The Urokinase Plasminogen Activator System in Human Cancers: An Overview of Its Prognostic and Predictive Role

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Abstract

Keywords
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► extracellular matrix
► cancer biomarker
► metastasis
► oncotherapy

Urokinase plasminogen activator (uPA) system regulates extracellular matrix remodelling by activating ubiquitous protease plasmin in many important physiological processes. The system components include uPA, plasminogen activator inhibitors (PAIs) and uPA receptor (uPAR). Besides its role in physiological processes, uPA system is active in most tumour types where its aberrant regulation has been associated with the development of metastatic phenotype. In vitro and in vivo studies have shown that the over-expression of uPA, PAI-1 and uPAR not only enhances tumour cell invasion capacity and metastasis, but also corresponds to a higher risk of disease correlating with traditional clinicopathological features which makes them potential prognostic biomarkers and therapeutic targets in a wide range of human malignancies. This review focuses on uPA system's prognostic and predictive role in several types of human cancers, summarizing its activities in cancer development and highlighting the importance of addressing all unanswered questions before bridging the gap between laboratory findings to clinic use of uPA system's components as cancer biomarkers.

Introduction

The urokinase plasminogen activator (uPA) system, active in most tumour types, is a system that controls extracellular matrix (ECM) degradation by activating the ubiquitous protease plasmin.[1–5] The key components of uPA system (uPAS) are uPA, plasminogen activator inhibitor-1 and -2 (PAI-1, PAI-2) and uPA-associated receptor (uPAR), all of which play an essential role in the remodelling of ECM following various physiological processes such as embryogenesis, wound healing and post-lactation involvement of breast tissue.[6–10] Plasminogen is cleaved and activated to plasmin by several proteases, but uPA, a highly specific and substrate-restricted extracellular serine protease,[11,12] is the most important plasminogen activator.[13] uPAS regulation is strict and cell-specific involving the regulation of proenzyme, its inhibitors and receptor on several levels; through controlled transcription of individual system elements, degradation of their messenger ribonucleic acid (mRNA) and translation into proteins.[14–19] The amount of uPA is precisely regulated through its interaction with inhibitors and uPAR. When uPA binds to uPAR, its activity becomes localized on the cell surface.[4] On the other hand, if it reacts with uPAR as uPA/PAI complex, the whole structure undergoes endocytosis. Such translocation is dependent on the interaction with specific endocytosis receptors, like low-density lipoprotein receptor-related protein-1 (LRP-1).[5] After endocytosis, uPA/PAI complex is degraded, while the uPAR returns to the surface (►**Fig. 1**).[20–23] In addition to its plasminogen-activating role, uPAS also stimulates many downstream signalling pathways and the regulation of the entire system is cell-specific.[22,24]

Participation in cell signalling is achieved through binding of uPA to uPAR and subsequent interaction of that complex with other proteins. uPAR, consisting of three homologous domains (DI, DII and DIII), is located on the plasma membrane and acts as
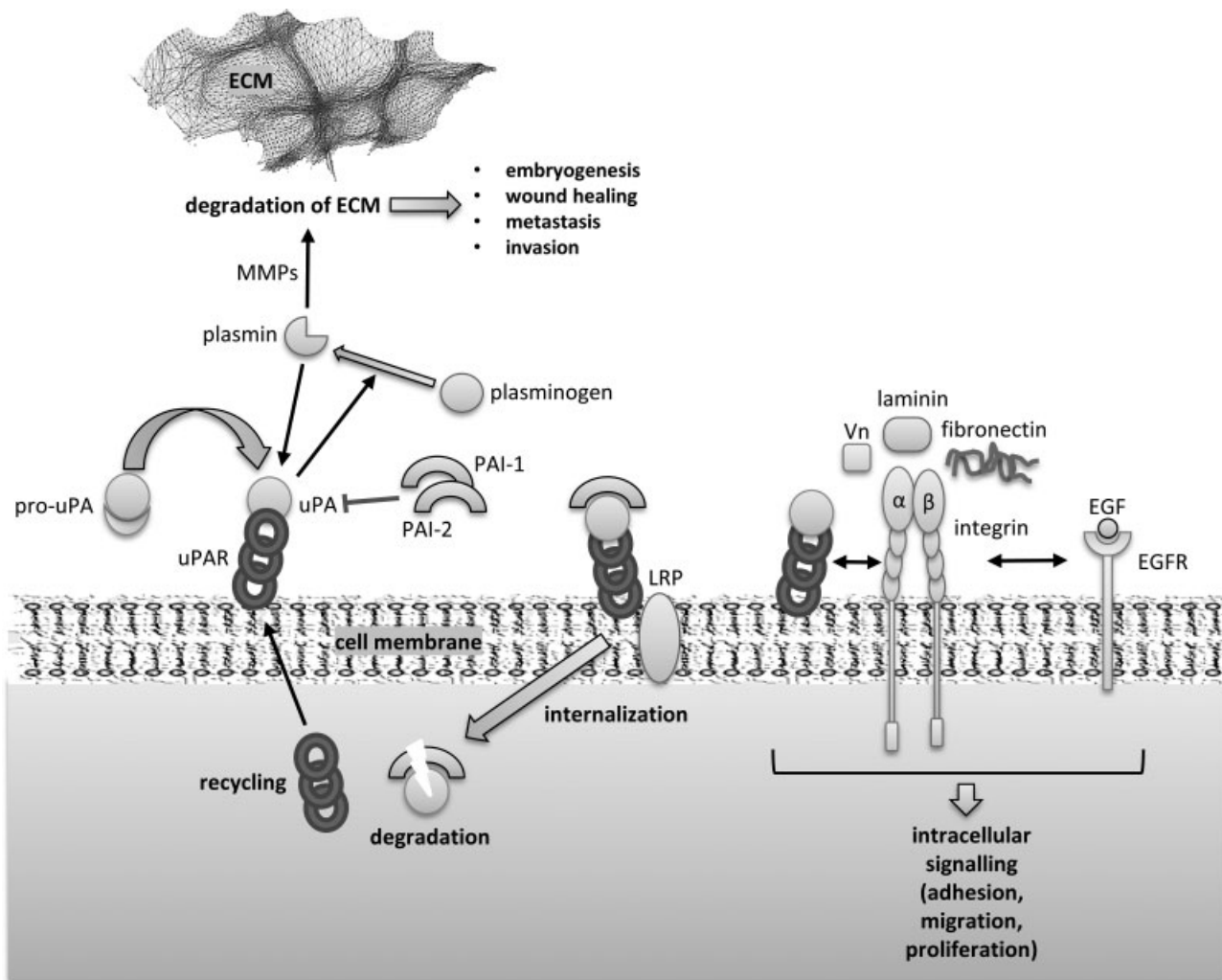
Fig. 1 The uPA system. The scheme of uPA system with its main components: uPA, inhibitors PAI-1, PAI-2 and receptor uPAR. Plasminogen is cleaved and activated to plasmin by uPA which facilitates ECM remodelling in important physiological processes as well as in pathological processes of cancer development. The amount of uPA is regulated through its interaction with inhibitors and uPAR. When uPA binds to uPAR, its activity is localized on the cell surface. In contrast, interaction of uPA/PAI complex with uPAR and proteins like LRP causes the whole structure to internalize and degrade while uPAR recycles. Association of uPAR with its interacting partners mediates intracellular signal transduction in processes of cell proliferation, adhesion, migration and survival (see the "Introduction" section for detailed description). ECM, extracellular matrix; EGF, epidermal growth factor; LRP, low-density lipoprotein receptor-related protein; MMPs, matrix metalloproteinases; PAI-1, plasminogen activator inhibitor; uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor; Vn, vitronectin.

