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REVIEW

Further Understanding of Urokinase Plasminogen Activator Overexpression in Urothelial Bladder Cancer Progression, Clinical Outcomes and Potential Therapeutic Targets

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Purpose: The Plasminogen Activation System (PAS) plays a role in tumor growth, invasion and metastasis and has been associated with oncological outcomes in urinary bladder carcinoma (UBC). The use of the different components of this system as molecular markers could improve our understanding of the heterogeneous behavior of UBC and might enable earlier disease detection, individual risk stratification, more accurate outcome prediction and be a rationale for new targeted therapies.

Methods: A comprehensive literature search including relevant articles up to October 2020 was performed using the MEDLINE/PubMed database.

Results: The components of the PAS axis are involved in tumor progression through their signaling processes during angiogenesis, cell migration, metastasis and adhesion. The body of evidence shows an association of PAS component overexpression with adverse pathological features and clinical outcome in UBC. Overexpressed PAS components correlate with a higher pathological tumor grade and advanced tumor stage. In non-muscle-invasive bladder cancer (NMIBC), the PAS components were associated with disease outcome while in muscle-invasive bladder cancer (MIBC), it was associated with disease outcome and pathological features. Possible therapeutic approaches in the PAS for the treatment of UBC have only been sparsely investigated in in vitro and in vivo studies. Intravesical plasminogen activator inhibitor 1 (PAI-1) instillation in animal models yielded interesting results and warrant further exploration in Phase II studies.

Conclusion: The overexpression of PAS components in UBC tumor tissue is associated with adverse pathological features and worse oncological outcomes. These findings are mainly based on preclinical studies and retrospective series, which requires further prospective studies to translate the PAS into clinically useful biomarkers and therapeutic targets.

Keywords: urothelial cancer, uPA, uPAR, PAI, therapy, review

Introduction

Urinary bladder carcinoma (UBC) is the fourth most common cancer in men and the sixth most common cause of cancer-related death in Europe.[1] In general, UBC is an aggressive disease with poor prognosis. About 75% of patients are diagnosed with an early stage non-muscle-invasive disease (NMIBC, staged Ta, Tis/CIS, T1), which has an up to 78% risk of recurrence and 45% risk or of progression to muscle-invasive disease (MIBC, stage T2-T4) within 5 years.[2] Moreover, about

50% of patients diagnosed with MIBC will die within 5 years despite surgery in curative intent.[3–5] Risk stratification and patient selection is of paramount importance in order to identify those patients who are more likely to fail therapy and counselling for additional systemic therapies. However, several predictive and prognostic models did not reach a sufficient level of discrimination to allow implementation in clinical practice.[3,6–10] To improve the accuracy of such a clinical model, several biomarkers have been investigated and implemented in these models.[11–14] The main objective in biomarker research is to identify a marker which is cheap, fast, accurate and can be used at several stages of the disease in order to aid clinicians in patient counselling regarding individualized treatment strategies.[14–18] Furthermore, biomarkers could serve potential targets for therapeutic approaches.[19] In the plethora of biomarkers discovered in UBC, the plasminogen activation system (PAS) has recently emerged as such a candidate biomarker. The PAS consists of urokinase, also known as urokinase-type plasminogen activator (uPA), the uPA receptor (uPAR) and plasminogen activator inhibitors 1 and 2 (PAI-1 and 2). The uPA activates plasminogen to plasmin by binding to its receptor uPAR and degrades the basement membrane and the extracellular matrix through a proteolytic cascade.[20] These components are involved in tumor growth, invasion and metastasis, through their effect on angiogenesis and cell migration.[20]

The aim of this review is to provide a comprehensive overview of the current literature on PAS as tissue biomarker and its association with UBC outcomes as well as potential treatment strategies.

