete remission and group of patients with disease progression.

In our group of patients the expression of uPA, uPAR, PAI 1 and 2 in tumor tissue in relation to response to treatment was also assessed. Our results suggest that the greater expression of these parameters in tumor tissue is linked to a worse response to therapy. However, statistical significance was confirmed for all parameters (uPA, uPAR, PAI 1 and PAI 2) only in the case of complete remission. The statistical significance for an over-expression of PAI 2 in progressive patients was also positively confirmed. The statistical significance of individual parameters in groups of patients in partial remission or stable disease is probably accidental because of the small number of patients in these groups (Tab. 6).

Tab. 6. Relationship between expression of PAI 1, PAI 2, uPA, uPAR in tumorous tissue and treatment response

	Treatment response				p[1] (negative vs. positive in all grades)
	complete remission	partial remission	stabilization	progression	
PAI 1 expression in tumor					
negative – N (%)	37 (78.7%)	4 (8.5%)	0 (0.0%)	6 (12.8%)	
positive – N (%)	16 (48.5%)	4 (12.1%)	4 (12.1%)	9 (27.3%)	
p (negative vs. positive in the same grade)[1]	**0.008**	0.711	**0.026**	0.146	**0.007**
PAI 2 expression in tumor					
negative – N (%)	33 (91.7%)	1 (2.8%)	0 (0.0%)	2 (5.6%)	
positive – N (%)	20 (45.5%)	7 (15.9%)	4 (9.1%)	13 (29.5%)	
p (negative vs. positive in the same grade)[1]	**p<0.001**	0.067	0.123	**0.008**	**p<0.001**
uPA expression in tumor					
negative – N (%)	40 (78.4%)	2 (3.9%)	1 (2.0%)	8 (15.7%)	
positive – N (%)	13 (44.8%)	6 (20.7%)	3 (10.3%)	7 (24.1%)	
p (negative vs. positive in the same grade)[1]	**0.003**	**0.024**	0.133	0.383	**0.009**
uPAR expression in tumor					
negative – N (%)	35 (87.5%)	1 (2.5%)	0 (0.0%)	4 (10.0%)	
positive – N (%)	18 (45.0%)	7 (17.5%)	4 (10.0%)	11 (27.5%)	
p (negative vs. positive in the same grade)[1]	**p<0.001**	0.057	0.116	0.083	**p<0.001**

[1] Tested with maximum likelihood Chi-square test (more than two categories) and Fisher's exact test

Discussion

A number of studies explored the importance of the individual plasminogen activator system elements for oncology patients' survival. An increased uPA, uPAR, PAI 1 and PAI 2 expression in tumor tissue was confirmed in a number of tumors, such as breast, ovarian, kidney and stomach [26-32] and it is certain that these factors play an important role in tumor invasiveness and metastasizing.

Concerning this, the result of our work demonstrated significantly increased expression of PAI 2 with increasing tumor grade. It is interesting that PAI 1 expression is high in grade 3 + 4, in more aggressive types of tumors, and low uPA expression is in grade 1, i.e. the less aggressive types, which correlates other documented results to our work. A comparison of our results is possible only in the expression of uPA and PAI 1, where we did not demonstrate relation to tumor grade in our group of patients, in contrast to the studies of Papadopoulou et al. [33], which made a positive correlation to tumor grade. A comparison of the correlation of uPAR and PAI 2 to grade in colorectal cancer is not possible, because the work, focused on this topic, has not been published yet.

Not only for cancer of the colon or rectum, but also in breast cancer the results are inconclusive and controversial. Minisini et al. indicate, that the negative expression of uPA in breast cancer is associated with a higher grade of tumor [34]. Dublin et al. published that higher expression of uPA and PAI 1 is significantly linked with higher grades of tumor [35]. Cai et al. did not prove a relationship of uPA and PAI 1 to the grade of ovarian cancer [36]. In contrast, increasing expression of uPA and PAI 1 was statistically significantly demonstrated for a higher grade of thyroid cancer [37] as well as for high grade gliomas [38]. For hepatocellular carcinoma a statistically significant association of tumor grade and expression of uPA, PAI 1 and PAI 2 in tumor was shown [39]. The reason for these contradictory results remains unclear, possible explanations could be a hypothesis that the relation of the various components of PAS and tumor grade could depend on the type of dissemination, namely, whether the cancer has spread mainly through hematogenous or lymphogenous way. For such conclusions, however, we still have little data.