a signalling receptor, but due to a lack of transmembrane and intracellular domains, it is unable to directly mediate intracellular signalling without interacting with other proteins.[11] Its interacting partners are not limited to uPA but include membrane proteins like certain integrins (i.e. β1, β2 and β3), G protein-coupled chemotaxis receptors, LRP, caveolin, insulin-like growth factor receptor (IGFR), epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor and other receptor tyrosine kinases, as well as ECM components like vitronectin (Vn), fibronectin and collagen.[9,12,25,26]

Integrins are αβ-heterodimeric transmembrane receptors which connect ECM components to cytoskeletal proteins. They are responsible for cell–ECM adhesion and have been shown to be an important signalling co-receptors of uPAR.[24] uPAR interacts with integrins through binding sites located on its two domains: DII and DIII. uPAR's ability to regulate integrin activity is crucial for the processes of cell prolifera-

tion, adhesion, migration and survival[23,27] (►**Fig. 1**). Studies have shown that uPAR exhibits a strong affinity for α5β1 (fibronectin receptor), α3β1 (laminin receptor) and for αvβ5 and αvβ3 (Vn receptors) integrins when it is bound to uPA due to stabilization of its active conformation.[22,27,28] Association of uPAR with α5β1 integrin induces cell growth by recruiting EGFR and activating extracellular signal-regulated kinases (ERKs).[5] Moreover, integrins steer uPAR signalling: interaction with β1 integrins triggers proliferation by activating focal adhesion kinase (FAK) and ERK, while the uPAR-β3 complex activates Rac Rho GTPase and induces cell migration. Vn is an adhesive ECM protein which is another important ligand for uPAR. In its multimeric conformation, it is able to interact with other proteins of ECM and integrin receptors. It has a different uPAR-binding site than uPA which enables uPAR to simultaneously interact with both, uPA and Vn.[29]

Furthermore, the components of uPAS have been found to be over-expressed in cancer cells and the surrounding tissue,[30,31] where their aberrant regulation is associated with metastatic capacity and poor clinical outcome.[4,9,25] The process of cancer metastasis corresponds to the growth of a tumour at a site not adjacent to the primary tumour. To acquire invasive and metastatic properties, tumour cells have to pass through cellular and ECM barrier.[28,32] An initial step that precedes this is local invasion consisting of extensive degradation of ECM (proteins like fibronectin) surrounding the tumour, which allows tumour cells to detach from an original site, enter blood or lymph circulation and spread to other organs or tissues in the body.[33–35] Many studies have reported that tumour cells secrete increased levels of several proteolytic enzymes which facilitate tumour invasion and metastasis.[36] Among those enzymes, uPA and matrix metalloproteinases (MMPs) are the most essential proteases in proteolytic cleavage of ECM.[37,38]

Surprisingly and contradictory to its uPA-inhibiting function, PAI-1, a serine proteinase, has been shown to positively regulate tumour development by promoting cell migration, enhancing angiogenesis and enabling apoptosis evasion in certain tumour types.[11,29] uPAR signalling activities are regulated by PAI-1, which is another uPAS component capable of interacting with Vn and integrins.[5,23,39] It has been shown that PAI-1 interferes with α5β3 integrin–Vn interaction which attenuates cell migration. Binding of PAI-1 to Vn can also contribute to the metastasis of cancer cells by blocking the uPAR- and integrin-dependent attachment of cells to Vn, which detaches cells from ECM.[40] Interestingly, in a complex with uPA, PAI-1 loses the affinity for Vn and migration inhibitory action.[41] Furthermore, the interaction of Vn and PAI-1 maintains inhibitor's active state.[5] Studies have also observed elevated uPAR expression following the processes of inflammation and tumour growth. Some authors even suggest that tumour cells assimilate uPAR signalling to further their metastatic potential. Upon uPA binding, full-length uPAR is cleaved and shed from cell membrane, releasing its soluble forms (suPAR), which are also considered to be cancer biomarkers.[42] Many studies have shown that cells in the invasive fronts of several cancer types exhibit over-expressed uPA and uPAR. In vivo and in vitro studies have demonstrated the importance of uPAS in both proteolytic and non-proteolytic processes during cancer development such as tumour cell proliferation, adhesion and migration, intravasation and extravasation, metastasis and neo-angiogenic growth.[1,30,43]