Materials and Methods

We performed a comprehensive literature search including relevant articles up to October 2020 using the MEDLINE/PubMed database. We included original articles only and restricted the search to the English literature. For the identification of appropriate publications with respect to the general knowledge about the PAS as well as its role in UBC, the search terms plasminogen activator system, urokinase-type plasminogen activator system, uPA, uPAR, PAI, urothelial cancer, transitional cell carcinoma, bladder cancer, bladder neoplasms, therapeutical targets, pharmacology, therapy, immunotherapy were used singularly and combined using a Boolean operator. References of the found articles were reviewed to additionally expand search results. The article selection process was performed by two reviewers. Disagreements were resolved by a third reviewer.

Evidence Synthesis

Preclinical Data

The PAS plays a role in angiogenesis, cell migration and adhesion in various carcinomas.[21–23] This is mainly achieved through remodeling of the extracellular matrix, which allows endothelial cells to invade the tumor stroma and tumor cells to metastasize. In vitro studies have shown a higher expression of uPAR in invasive UBC cell lines and a >60% ($p<0.005$) and >80% ($p<0.005$) reduction in cell migration and invasion, respectively, after uPAR gene-silencing.[24] McGarvey et al examined the PAS components in UBC cell lines and tumor tissue. They found that in UBC cell lines uPA, uPAR and PAI-1 showed a significant higher expression rates in invasive cells ($p<0.05$) and a 3-fold increase of uPA ($p<0.003$) as well as a 2.7-fold increased uPAR expression ($p<0.008$) in MIBC compared to NMIBC tumor tissue, but not difference in PAI-1 expression.[25] These findings are in line with those reported by Champelovier et al They found that uPAR overexpression was associated with increased cell motility in cell culture ($p=0.014$) and with higher tumor stage in tumor tissue analyses ($p=0.02$).[26] These data suggest that PAS components are involved in carcinogenesis and progression of UBC, and could, therefore, serve as candidate biomarkers. Table 1 summarizes the findings in in vitro studies.

Association of the PAS with Pathologic Features in UBC

The current literature suggests that tissue overexpression of PAS components correlates with higher pathologic grade and more advanced tumor stage in UBC.

In a retrospective series of 939 patients with UBC, for example, authors investigated the association of angiogenin, MMP-2, p53, RB and PAI-1 expression using immunohistochemistry (IHC). They found a significantly higher rate of high-grade (65% vs 35%) and advanced (T3-T4) tumors (78% vs 23%) in patients with high PAI-1 expression.[27] Similarly, in an IHC study of 827 patients with NMIBC, Iwata et al found a significant association of uPA (odds ratio (OR) 1.19, $p = 0.005$), uPAR (OR 1.22; $p = 0.001$) and PAI-1 (OR 1.25, $p = 0.0003$) with tumor grade 3 disease.[28]

Intratumoral heterogeneity is a known feature of UBC.[29] Therefore, measurements of the PAS on IHC may be affected

Table 1 Overview of the Studies Investigating the Plasminogen Activation System (PAS) in vitro