Previous studies established a direct relationship between plasma levels of the soluble uPAR protein; pre-operative levels of this protein correlated closely with the CEA levels, disease stage according to Dukes and prognosis of the colorectal carcinoma patient [40]. Based on these studies, we focused on PAI 1 plasma levels and their correlation with PAI 1 expression in tumor tissue and thus also the patient's prognosis. We have shown the statistically significant relationship between soluble PAI 1 plasma levels and tumor progression expressed through TNM and Dukes classification. This provides evidence for direct association between PAI 1 plasma levels and disease progression. This means that plasmatic PAI 1 levels decrease within weeks of a surgical procedure or treatment initiation, i.e. that PAI 1 occurs in the organism in close association with the tumor process and its levels in peripheral blood decrease when the tumor is eliminated. We have further shown a significant association between declining plasmatic PAI 1 levels and treatment response. We were able to divide the patients' samples into 3 homogenous groups; the group of patients in partial remission or with stabilized disease was clearly separated from the group of patients in complete remission and the group of patients with progressing disease.

An interesting situation is in the relationship of PAI 1 to tumor grade, when before initiation of therapy the baseline level of PAI 1 is significantly different in the group of patients with grade 1+2 to the group with grade 3+4. Moreover, a positive correlation between the expression of uPA and PAI 1 in tumor tissue and clinico-pathological factors, such as grade, has been also shown by Papadopoulou et al. [33] and has been demonstrated for other cancers such as cancer of the prostate [41] or breast [42].

The findings summarized above could lead to the hypothesis that the biological characteristics of a tumor do not correspond with the usual classification based on the currently accepted parameters. However, soluble level of PAI 1 cannot be used in isolation from other parameters employed in patient prognosis evaluation; it should, instead, be correlated with them.

In our group of patients, we also demonstrated a statistically significant relationship between plasma levels of PAI 1 and response to treatment, when levels of PAI 1 decreases after initiation of treatment. Similar studies focusing on colorectal carcinoma were not found in the available literature. But results have been reported with tamoxifen treatment in patients with metastatic breast cancer, where the expression of uPA in tumor tissue was a predictive factor of response, duration of response and overall survival. For tumors with low expression of uPA, a better response to tamoxifen was observed, than in those with high expression of uPA [43]. Likewise, there was observed a poorer response to tamoxifen therapy in patients with recurrence of breast cancer, without previous treatment with tamoxifen, where an increased expression of PAI 1 in tumor tissue was found [43].

Heiss et al. described that patients with gastric cancer and negative lymph nodes, in which a high expression of uPA and PAI 1 was found, have a benefit from adjuvant chemotherapy compared to patients with positive lymph nodes and a low PAI 1 expression [44]. These findings suggest that individual components of PAS could serve as a predictive factor of response to treatment.

Furthermore, in our group of patients we significantly demonstrated a common decline in plasma levels of PAI 1 in relation to the response to the treatment by division into 3 homogeneous groups – where the first group of patients is in complete remission and the third group of patients in progression- are separated from a completely homogeneous group of patients in partial remission and stabilization of the disease. It may be a sign of "artificial" division of the individual responses, based on RECIST criteria, which do not correspond to the biology of the tumor and its response to the therapy.

The question therefore remains for the future, if the evaluation of response to treatment, as currently used, would not be modified by other biological markers of tumors with clear and predictive values.

In the literature, data can be found focusing on this relationship in colorectal carcinoma in the study of Mytnik et al. In an evaluation regarding the type of tumor, it was found that the highest pre-operative levels of PAI-1 were present in non-differentiated and mucinous carcinoma. According to the evaluation of clinical stage based on the TNM classification, the plasma levels increased in relation to the progress/advance of clinical stage and reflected the size of tumor and infiltration of lymph nodes. The highest measured values were observed in the III. and IV. clinical stages [45,46].