Clinically relevant cancer biomarkers are invaluable in improving patient's outcome and in selecting an appropriate therapy. To be applicable in clinical medicine, biomarker has to meet certain requirements (for more details, see reviews[11,40]). A prognostic biomarker is a biological or clinical characteristic which informs about patient's outcome (prognosis), in the absence of any therapy.[44] In contrast, predictive biomarker is able to predict a difference in benefit from a specific therapeutic intervention.[45,46] The prognostic importance of uPAS was first suggested by Duffy et al in 1988,[47] when the authors showed that uPA activity in breast cancer correlated with tumour size and number of axillary nodes with metastases. Afterwards, additional studies have reported that besides uPA,

PAI-1 is also prognostically relevant, particularly in breast cancer patients.[39,40] Determination of both factors, uPA/PAI-1 (both low vs. high uPA and/or PAI-1), has been proved to be superior to assessing a single factor or using conventional prognostic markers for patient risk-group stratification.[48] Various retrospective and prospective studies, including multicentric clinical trials (Chemo N0[10,49] and NNBC-3[9,50]) and pooled meta-analyses,[51] have since validated the prognostic and predictive value of uPA and PAI-1 at the highest level of evidence (LOE-1[11]) in breast cancer[4,9,23] (for a summary of clinical milestones see Schmitt et al[49]). Interestingly, unlike any other cancer biomarker, there are no contradictions regarding the prognostic relevance of uPA/PAI-1 in breast cancer.[23,39] Furthermore, predictive information obtained from uPA/PAI-1 status can support individualized therapy selection and is being recommended and used routinely for treatment decision, particularly in node-negative breast cancer.[10,39,52]

From the available literature, it is evident that most of the research on the clinical relevance of uPA and PAI-1 has been performed in breast cancer.[49] Nowadays, uPAS is considered as a potential prognostic and predictive factor in many different human cancers (►Table 1), including those of breast, lung, bladder, stomach, intestine, uterus, kidney, thyroid, head and soft tissue (previously reviewed by Ulisse et al[3] and Schmitt et al[40]). The aim of this article is to present an overview of recent research focusing on the role of uPAS in the development of human cancers.

Methods

The PubMed (www.ncbi.nlm.nih.gov/pubmed) database was searched on 13 November 2017 for all articles published in English in the past 10 years using the search term "cancer" AND "(uPA OR urokinase)" and applying filter Search Field "Title." Resulting 272 articles were afterwards methodically filtered in the next 3 weeks based on their relevance, redundancy in data, publication in respected journals and after reading them all and focusing on the ones reporting of uPAS activities in cancer development—reduced to 144 articles reviewed here in this article.

The Role of uPA System in Cancer Invasion and Metastasis

Breast Cancer

As mentioned previously, it was in breast cancer where the relationship between uPAS and tumourigenesis and metastasis was first proposed.[47] The authors have studied the connection between uPA in primary breast carcinomas and different prognostic parameters and found that uPA activity was associated with the stage of disease and axillary node metastasis status. Also, primary carcinoma patients with observed high levels of uPA activity had much shorter disease-free survival (DFS) than the ones with low uPA activity. Furthermore, by performing (multivariable) analyses, previous clinical studies revealed that, aside from lymph node status, uPA and PAI-1 had the strongest independent prognostic effect on DFS and overall survival (OS).[51,53]