In vitro Studies						
Author	**Year**	**Pat. No.**	**Study Design**	**Marker Investigated**	**Cell Line/Source**	**Findings**
McGarvey et al[25]	1998	6 cell lines, 29 tissue specimens	retrospective	uPA, uPAR, PAI-1	UBC cell lines (RT4, HT-5637, HT-1376, TCCsup, J82, T24), tissue	• uPA, uPAR, PAI-1 overexpression in UBC cell lines is associated with higher tumor stage ($p<0.05$) • uPA overexpression in tissue is associated with higher tumor stage ($p<0.003$) • uPAR overexpression in tissue is associated with higher tumor stage ($p<0.008$)
Champelovier et al[26]	2002	Preliminary experiment: 7 cell lines, 15 tissue specimens Validation: 129 tissue specimens	retrospective	uPA, tPA, uPAR, PAI-1, PAI-2	Exploratory analysis: UBC cell lines (RT4, RT112, CHA 89, T24, J82S, DAG-1, TCCsup), tissue Validation cohort: tissue	Exploratory analysis: • uPAR overexpression in UBC cell lines is associated with higher tumor grade ($p=0.02$) and cell motility ($p=0.014$) • tPA overexpression in tissue is associated with higher tumor grade ($p=0.05$) • uPAR overexpression in tissue is associated with higher tumor stage ($p=0.02$) and higher tumor ploidy ($p=0.04$) • PAI-2 overexpression in tissue is associated with higher tumor stage ($p=0.02$), higher tumor grade ($p=0.03$) and higher tumor ploidy ($p=0.04$) Validation cohort: • uPAR overexpression is associated with higher tumor grade ($p=0.002$), higher tumor stage ($p=0.003$), higher tumor ploidy ($p=0.05$) and shorter OS ($p=0.045$) • PAI-2 overexpression is associated with higher tumor grade ($p=0.005$) and shorter OS ($p=0.038$)
Hau et al[24]	2017	5 cell lines	not reported	uPAR	UBC cell lines (UROtsa, RT4, UM-UC-3, T24, J82)	• uPAR overexpression is associated with higher tumor stage ($p<0.05$) • uPAR gene-silencing inhibited cell migration >60% ($p<0.005$) and cell invasion >80% ($p<0.005$)

Abbreviations: UBC, urinary bladder carcinoma; uPA, urokinase-type plasminogen activator; tPA, tissue-type plasminogen activator; uPAR, uPA receptor; PAI-1, plasminogen activator inhibitor 1; PAI-2, plasminogen activator inhibitor 2; OS, overall survival.

by the location of the region examined. A retrospective series of 149 patients treated with radical cystectomy addressed this. Authors found a uPAR positivity in 89% and 74% of the tumors at the invasive front and tumor core, respectively. Patients with higher tumor stage (≥T2) had higher uPAR expression at the tumor core compared to those with NMIBC disease ($p < 0.04$).[30] The findings of PAS on pathological features are summarized in Tables 1–3.

Association of PAS with Oncologic Outcomes in NMIBC

Current evidence on the association of PAS with oncologic outcomes in NMIBC is mainly based on retrospective studies. Two small series by Hasui et al showed that overexpressed uPA is associated with a shorter progression-free survival (PFS) ($p<0.005$)[31] and a worse overall survival (OS) ($p<0.005$).[32] Furthermore, on multivariable analysis, uPA was independently associated with PFS (relative risk (RR) 5.93; p=0.011) and OS (RR 6.22; p=0.020).[32] In the retrospective series of Iwata et al, authors found on multivariable analyses a significant association of uPA (Hazard ratio (HR): 1.40, $p = 0.006$), uPAR (HR: 1.70, $p < 0.001$) and PAI-1 (HR: 1.35, $p = 0.014$) with recurrence-free survival (RFS).[28] This association was independent of tumor stage and grade. Indeed, in subgroup analysis of TaG1-2 and T1G3 tumors, all three markers were independently associated with RFS. However, only uPA was associated with PFS (HR 1.68, $p = 0.03$).

Controversially, in the series by Chan et al on multivariable analysis, PAI-1 was not associated with RFS but with OS (HR: 2.58, $p = 0.0004$).[27]

Both series are limited by the retrospective design and missing prognostic pathologic features like lymph vascular invasion and variant histology.[33] Table 2 summarizes the findings for NMIBC.

Table 2 Overview of the Studies Investigating the Plasminogen Activation System (PAS) on Non-Muscle-Invasive Bladder Cancer (NMIBC)