Furthermore, we statistically proved a relationship between treatment response and the expression of uPA, uPAR, PAI 1 and PAI 2 in tumor tissue. Patients with negative expression of these factors had better response rates than patients with higher expressions. And not only worse response rates but a shorter overall survival was significantly demonstrated in patients expressing uPAR and PAI 2 in tumor tissue, which is already published by Kammori et al. in their study concerning patients with stomach cancer, where patients with higher expression of PAI 2 in tumor tissue were more likely to relapse, even in the early stages of tumor [47]. The role of the PAS in colorectal cancer appears to be complex. uPA, uPAR and PAI 1 were upregulated in tumor tissue and the degree of this upregulation correlates to the Dukes stage and lymphatic invasion [48].

An evidence of negative uPA, uPAR, PAI 1 and PAI 2 expression in tumor tissue of colorectal carcinoma in relation to the treatment response can then be considered on the basis of our results as a favourable predictive factor.

In conclusion, some PAS factors should act as the prognostic indicators and could also act as a predictive factor, this being of particular importance for patients who are not indicated -according to current criteria – for adjuvant chemotherapy, in patients with locally advanced tumors, but without lymph node involvement or eventually in patients with an insufficient number of collected lymph nodes. In these cases increased plasma levels of PAI 1 or expression of PAS components in tumor tissue should lead to indication of intensive adjuvant treatment, which could improve their prognosis.

The social implications of developing such an improved form of prognosis would mean a reduction of the risk of relapse in patients, by a more effective screening of individual cases for the presence of risk factors than those treated according to standard criteria. This in turn would reduce patient trauma, whether psychological or physical, in the form of adverse side effects of adjuvant chemotherapy and/or radiotherapy while also providing considerable economic savings.

An increased expression of PAS components in tumor tissue could be utilized when planning adjuvant treatment of patients with colorectal carcinoma as well as in the planning of a tailored therapy. Its individual components could become the target of targeted therapies.

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References

[1] DUSEK L, MUZIK J, KUBASEK M, KOPTIKOVA J, ZALOUDIK J et al. Epidemiology of malignant tumors in the Czech Republic [online]. Masaryk University, [2005], [cit. 2010-3-22]. Available WWW: http://www.svod.cz. Version 7.0 [2007], ISSN 1802-8861.

[2] BRUMMEL-ZIEDINS K, ORFEO T, SWORDS JENNY N, EVERSE SJ, MANN KG. Blood coagulation and fibrinolysis. In: Greer JP, Foerster J, Rodgers JM, editors. Wintrobe's Clinical Hematology, 12th edition. Baltimore: Lippincott Wiliams & Wilkins, 2009: 528-619.

[3] SCHMITT M, HARBECK M, THOMSSEN C, WILHELM O, MAGDOLEN V et al. Clinical impact of the plasminogen activation system in tumor invasion and metastasis: Prognostic relevance and target therapy. Thromb Haemost 1997; 78: 285-296.

[4] DE BRUIN PA, GRIFFIOEN G, VERSPAGET HW, VERHEIJEN JH, LAMERS CB. Plasminogen activators and tumor development in the human colon: activity levels in normal mucosa, adenomatous polyps, and adenocarcinomas. Cancer Res 1987; 47: 4654-4657.

[5] DUFFY MJ. The urokinase plasminogen activator system: role in malignancy. Curr Pharm Des 2004; 10: 39-49. doi:10.2174/1381612043453559

[6] FAY WP, SHAPIRO AD, SHIH JL, SCHLEEF RR, GINSBURG D. Complete deficiency of plasminogen-activator inhibitor type 1 due to a frame-shift mutation. N Engl J Med 1992; 327: 1729-1733. doi:10.1056/NEJM199212103272406

[7] CARMELIET P, STASSEN JM, SCHOONJANS L, REAM B, VAN DEN OORD JJ et al. Plasminogen activator inhibitor -1 gene-deficient mice. II. Effect on haemostasis, thrombosis and thrombolysis. J Clin Invest 1993; 92: 2756-2760. doi:10.1172/JCI116893

[8] HILL SA, SHAUGHNESSY SG, JOSHUA P, RIBAU J, AUSTIN RC et al. Differential mechanisms targeting type 1 plasminogen activator inhibitor and vitronectin into the storage granules of a human megakaryocytic cell line. Blood 1996; 87: 5061-5073.