Table 1 Activities of uPA system components in human cancers

Cancer type	uPA system component	Observed effect	Study type	References
Breast	uPA, uPAR, PAI-1, -2	uPAS components correlate with traditional prognostic factors in patients	In vivo	56,61,62,64
	uPA, PAI-1	uPA and PAI-1 are independent prognostic markers for DFS and OS	In vivo	51,53,58
	uPA, PAI-1	uPA and PAI-1 are predictive factors used in therapy decision	In vivo	10,39,52,72
	uPAR	Splice variant uPAR-del4/5 is an independent prognostic marker for DFS, DMFS and OS	In vitro/In vivo	38,42,63
	uPAR	uPAR-del4/5 over-expression reduces breast cancer cell adhesion and invasion	In vitro/In vivo	28,66
	uPA, uPAR	uPA down-regulation and uPAR signalling inhibition reverse EMT	In vitro	69
	uPAR	uPAR signalling induces CSC-like properties in breast cancer cells	In vitro/In vivo	70
	uPA	miRNAs target uPA and modulate invasion of breast cancer cells	In vitro/In vivo	78,79
	uPAR	miR-221 targets uPAR7b and up-regulates its protein expression in breast cancer cells	In vitro	80
	uPA	Reduction of uPA expression via NF-κB inactivation inhibits invasion of cancer cells	In vitro	81
	uPA, PAI-1	Active TGF-β signalling correlates with high expression of uPA and PAI-1 in cancer tissue	In vivo	82
	uPA, PAI-1	uPA/PAI-1 prognostic value is primarily detected in HER2-positive breast cancer patients	In vivo	59
	uPA, PAI-1	PAI-1 expression determination improves the prognostic value of tumour size in patients	In vivo	60
	uPAR	Cleaved uPAR is better biomarker than elevated uPAR expression in breast cancer-bearing mice	In vivo	65
	uPAR	Interactions of uPAR with uPA and IGFR-1 stimulate migration and invasion in cancer cells	In vitro	26
	uPAR	Cysteine-rich angiogenic inducer-61 interacts with uPAR in breast cancer cell invasion	In vitro/In vivo	25
	uPA	Concurrent suppression of uPA and MMP-9 down-regulates migratory capacity of cancer cells	In vitro/In vivo	71
	uPAR	Depletion of uPAR from cancer cell membranes inhibits invasion and slows tumour growth	In vitro/In vivo	67
	uPA, PAI-1	Inhibition of uPA activity accompanied with TGF-β activation arrests cancer cell growth	In vitro	83
Prostate	uPA	Demethylation-associated induction of uPA contributes to development of prostate cancer	In vivo	19
	uPA, uPAR, PAI-1, -2	uPAS components correlate with main prognostic markers in prostate cancer patients	In vivo	84,87,88
	uPA, uPAR	Suppression of uPA/uPAR inhibits prostate cancer cell growth and migration	In vitro	92
	uPAR	Serum uPAR is associated with cancer extra-prostatic extension in cancer patients	In vivo	85
	uPAR	Anti-uPAR antibody reduces cancer cell invasion and decreases xenograft tumour volume	In vitro/In vivo	93
	uPAR	Intact and cleaved uPAR correlate with OS in prostate cancer patients	In vivo	86
	uPA, uPAR	uPA$^{-/-}$ and uPAR$^{-/-}$ mice exhibit reduced xenograft tumour development	In vivo	91

(*Continued*)

Table 1 (*Continued*)

Cancer type	uPA system component	Observed effect	Study type	References
	uPA, uPAR	Protein kinases D2/3 induce cancer cell invasion via p65 NF-κB-associated activation of uPA	In vitro	35
	uPA, PAI-1	uPA/PAI ratio distinguishes prostate cancer and benign prostatic hyperplasia	In vivo	89
	uPA, uPAR	Snail over-expression increases cancer cells invasion through uPA and uPAR up-regulation	In vitro	96
	uPA, PAI-1	Increased methylation of PAI-1 promoter leads to an increased proteolysis in cancer cells	In vitro/In vivo	90
	uPA	CFTR over-expression inhibits uPA through miR-193b induction	In vitro/In vivo	97
	uPA	ETV4 inactivation supresses uPA expression and inhibits invasion of prostate cancer cells	In vitro	94
	uPA	Aspirin down-regulates uPA expression and decreases cancer cell invasion through NF-κB	In vitro	95
Colorectal	*uPA, PAI-1*	uPA and PAI-1 levels are better prognostic factors than commonly used CRC markers	In vivo	36
	uPA, PAI-1	uPA and PAI-1 prognostic value is different in colon and rectal cancers	In vivo	98
	uPAR	suPAR forms are up-regulated in CRC patients and correlate with CRC metastasis	In vivo	100–102
	uPAR	uPAR antibody suppresses CRC cell migration and reduces tumour growth in xenograft model	In vitro/In vivo	109
	uPA, uPAR	Lipopolysaccharide induces CRC cell invasion by stimulating uPA and uPAR	In vitro	32
	uPA	GATA6 induces CRC cell invasion through Sp1-associated activation of uPA promoter	In vitro	34
	uPA, uPAR	uPA is predictive marker for MMR-proficient CRC correlating with pT stage and OS	In vivo	99
	uPA, PAI-1	uPA and PAI-1 levels are associated with tumour budding in colon cancer	In vivo	111,112
	uPA	Stromal cell-derived factor-1 induces uPA via different pathways in CRC cells	In vitro	106
	uPA, uPAR	uPAR mRNA levels correlate with CRC stage	In vivo	103
	uPAR	uPAR-positive macrophages are markers of poor OS in CRC patients	In vivo	104
	uPAR	Inactivation of uPAR promotes TRAIL-mediated apoptosis in colon cancer cells	In vitro	107
	uPA	uPA inhibition mediated by 3,3′-diindolylmethane suppresses CRC cell invasion	In vitro	108
	uPAR	uPAR is differently expressed in epithelium and stroma-related rectal cancer cells	In vivo	105
Gastric	*uPAR*	Elevated uPAR by lysophosphatidic acid induces the invasiveness of AGS cancer cells	In vitro	113
	uPAR	Invasive cancer cells over-expressing uPAR are a predictive factor for poor OS	In vivo	117
	uPA, uPAR, PAI-1	Expression of uPAS correlates with peritoneal metastasis in gastric cancer patients	In vivo	118,119
	uPA	Semaphorin 5A-mediated uPA increase enhances the invasiveness of gastric cancer cells	In vitro	116
	uPAR	Cadmium induces migration and invasion of AGS gastric cancer cells by up-regulating uPAR	In vitro	114

Table 1 (*Continued*)