NMIBC Tissue						
Author	**Year**	**Pat. No.**	**Study Design**	**Marker Investigated**	**Source**	**Findings**
Hasui et al[31]	1994	42	not reported	uPA	Tissue	• uPA overexpression is associated with shorter PFS (p<0.005)
Hasui et al[32]	1996	52	prospective	uPA	Tissue	• uPA overexpression is associated with shorter OS (p<0.005) and is an independent predictor for worse OS (RR 6.22; p=0.020) and shorter PFS (RR 5.93; p=0.011) • Muscle invasion occurred in 2 (12%) patients in the uPA overexpression group versus 1 (3%) in the low-expression group (p-value not shown)
Iwata et al[28]	2019	827	retrospective	uPA, uPAR, PAI-1	Tissue	• uPA overexpression is associated with higher tumor grade (p=0.016) and is an independent predictor for shorter RFS (HR 1.4, 95% CI: 1.10–1.78; p=0.006) and shorter PFS (HR 1.68, 95% CI: 1.04–2.74; p=0.035) in all NMIBC tumors and shorter RFS (HR 1.64, 95% CI: 1.07–2.51; p=0.022) and shorter PFS (HR 2.19, 95% CI: 1.15–4.18; p=0.018) in T1G3 tumors • uPAR overexpression is associated with higher tumor grade (p=0.004) and is an independent predictor for shorter RFS (HR 1.7, 95% CI: 1.33–2.17; p<0.001) in all NMIBC tumors and shorter RFS in TaG1-2 (HR 1.54, 95% CI: 1.13–2.09; p=0.006) and T1G3 tumors (HR 2.0, 95% CI: 1.29–3.12; p=0.002) • PAI-1 overexpression is associated with higher tumor grade (p=0.001) and is an independent predictor for shorter RFS (HR 1.35, 95% CI: 1.06–1.71; p=0.014) in all NMIBC tumors and shorter RFS (HR 1.46, 95% CI: 1.08–1.97; p=0.013) in TaG1-2 tumors • Combination of 3 overexpressed marker is associated with higher tumor grade (p=0.000), a higher tumor number (p=0.04) and is an independent predictor for shorter RFS (HR 3.38, 95% CI: 2.04–5.60; p<0.001) and PFS (HR 8.79, 95% CI: 1.96–39.4; p=0.005) in all NMIBC tumors and shorter RFS in TaG1-2 (HR 3.48, 95% CI: 1.93–6.27; p<0.001) and T1G3 tumors (HR 6.12, 95% CI: 1.70–22.1; p=0.006)

Abbreviations: uPA, urokinase-type plasminogen activator; tPA, tissue-type plasminogen activator; uPAR, uPA receptor; PAI-1, plasminogen activator inhibitor 1; RFS, recurrence free survival; PFS, progression free survival; OS, overall survival; RR, relative risk; HR, hazard ratio; 95% CI, 95% confidence interval.

Table 3 Overview of the Studies Investigating the Plasminogen Activation System (PAS) on Non-Muscle-Invasive Bladder Cancer (NMIBC) and Muscle-Invasive Bladder Cancer (MIBC)