[9] STEFANSSON S, PETICLERC E, WONG MK, MCMAHON GA, BROOKS PC et al. Inhibition of angiogenesis in vivo by plasminogen activator inhibitor-1. J Biol Chem 2001; 276: 8135-8141. doi:10.1074/jbc.M007609200

[10] STEFANSSON S, LAWRENCE DA. The serpin PAI-1 inhibits cell migration by blocking integrin alpha V beta 3 binding to vitronectin. Nature 1996; 383: 441-443. doi:10.1038/383441a0

[11] NYKJAER A, CONESE M, CHRISTENSEN EI, OLSON D, CREMONA O et al. Recycling of the urokinase receptor upon internalization of the uPA: serpin complexes. EMBO J 1997; 16: 2610-2620. doi:10.1093/emboj/16.10.2610

[12] SCHMITT M, WILHELM O, REUNING U, KRÜGER A, HARBECK N et al. The urokinase plasminogen activator sys-

tem as a novel target for tumour therapy. Fibrinolysis Proteol 2000; 14: 114-132. doi:10.1054/fipr.2000.0079

[13] MORSON BC Precancerous lesions of the colon and rectum. JAMA 1962; 179: 316-321.

[14] GANESH S, SIER CF, HEERDING MM, GRIFFIOEN G, LAMERS CB et al. Urokinase receptor and colorectal cancer survival. Lancet 1994; 344: 401-402. doi:10.1016/S0140-6736(94)91427-3

[15] VERSPAGET HW, SIER CF, GANESH S, GRIFFIOEN G, LAMERS CB. Prognostic value of plasminogen activators and theirs inhibitors in colorectal cancer. Eur J Cancer 1995; 31A: 1105-1109. doi:10.1016/0959-8049(95)00170-N

[16] WALTZ DA, NATKIN LR, FUJITA RM, WEI Y, CHAPMAN HA. Plasmin and plasminogen activator inhibitor type 1 promote cellular motility by regulating the interaction between the urokinase receptor and vitronectin. J Clin Invest 1997; 100: 58-67. doi:10.1172/JCI119521

[17] RESNATI M, GUTTINGER M, VALCAMONICA S, SIDENIUS N, BLASI F. et al. Proteolytic cleavage of the urokinase receptor substitutes for the agonist-induced chemotactic effect. EMBO J 1996; 15: 1572-1582.

[18] BUSSO N, MASUR SK, LAZEGA D, WAXMAN S, OSSOWSKI L. Induction of cell migration by pro-urokinase binding to its receptor: possible mechanism for signal transduction in human epitelial cells. J Cell Biol 1994; 126: 259-270. doi:10.1083/jcb.126.1.259

[19] NGUYEN DH, HUSSAINI IM, GONIAS SL. Binding of urokinase-type plasminogen activator to its receptor in MCF-7 cells activates extracellular signal regulated kinase 1 and 2 which is required for increased cellular motility. J Biol Chem 1998; 273: 8502-8507. doi:10.1074/jbc.273.14.8502

[20] DUMLER I, WEIS A, MAYBORODA OA, MAASCH C, JERKE U et al. The Jak/Stat pathway and urokinase receptor signaling in human aortic vascular smooth muscle cells. J Biol Chem 1998; 273: 315-321. doi:10.1074/jbc.273.1.315

[21] DURAND MK, BØDKER JS, CHRISTENSEN A, DUPONT DM, HANSEN M et al. Plasminogen activator inhibitor 1 and tumour growth, invasion and metastasis. Tromb Haemost 2004; 91: 438-449.