Cancer type	uPA system component	Observed effect	Study type	References
	uPA	uPA together with VEGF expression possesses a prognostic value in invasive gastric cancer	In vivo	120
	uPA	Elevated uPA is linked to gastric cancer stage and negatively correlates with OS	In vivo	121
	uPA	uPA in cancer-associated fibroblasts stimulates migration of oesophageal carcinoma cells	In vitro/In vivo	122
	uPAR	Prostaglandin E_2 increases gastric cancer cell invasiveness by up-regulating uPAR expression	In vitro	115
	uPA, uPAR	Quercetin suppresses cancer cells migration by inhibiting uPA and uPAR expression	In vitro/In vivo	123
Ovarian	*uPAR*	Cleaved uPAR forms are differentiating factors between benign and malignant ovarian cancer	In vivo	128
	uPA, uPAR	uPA and uPAR are over-expressed in cancer cells and correlate with other prognostic factors	In vitro/In vivo	130
	uPA	Co-expression of uPA and MDR-1 correlates with the invasion of ovarian cancer	In vitro/In vivo	131
	uPA	Basic fibroblast growth factor stimulates cancer cell progression by up-regulating uPA expression	In vitro	124
	uPAR	uPAR inhibition supresses the invasion of cancer cells and reduces xenograft tumour development	In vitro/In vivo	125
	uPA, PAI-1	uPA and PAI-1 levels are higher in malignant than in benign ovarian cancers	In vitro/In vivo	126
	uPA	Leptin-mediated uPA over-expression contributes to ovarian cancer cell migration and invasion	In vitro	127
Lung	*uPAR*	Intact and cleaved forms of uPAR are significant prognostic factors in lung cancer patients	In vivo	132–134
	uPA	uPA expression is crucial for invasion induction in A549 lung cancer cells by peroxiredoxin-6	In vitro	135
	uPA, uPAR	uPA and uPAR SNPs have a prognostic value for the predisposition and stage of NSCLC	In vivo	138
	uPA	Kappa elastin induces lung cancer cell invasion by stimulating uPA secretion	In vitro	136
	uPA, uPAR	NHE-1 plays a crucial role in uPA/uPAR-mediated metastasis of NSCLC cells	In vitro/In vivo	137
Pancreas	*uPAR*	Hypoxia-activated HIF induces uPAR, enabling angiogenesis and invasion of cancer cells	In vitro/In vivo	139
	uPA, uPAR	uPA and uPAR suppression weakens metastatic capacity of pancreatic cancer cells	In vitro/In vivo	140
	uPA, PAI-1, uPAR	PARK-7 controls pancreatic cancer cell invasiveness through uPAS	In vitro/In vivo	143
	uPA	uPA induces stem-like characteristics to pancreatic cancer cells	In vitro/In vivo	141
	uPA	Polyserase-1 enhances PaCC invasion by stimulating active uPA conversion	In vitro	142
	uPAR	uPAR expression correlates with OS in pancreatic adenocarcinoma patients	In vivo	144

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; CRC, colorectal cancer; CSC, cancer stem cells; DFS, disease-free survival; DMFS, distant metastasis-free survival; EMT, epithelial-mesenchymal transition; HER2, human epidermal growth factor receptor 2; HIF, hypoxia-inducible factor; IGFR, insulin-like growth factor receptor; MAPK, mitogen-activated protein kinase; MDR-1, multiple drug-resistance-1; miRNA, micro ribonucleic acid; MMP, matrix metalloproteinase; MMR, mismatch repair; mRNA, messenger ribonucleic acid; NF-κB, nuclear factor kappa B; NHE-1, sodium hydrogen exchanger isoform-1; NSCLC, non-small cell lung cancer; OS, overall survival; PaCC, pancreatic cancer cells; PAI, plasminogen activator inhibitor; PARK-7, Parkinson's disease-associated protein-7; PI3K, phosphatidyl-inositol-3 kinase; SNP, single-nucleotide polymorphism; suPAR, soluble urokinase plasminogen activator receptor; TGF-β, transforming growth factor-β; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand; uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor; uPAS, urokinase plasminogen activator system; VEGF, vascular endothelial growth factor.

A particularly aggressive type of breast cancer cells are so-called triple-negative breast cancer (TNBC) cells,[50] which do not express oestrogen and progesterone receptors or human EGFR 2 (HER2), making them unsusceptible to targeted therapy.[54,55] Studies have shown that uPAS components were over-expressed in this sub-class of breast cancer cells.[56] Metastatic breast cancer cells, such as TNBC MDA MB-231, are known to express higher levels of uPA and uPAR at cell surface enabling them to bind and activate larger amount of plasminogen.[57] A study by Stillfried et al[13] confirmed uPA as a crucial facilitator of breast cancer progression. The authors analysed plasminogen binding and activation at the surface of two breast cancer cell lines: MDA MB-231 and MCF-7, which differed in uPAR/uPA expression. Their results revealed that plasmin activity promoted plasminogen binding on the surface of breast cancer cells and was reliant on the presence of active uPA. Moreover, it was shown that increased uPA expression on the surface of metastatic breast cancer cells, such as MDA MB-231, up-regulated plasmin activation which in turn enhanced their invasive proteolytic capacity.

After the long-term follow-up of lymph node-negative (LNN) breast cancer patients, Zemzoum et al[58] have shown that uPA/PAI-1 levels were significantly associated with tumour aggressiveness, independently of HER2 status which was evaluated as well. Among conventional biomarkers, uPA/PAI-1 status has been reported to be the only independent prognostic factor for DFS in uni- and multivariate analyses. Regarding the OS, a significant effect of both uPA/PAI-1 and HER2 status has been observed suggesting the complementary clinical use of both factors. In another study, Witzel et al[59] evaluated the prognostic value of uPA and PAI-1 mRNA levels in molecular sub-types of breast cancer. The authors have observed a discrepancy in the prognostic role of uPA/PAI-1 mRNA in different sub-types. A meaningful prognostic value was primarily detected in patients with HER2-positive cancers, where the (uni- and multivariate) analysis showed a strong prognostic association between elevated uPA/PAI-1 and shorter DFS. Similarly, a recent study suggested that PAI-1 expression determination can improve the prognostic value of tumour size in post-menopausal, LNN breast cancer patients and thus differentiate patients with low- and high-risk of disease recurrence during an early follow-up.[60] Furthermore, a survey of 606 primary breast cancer patients established that the patients with high uPA and PAI-1 expression had larger tumours, of higher malignancy grade, capable of ductal invasion, but hormone-independent.[61] Authors also observed that there was no substantial correlation between uPA and age or menopausal status. In another study, according to both uni- and multivariate DFS analyses, same group demonstrated that uPA/PAI-1 expression represented independent prognostic value irrespective of HER2 status in LNN breast cancer patients during the long follow-up period of 8 years.[62]