NMIBC and MIBC Tissue						
Author	**Year**	**Pat. No.**	**Study Design**	**Marker Investigated**	**Source**	**Findings**
Hasui et al[59]	1992	46	not reported	uPA	Tissue	• uPA overexpression is associated with higher tumor grade (p<0.01), tumor stage (p<0.01) and a worse CSS (p<0.005)
Seddighzadeh et al[37]	2002	194	prospective	uPA, uPAR	Tissue	• uPA overexpression is associated with worse CSS (RHR 1.8, 95% CI: 1.0–3.3; p=0.036) and worse OS (RHR 1.9, 95% CI: 1.2–3.0; p=0.006) • uPAR overexpression is associated with higher tumor stage (p<0.001), higher risk for metastasis (RHR 4.0, 95% CI: 1.6–9.9; p=0.003), worse CSS (RHR 2.2, 95% CI: 1.3–4.0; p=0.005) and worse OS (RHR 2.2, 95% CI: 1.4–3.5; p=0.001) • Combination of overexpressed uPA and uPAR is associated with higher risk for metastasis (RHR 3.2, 95% CI: 1.0–10.2; p=0.049), worse CSS (RHR 2.5, 95% CI: 1.3–4.9; p=0.008) and worse OS (RHR 2.7, 95% CI: 1.6–4.7; p<0.001)
El Kott et al[36]	2004	100	retrospective	uPA, uPAR	Tissue	• uPA overexpression is associated with higher tumor stage (p=0.00013), lymph node metastasis (p=0.00011) and distant metastasis (p=0.0015) • uPAR overexpression is associated with higher tumor stage (p=0.0024), lymph node metastasis (p=0.026), distant metastasis (p=0.0017) and is an independent predictor for worse OS (OR 3.1386; p<0.000001)
Gotanda et al[60]	2006	72	retrospective	uPA, PAI-1	Tissue	• uPA overexpression is associated with higher tumor stage (p<0.05)
Dohn et al[30]	2015	149	retrospective	uPAR	Tissue	• uPAR overexpression in cancer cells, macrophages and myofibroblasts is associated with higher tumor grade, higher tumor stage (all p<0.02) and worse OS (HR 2.39, 95% CI: 1.15–5.01; p=0.020) • uPAR overexpression in tumor core was associated with female gender (p=0.01)
Dohn et al[35]	2015	186	retrospective	uPAR	Tissue	• uPAR overexpression in macrophages and myofibroblasts in the tumor core is associated with higher tumor stage and lymph vascular invasion (all p<0.04) • uPAR overexpression in macrophages and myofibroblasts at the invasive tumor front is associated with higher tumor stage, higher tumor grade and concomitant CIS (all p<0.03) • uPAR overexpression in myofibroblasts at the invasive tumor front is associated with lymph node metastasis (p=0.021)
Chan et al[27]	2017	939	retrospective	PAI-1	Tissue	• PAI-1 overexpression is associated with higher tumor grade (65% in high grade vs 49% in low grade disease; p<0.001) • PAI-1 overexpression independently associated with OS (HR 2.58, 95% CI: 1.52–4.38; p=0.0004) in NMIBC • PAI-1 overexpression is independently associated with OS (HR 1.69, 95% CI: 1.09–2.62; p=0.02) in MIBC

(*Continued*)

Table 3 (Continued).

NMIBC and MIBC Tissue						
Author	**Year**	**Pat. No.**	**Study Design**	**Marker Investigated**	**Source**	**Findings**
Janisch et al[38]	2020	272	retrospective	uPA, uPAR, PAI-1	Tissue	• uPA overexpression is associated with lymphovascular invasion (p=0.034), lymph node metastasis (p=0.013), shorter RFS (p=0.004), shorter CSS (p=0.012) and is independently associated with RFS (HR 1.75, 95% CI: 1.02–3.00; p=0.041) • uPAR overexpression is associated with shorter RFS (p=0.005) and shorter CSS (p=0.007) • PAI-1 overexpression is associated with primary muscle invasiveness (p=0.015) and lymphovascular invasion (p=0.024) • The number of overexpressed markers is associated with shorter RFS (p<0.001) and worse CSS (p<0.001) while the combination of 2 overexpressed markers is independently associated with RFS (HR 2.25, 95% CI: 1.19–4.27; p=0.013)

Abbreviations: CIS, carcinoma in situ; uPA, urokinase-type plasminogen activator; tPA, tissue-type plasminogen activator; uPAR, uPA receptor; PAI-1, plasminogen activator inhibitor 1; RFS, recurrence free survival; CSS, cancer specific survival; OS, overall survival; HR, hazard ratio; RHR, relative hazard ratio (adjusted HR for specified covariates); OR, odds ratio; 95% CI, 95% confidence interval.