[22] PRAGER GW, BREUSS JM, STEURER S, OLCAYDU D, MIHALY J et al. Vascular endothelial growth factor receptor-2-induced initial endothelial cell migration depends on the presence of the urokinase receptor. Circ Res 2004; 94: 1562-1570. doi:10.1161/01.RES.0000131498.36194.6b

[23] BALSARA RD, PLOPLIS VA Plasminogen activator inhibitor-1: the double-edged sword in apoptosis. Thromb Haemost 2008; 100: 1029-1036.

[24] SCHNEIDER DJ, CHEN Y, SOBEL BE. The effect of plasminogen activator inhibitor type 1 on apoptosis. Thromb Haemost 2008; 100: 1037-1040.

[25] THERASSE P, ARBUCK SG, EISENHAUER EA, WANDERS J, KAPLAN RS et al. New guidelines to evaluate the response to treatment in solid tumors. JNCI 2000; 92: 205-216. doi:10.1093/jnci/92.3.205

[26] WANG L, MADIGAN MC, CHEN H, LIU F, PATTERSON KI et al. Expression of urokinase plasminogen activator and its receptor in advanced epithelial ovarian cancer patients. Gynecol Oncol 2009; 114: 265-272. doi:10.1016/j.ygyno.2009.04.031

[27] ZEMZOUM I, KATES RE, ROSS JS, DETTMAR P, DUTTA M et al. Invasion factors uPA/PAI-1 and HER2 status provide independent and complementary information on patient outcome in node-negative breast cancer. J Clin Oncol 2003; 21: 1022-1028. doi:10.1200/JCO.2003.04.170

[28] HILDENBRAND R, SCHAAF A, DORN-BEINEKE A, ALLGAYER H, SÜTTERLIN M et al. Tumor stroma is the predominant uPA-, uPAR-, PAI-1-expressing tissue in human breast cancer: prognostic impact. Histol Histopathol 2009; 24: 869-877.

[29] SAKAKIBARA T, HIBI K, KOIKE M, FUJIWARA M, KODERA Y et al. Plasminogen activator inhibitor-1 as a potential marker for the malignancy of colorectal cancer. Br J Cancer 2005; 93: 799-803. doi:10.1038/sj.bjc.6602743

[30] OHBA K, MIYATA Y, KANDA S, KOGA S, HAYASHI T et al. Expression of urokinase-type plasminogen activator, urokinase-type plasminogen activator receptor and plasminogen activator inhibitors in patients with renal cell carcinoma: correlation with tumor associated macrophage and prognosis. J Urol 2005; 174: 461-465. doi:10.1097/01.ju.0000165150.46006.92

[31] PEDERSEN H, GRØNDHAL-HANSEN J, FRANCIS D, OSTERLIND K, HANSEN HH et al. Urokinase and plasminogen activator inhibitor type 1 in pulmonary adenocarcinoma. Cancer Res 1994; 54: 120-123.

[32] KONECNY G, UNTCH M, PIHAN A, KIMMIG R, GROPP M et al. Association of urokinase-type plasminogen activator and its inhibitor with disease progression and prognosis in ovarian cancer. Clin Cancer Res 2001; 7: 1743-1749.

[33] PAPADOPOULOU S, SCORILAS A, YOTIS J, ARNOGIANAKI N, PLATANIOTIS G et al. Significance of urokinase-type plasminogen activator and plasminogen activator inhibitor-1 (PAI-1) expression in human colorectal carcinomas. Tumour Biol 2002; 23: 170-178. doi:10.1159/000064033

[34] MINISINI AM, FABBRO D, DI LORETO C, PESTRIN M, RUSSO S et al. Markers of the uPA system and common prognostic factors in breast cancer. Am J Clin Pathol 2007; 128: 112-117. doi:10.1309/M0GXVXA89BVLJ5C9

[35] DUBLIN E, HANBY A, PATEL NK, LIEBMAN R, BARNES D. Immunohistochemical expression of uPA, uPAR, and PAI-1 in breast carcinoma. Fibroblastic expression has strong associations with tumor pathology. Am J Pathol 2000; 157: 1219-1227. doi:10.1016/S0002-9440(10)64637-8

[36] CAI Z, LI YF, LIU FY, FENG YL, HOU JH et al. Expression and clinical significance of uPA and PAI-1 in epithelial ovarian cancer. Ai Zheng 2007; 26: 312-317.