Various forms of uPAR, differently cleaved or having diverse splicing, have been reported previously.[1,3] By using univariate analysis, Luther et al[42] discovered a significant association between higher expression of novel uPAR splice variant, uPAR-del4/5 (lacking exons 4 and 5) and shorter DFS in breast cancer patients. At the same time, wild-type uPAR

mRNA did not show such association with DFS which suggested a novel prognostic role of uPAR-del4/5 variant. These results were confirmed in a larger cohort of breast cancer patients using both uni- and multivariate analyses which showed that high uPAR-del4/5 level was an independent marker for shorter DFS.[38] Additionally, low expression of tissue inhibitor of MMP-3 (TIMP-3) was significantly associated with similar poor prognosis. By combining these two independent prognostic factors, authors showed that patients with both high uPAR-del4/5 and low TIMP-3 had significantly shorter DFS compared with other patient sub-groups. Another multivariate analysis revealed that the expression of uPAR-del4/5 and associated gene rab31 (Ras oncogene family) had a significant and independent prognostic impact on prognosis of untreated LNN breast cancer patients.[63] Concurrent analysis of both factors showed that patients with high levels of uPAR-del4/5 and rab31 mRNA exhibited the worst distant metastasis-free survival (DMFS) and OS. In another study, an assessment of the role of uPAS in women with primary breast cancer revealed that the levels of uPAR were significantly higher in breast cancer patients, with positive correlation with other prognostic factors such as the stage of disease and the size of the primary tumor.[64] Interestingly, the cleavage of uPAR has been recognized as a more specific cancer biomarker than the elevated expression of intact uPAR in breast cancer-bearing mice.[65] Cleaved uPAR forms exhibited a significant correlation to tumour volume and were not affected by the depletion of uPA indicating the presence of another uPAR-cleaving proteases.

An in vitro study of MDA MB-231 TNBC transfected with uPAR-del4/5 showed that the over-expression of this receptor variant led to uPA-independent inhibition in cell adhesion and invasion.[28] Moreover, an in vivo xenograft model demonstrated uPAR-del4/5-mediated suppression of breast cancer cell metastasis. Additional study by the same group demonstrated that the over-expression of uPAR-del4/5 diminished the invasive potential and adhesion of breast cancer cells in a dose-dependent manner.[66] As these effects were contrary to the activity of endogenous receptor, it has been suggested that uPAR-del4/5 could, by competition, adversely regulate wild-type uPAR activity. Aside from its over-expression, interactions of uPAR with uPA and IGFR-1 have also been reported to stimulate migration and invasion in TNBC cell lines.[26] The observed effect was annulled when the uPAR and uPA were silenced. A recent study by van Veen et al[67] investigated the mechanism of uPAR shedding from cell surface and identified a specific phospholipase, glycerophosphodiesterase-3 (GDE3), which was able to cleave and inactivate uPAR. Furthermore, over-expression of GDE3 exhausted uPAR levels on the membranes of MDA MB-231 breast cancer cells making them less invasive as evidenced by slower tumour development in mice xenograft model.

Epithelial-mesenchymal transition (EMT) is an important process in development but is also involved in cancer metastasis.[29,68] Previous studies have reported of hypoxia-induced EMT in cancer cells via uPAR cell signalling activation. Induction of EMT was also observed in cancer cells over-expressing uPAR. A study by Jo et al[69] examined if uPAR could be targeted to

reverse EMT in uPAR-over-expressing breast cancer cells. Their findings demonstrated reversal of EMT which was caused by down-regulation of endogenous uPA or through uPAR-activated cell signalling inhibition. The same research group suggested that the appearance of cancer stem cell (CSC)-like properties in breast cancer cells was connected to uPAR signalling. This was further confirmed by the development of mammospheres when uPAR was over-expressed in breast cancer cells.[70] A study by Moirangthem et al[71] observed that concurrent suppression of uPA and MMP-9 down-regulated migratory and invasive capacity of breast cancer cells followed with an increase in the expression of epithelial marker E–cadherin and down-regulation of mesenchymal markers, Snail and vimentin. The observe change in EMT genes was reminiscent of the expression modulation taking place in the mesenchymal–epithelial transition. Same effect was detected in vivo as well; human invasive breast tumour tissues displayed high expression of uPA and MMP-9, while the expression of E–cadherin was lower.

On the subject of potential predictive relevance of uPAS, retrospective analyses on a large number of breast cancer patients (for several studied patients see Schmitt et al[4]) have shown that high uPA/PAI-1 antigen levels correlate with an increased response to adjuvant chemotherapy with regard to patient's outcome like DFS.[9,48] Correspondingly, a study by Meijer-van Gelder et al[72] demonstrated that uPA, uPAR and PAI-1 provided predictive information regarding the hormone treatment of oestrogen receptor-positive breast cancer. Elevated expression of uPAS components was associated with a reduced tamoxifen efficacy (univariate analysis), where their values predicted shorter progression-free survival (PFS) in hormone-treated patients. Furthermore, multivariable model showed that uPA was an independent and significant predictive factor for tamoxifen therapy benefit.