Association of PAS with Oncologic Outcomes in MIBC

Several studies have investigated the association of tissue PAS components with oncologic outcomes in MIBC. However, the bulk of evidence remains limited to retrospective series.[27,34–36] One of these studies showed an association between uPAR overexpression (in macrophages and myofibroblasts) in the tumor core with existing lymph vascular infiltration (p=0.04, p=0.014, respectively) and at the tumor front with concomitant carcinoma in situ (CIS) (p=0.015, p=0.026, respectively).[35] Patients with metastasis at initial tumor resection showed a significantly increased uPA (p=0.0015)[36] and uPAR (p=0.0017)[36] expression with a 80% probability of metastases (p=0.003).[37] A similar observation was made for preexisting lymph nodes metastasis (uPA p=0.00011; uPAR p=0.026).[36] In terms of cancer-specific survival (CSS), the likelihood of patients dying from their disease was shown to be 64% (p=0.036) higher in case of overexpressed uPA and 69% (p=0.005) higher in case of uPAR overexpression compared to patients with normal expression levels.[37] For OS, the probability of premature death is described as 69% (p=0.001),[37] 71% (p=0.02)[34] and 76% (p<0.000001)[36] higher for uPAR overexpression. With an increased uPA, the probability is 66% (p=0.006)[37] and with an increased PAI-1 63% (p=0.02)[27] higher. Further, in an analysis of 272 patients treated with radical cystectomy, authors investigated the association of uPA, uPAR and PAI-1 with RFS and CSS. On multivariable analysis they found an association of uPA only with RFS (HR 1.75, p = 0.04). Interestingly, uPA overexpression was associated with established adverse prognostic features such as lymph vascular invasion (p = 0.034) and lymph node metastases (p = 0.013).[38] Table 3 provides the findings for MIBC besides NMIBC, from studies where both have been investigated.

Possible Therapeutic Approaches

The association of the PAS components with tumorigenesis, cell proliferation, migration, adhesion and angiogenesis sets the ground for potential therapeutic approaches and development of new drugs that could be used in combination with current standards.

The urinary bladder is a well accessible organ. Therefore, the intravesical administration of topic agents for the treatment of superficial bladder cancer is a widely used approach as it maximizes the local exposure by minimizing systemic effects. Based on this rationale, the effect of intravesical PAI-1 instillation has been investigated in an orthotopic rat bladder tumor animal model. Authors found that the intravesical administration of high concentrated recombinant mutant human PAI-1 reduced the tumor size by 53%. Moreover, 22% of PAI-1 treated tumors invaded the muscle compared to 79% in the control

group.[39] Development of a drug applicable in clinical trials is warranted.

A number of studies have investigated the antitumoral effect of modulating other molecules involved in the PAS cascade.

Oka et al compared the expression levels of mRNA and protease activity of uPA as well as plasmin formation between cell cultures of non-invasive and highly invasive UBC cell lines. Using an in vitro cell invasion assay, the authors evaluated the effects of amiloride, a sodium ion transporter that competitively inhibits the catalytic activity of uPA, and urinary trypsin inhibitor (UTI) on invasion in both cell lines. They observed a substantial decrease in plasmin formation and thus a significantly reduced cellular invasion in both cell lines among amiloride and UTI ($p < 0.001$).[40]

Flavonoids, bioactive compounds extracted from plants, have been shown to influence cancerogenesis, progression, invasion and metastasis.[41] In UBC cell lines, the flavonoids amentoflavone and apigenin have been associate with a reduction in cancer progression and invasion. Amentoflavones have shown an inhibitory effect on tumor progression-related proteins such as VEGF, MMP-2, MMP-9 and uPA/uPAR[42] while apigenin showed a significant suppression of uPAR expression after its application compared to control cells without apigenin treatment ($p<0.05$).[43] They also observed a reduction in the number of invading tumor cancer cells after treatment with apigenin compared to control cells ($p=0.025$). Thus, a promising suppression of tumor progression-related proteins in UBC cells in vitro is shown. Further, epidemiological studies have revealed that a flavonoid-rich diet reduces the risk of developing certain cancers, making these compounds attractive for cancer prevention.[44] Therefore, in vivo studies are needed to further investigate the anti-cancer effects of flavonoids in order to make them available for future clinical practice.