[37] HORVATIĆ HERCEG G, HERCEG D, KRALIK M, BENCE-ZIGMAN Z, TOMIĆ-BRZAC H et al.:Urokinase-type plasminogen activator and its inhibitor in thyroid neoplasms: a cytosol study. Wien Klin Wochenschr 2006; 118: 601-609. doi:10.1007/s00508-006-0703-1

[38] MURACCIOLE X, ROMAIN S, DUFOUR H, PALMARI J, CHINOT O et al. PAI-1 and EGFR expression in adult glioma tumors: toward a molecular prognostic classification. Int J Radiat Oncol Biol Phys 2002; 52: 592-598. doi:10.1016/S0360-3016(01)02699-2

[39] ITOH T, HAYASHI Y, KANAMARU T, MORITA Y, SUZUKI S et al. Clinical significance of urokinase-type plasminogen activator activity in hepatocellular carcinoma. J Gastroen Hepatol 2000; 15: 422-430. doi:10.1046/j.1440-1746.2000.02150.x

[40] HERSZÉNYI L, FARINATI F, CARDIN R, ISTVÁN G, MOLNÁR LD et al. Tumor marker utility and prognostic relevance of cathepsin B, cathepsin L, urokinase-type plasminogen activator, plasminogen activator inhibitor type-1, CEA and CA 19-9 in colorectal cancer. BMC Cancer 2008; 10: 194.

[41] COZZI PJ, WANG J, DELPRADO W, MADIGAN MC, FAIRY S et al. Evaluation of urokinase plasminogen activator and its receptor in different grades of human prostate cancer. Hum Pathol 2006; 37: 1442-1451. doi:10.1016/j.humpath.2006.05.002

[42] MANDERS P, TJAN-HEIJNEN VC, SPAN PN, GREBENCHTCHIKOV N, GEURTS-MOESPOT A et al. Complex of urokinase-type plasminogen activator with it's type 1 inhibitor predicts poor outcome in 576 patients with lymph node-negative breast carcinoma. Cancer 2004; 101: 486-494. doi:10.1002/cncr.20374

[43] FOEKENS JA, LOOK MP, PETERS HA, VAN PUTTEN WL, PORTENGEN H et al. Urokinase-typ plasminogen activator and its inhibitor PAI 1: predictors of poor response to tamoxifen therapy in recurrent breast cancer. J Natl Cancer Inst 1995; 87: 751-756. doi:10.1093/jnci/87.10.751

[44] HEISS MM, ALLGAYER H, GRUETZNER KU, BABIC R, JAUCH KW et al. Clinical value of extended biologic staging by bone marrow micrometastases and tumor-associated proteases in gastric cancer. Ann Surg 1997; 226: 736-745. doi:10.1097/00000658-199712000-00010

[45] MYTNIK M, STASKO J. D-dimer, plasminogen activator inhibitor-1, prothrombin fragments and protein C – role in prothrombotic state of colorectal cancer. Neoplasma 2011; 58: 235 -238. doi:10.4149/neo_2011_03_235

[46] MYTNIK M, KUBISZ P, STASKO J, SELIGA P, DANO J. Colorectal malignancies with respect to hemocoagulation. Rozhl Chir 2009; 88: 735-739.

[47] KAMMORI M, KAMINISHI M, KOBAYASHI K, OOHARA T, ENDO H et al. Immunohistochemical analysis of PAI-2 (plasminogen activator inhibitor type 2) and p53 protein in early gastric cancer patients with recurrence: a preliminary report. Jpn J Clin Oncol 1999; 29: 187-191. doi:10.1093/jjco/29.4.187

[48] BAKER EA, BERGIN FG, LEAPER DJ. Plasminogen activator system, vascular endothelial growth factor, and colorectal cancer progression. J Clin Pathol 2000; 53: 307-312. doi:10.1136/mp.53.6.307