As the patients with low uPA and PAI-1 are less likely to relapse and do not benefit from adjuvant therapy, such over-treatment could be avoided there,[11,49,50] even in patients with intermediate risk of recurrence.[52,73] Analysis of Chemo N0 trial showed that, even after extended follow-up (∼10 years), prognostic and predictive value of uPA/PAI-1 was still significant regarding long-term outcome; suggesting the omission of adjuvant chemotherapy in low-uPA/PAI-1 patients and indicating its necessity where needed.[10] By guiding clinicians to unnecessary chemotherapy avoidance, uPA/PAI-1 determination has been shown to significantly reduce costs in breast cancer health care.[74] Furthermore, study by Marguet et al[73] analysed this cost-effectiveness in more detail and elucidated that the use of uPA/PAI-1 testing in guiding chemotherapy decision was not so certain when the age of (node-negative) breast cancer patients was taken into consideration.

In most of the clinical studies, enzyme-linked immunosorbent assay (ELISA) is the standard method for determining uPA/PAI-1 antigen content in tumour tissues.[4,5,9,10,39,48] As the method demands rather large amounts (∼300 mg) of fresh or fresh-frozen tissue,[75,76] other ways of assessing the uPA/PAI-1 status have been explored.[4] Biermann et al[77] analysed the uPA and PAI-1 mRNA expression by quantitative reverse transcription polymerase chain reaction (qRT-PCR) as an alternative to ELISA. Their findings revealed that there was no significant correlation between the mRNA and antigen expression in breast cancer samples which challenges the use of qRT-PCR as a substitute for direct protein detection by ELISA assays. Regarding the ELISA sample quantity requirement, it has been shown that even small tumour samples (10–30 mg), obtained from preoperative core needle biopsies, give enough significant information about the uPA/PAI-1 status for patient stratification.[75] To bypass some of the disadvantages of ELISA, many research have also tried to quantify uPA and PAI-1 from formalin-fixed and paraffin-embedded material, commonly used format for tissue storage. Malinowsky et al[76] successfully extracted uPA and PAI-1 proteins from such samples and confirmed that their expression was comparable to that obtained with ELISA.

It has been reported that different microRNAs (miRNAs), whose abnormal expression has been associated with carcinogenesis, may directly target uPAS components. A research by Li et al[78] described miR-193b acting as a negative post-transcriptional regulator of uPA, and by doing so, supressing invasion of breast cancer cells. Similarly, another study showed that not only miR-193b, but miR-193a and miR-181a also targeted uPA mRNA.[79] In this study, authors observed that diminished miRNA processing led to the increase in uPA expression followed by the enhanced in vitro invasion of breast cancer cells. Furthermore, a decreased concentration of aforementioned miRNAs was detected in breast cancer cells-over-expressing uPA. Depletion of uPA levels attenuated miRNA knockdown-associated invasion in those cells. A study by Falkenberg et al[80] reported that miR-221 directly targets uPAR7b (secreted splicing variant) and up-regulates its protein expression in TNBC cells, an association which could be exploited in future therapies for invasive breast cancer. Potential candidates for breast cancer-targeted therapy are also cysteine-rich angiogenic inducer-61 and the Y-box-binding protein-1, whose interaction with uPAR has been recently identified and associated with TNBC invasion and migration.[25] Furthermore, multivariate analysis revealed Y-box-binding protein-1 as an independent prognostic factor for DMFS.

The promoter region of uPA contains the binding site for nuclear factor kappa B (NF-κB)[2,37] and constitutive activation of RelA, p65 sub-unit of NF-κB, is responsible for the over-expression of uPA in tumours.[14] A study by Zong et al[81] demonstrated that curcumin inhibited invasion of MCF-7 breast cancer cells by reducing uPA expression mediated through NF-κB inactivation. Another signalling pathway associated with uPAS and breast cancer is transforming growth factor-β (TGF-β) pathway. A correlation study using tissue microarray technique in human breast cancer tissues discovered that almost all cancer samples had both active TGF-β signalling and elevated uPA levels indicating a highly significant relationship between these two systems involved in breast cancer invasiveness.[82] Another recent study demonstrated inhibition of the uPA activity in MDA MB-231 breast cancer cells which was associated with the TGF-β pathway.[83] Treatment with non-steroidal anti-inflammatory drug sodium salicylate induced cellular reprogramming characterized by activation of TGF-β pathway elements' and change in uPA/PAI-1 expression ratio which led to breast cancer cell growth arrest.