Future Therapeutic Approaches

A promising therapeutic goal is the antagonization of uPA–uPAR interaction by antisense therapy, gene therapy or mainly by immunotherapy.[45] These therapeutic targets have been investigated in human cell lines and animal models. The resulting downregulation of uPAR by antisense and gene therapy has been associated with increased tumor dormancy,[46] reduced tumorigenesis,[47] inhibition of angiogenesis[48] and increased survival[49] in different malignancies. The use of monoclonal antibodies as inhibitors of the uPA–uPAR interaction in various solid tumors have shown to inhibit cell proliferation, invasion, migration and adhesion in vitro. In vivo, they reduced cell proliferation, invasion, tumor count, metastasis, tumor as well as metastasis volume and improved survival.[50–54] Contrary to antisense and gene therapy, which are limited in their practicability due to a difficult delivery and the instability of these vectors, immunotherapy is an already established therapeutic procedure. Therefore, further investigations on the effects of immunotherapy targeting uPAR in UBC cell lines, in corresponding animal models and thereafter in clinical trials are necessary.

Discussion

Through the progressive understanding of the molecular landscape of UBC, a number of candidate biomarkers have emerged. In this context, the association of PAS components with pathologic features and clinical outcomes has been investigated in several series showing promising results. However, the current body of evidence is mainly based on preclinical trials and retrospective series.

uPA and uPAR have shown to be associated with tumor cell adhesion, migration and metastasis by supporting angiogenesis.[21–23] PAI-1 is mainly associated with tumor cell differentiation and is partially involved in cell migration and adhesion by its binding to ECM proteins.[27,55] While the overexpression of PAS components is in general associated with higher tumor grade and stage, the literature presents conflicting results regarding the single PAS components.[27,28,31,32,34–38] Explanations for this are manifold and are mainly inherent to the retrospective design of clinical studies. First and foremost, there is a case mix in clinical studies including UBC across all tumor stages. Second, there is a variety of different antibodies used for staining and the cut-off values for positivity are arbitrary set. Third, sample selection, collection, storage and handling are not standardized, even within studies. Fourth, clinical factors such as age, preexisting disease, medication or nutrition, which could influence the expression of these proteins, are not consistently evaluated. Finally, tumor heterogeneity leads to different results dependent of the region analyzed.[30] Altogether, limits the reproducibility and validation of the studies and, therefore, the applicability of PAS components as biomarkers in clinical routine.

In the era of personalized medicine, several aspects in translation from biomarker discovery to clinical applicability should be considered. It is highly unlikely that the use

of a single biomarker will be sufficient to predict the behavior of an individual malignancy. In several studies, it has been shown that the use of several biomarkers is superior to a single one.[14,16,56,57] However, before a biomarker is clinically useful, it needs to be demonstrated that adding a biomarker to an existing model, based on the most important clinical and pathological factors, significantly improves its predictive accuracy.[18,58] Further, before such a model can be used for individual patient care in diagnosis or treatment, its true clinical implications must be investigated in prospective and well-designed studies.

Possible therapeutic approaches to PAS in UBC have only been sparsely investigated so far. Most of the current knowledge is based on in vitro studies that have not been further investigated in vivo. Only the investigation of intravesical PAI-1 instillation[39] and the use of monoclonal antibodies targeting uPAR[50–54] in animal models were performed in vivo and yielded interesting results.

Conclusion

The tissue expression of PAS components has shown to be associated with adverse pathological features and outcomes in UBC. However, these findings are based on a low level of evidence, making its further exploration in prospective trial necessary to translate the PAS components to clinically applicable biomarkers and therapeutic targets.

Abbreviations

PAS, plasminogen activation system; UBC, urinary bladder carcinoma; NMIBC, non-muscle-invasive bladder cancer; MIBC, muscle-invasive bladder cancer; uPA, urokinase-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor; PAI, plasminogen activator inhibitor; IHC, immunohistochemistry; OR, odds ratio; RR, relative risk; HR, hazard ratio; RFS, recurrence-free survival; PFS, progression-free survival; OS, overall survival; CSS, cancer-specific survival; CIS, carcinoma in situ; UTI urinary trypsin inhibitor.

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