Prostate Cancer

One of the most diagnosed cancers in men is prostate cancer, and according to statistics, it is a second most frequent cause of cancer-related death among men. Elevated expression of uPA has been found in prostate cancer cells, as well as in neighbouring stroma cells and metastatic lymph nodes.[40] It has been reported that demethylation-associated induction of uPA expression played a role in the development and metastasis of prostate cancer.[19] Human prostate cancer tissue samples exhibited abnormally expressed uPA which was not detected in adjacent tissue or benign prostatic hyperplasia (BPH). Furthermore, a study by Kumano et al[84] showed that almost all uPAS components were associated with main prognostic markers in patients which had undergone radical prostatectomy. Expression of uPA, uPAR and PAI-1 strongly correlated with pathological stage, Gleason grading and lymph node metastasis. Additionally, multivariate analysis established that uPA expression provided independent prognostic information for DFS.[84] Similarly, evaluation of serum uPAR and EGFR in patients suspected to have prostate cancer showed that the up-regulation of serum uPAR was associated with cancer extra-prostatic extension and could be used as a prognostic serum marker.[85] Interestingly, serum levels of both intact and cleaved uPAR have been established to be good prognostic factors which correlated significantly in univariate analysis with OS of prostate cancer patients. Moreover, OS multivariate analysis identified uPAR(I–III) + uPAR(II–III) as an independent prognostic factor in androgen-blocked patients.[86] Also, suPAR has been prognostically associated with shorter OS (in both uni- and multivariate analyses) in prostate cancer patients where high serum suPAR corresponded to multi-fold increase in death risk.[87] The same authors broadened the research on suPAR and included miR-375 to analyse their combination as possible prostate cancer prognostic marker.[88] Their uni- and multivariate survival analyses revealed a strong link between high suPAR/miR-375 levels and poor OS in patients which suggests that a specific combination of miRNA and protein could be a valuable prognostic biomarker in prostate cancer. Another study has reported that PAI-1 protein concentrations were considerably higher than uPA concentrations in prostate cancer and BPH tissue sections. Since the resulting uPA/PAI-1 ratios were distinctly higher in prostate cancer samples, it was suggested that uPA/PAI ratio could be used as differentiating marker between prostate cancer and BPH.[89] Similarly, Hagelgans et al[90] observed a change in the ratio of uPA/PAI-1 expression which corresponded to increased proteolysis in prostate cancer cells. One of the mechanisms behind such expression ratio shift was PAI-1 down-regulation due to increased methylation of its promoter, detected both in invasive prostate cancer cell lines (DU-145 and LNCaP) and prostate tumour samples.

Tumour-associated macrophages stimulate invasion, migration and metastasis in tumour microenvironment. As uPA and uPAR participate in macrophage chemotaxis, Zhang et al[91] tested the link between uPA/uPAR and tumour-associated macrophages in prostate cancer. Their results revealed that RM-1 prostate cancer cell xenograft tumour development was significantly reduced in uPA$^{-/-}$ and uPAR$^{-/-}$ mice with fewer tumour-penetrating macrophages when compared with control mice. An in vitro study revealed that suppression of uPA and uPAR diminished cell growth and migration by inhibiting vascular endothelial growth factor (VEGF) and MMP-9 expression in prostate cancer cell lines.[92] PC-3 prostate cancer cells incubated with monoclonal anti-uPAR antibody exhibited reduced invasion and migration due to the inactivation of FAK, Akt and mitogen-activated protein kinase (MAPK) signalling pathways. Moreover, treatment with uPAR antibody decreased tumour volume and attenuated skeletal lesions in in vivo models.[93] In another study, PC-3 cells exhibited diminished migration and invasion caused by inhibited uPA expression due to the inactivation of Ets transcription factor ETV4.[94]

Constitutive activity of NF-κB pathway is one of the mechanisms behind prostate cancer metastasis. Protein kinases D2 and D3 were suggested to induce prostate cancer cell invasion via p65 NF-κB-associated activation of uPA. Suppression of kinases down-regulated uPA and uPAR levels and concurrently increased the expression of PAI-2.[35] It has been recently shown that aspirin down-regulates uPA expression, which has led to a decrease in prostate cancer cell invasion.[95] Detected decline in uPA expression was attributed to aspirin-mediated interference of NF-κB activation. In other study, prostate cancer cells transfected with EMT-inducing factor Snail exhibited increased invasion and migration which was a result of Snail-mediated up-regulation of uPA and uPAR levels.[96] As reported previously in breast cancer cells,[78] miRNA-193b targeted uPA and suppressed its expression. A study by Xie et al[97] discovered reduction in prostate tumour development associated with change in miR-193b-mediated modulation of uPA. Specifically, over-expression of ion channel protein cystic fibrosis transmembrane conductance regulator inhibited uPA expression via miR-193b induction in prostate cancer cell lines and xenograft tumours.

Colorectal Cancer

Colorectal cancer (CRC) is the most prevalent type of gastrointestinal cancer, affecting men and women equally. A high CRC-related mortality rate is the consequence of recurring metastases. So far, the most commonly used prognostic markers were carcinoembryonic antigen and the gastrointestinal cancer-associated carbohydrate antigen-19–9. Herszényi et al[36] analysed blood samples from CRC patients and discovered that uPA and PAI-1 levels were better CRC prognostic factors than frequently used serum markers. In addition, PAI-1 contributed independent prognostic information in multivariate statistical analysis for OS. Interestingly, a study designed to analyse uPA and PAI-1 expression in large intestine cancers showed differing prognostic impact of these uPAS components in colon and rectal cancers; with uPA and PAI-1 being associated only with rectal cancer OS (univariate analyses), suggesting that their prognostic value was not generally relevant for all CRC types.[98] Analysing two large CRC patient cohorts, characterized by their mismatch repair (MMR) status, Minoo et al[99] found, by using univariate and multivariable model, that uPA was an independent prognostic marker applicable particularly for MMR-proficient CRC where its up-regulation correlated with tumour stage, invasive margin and OS.

Previous studies have suggested that the amount of cleaved uPAR in tumours corresponds to the activity of uPA and represents a better prognostic value than intact uPAR form. Similar to findings of studies in breast[42,65,67] and prostate cancers,[86,87] various suPAR forms have been shown to be up-regulated in CRC patients and to correlate with colorectal carcinogenesis.[100] Additional study by the same group confirmed that all three suPAR types, suPAR(I-III), suPAR(II-III) and uPAR(I), were independent markers of patient's OS and were more effective CRC prognostic markers as individual forms when compared with their sum, as demonstrated by uni- and multivariable analyses.[101] Recently, the same authors reported that soluble intact and cleaved uPAR (suPAR(I-III) + (II-III)) was a valuable independent prognostic and predictive factor for metastatic CRC, which correlated well with patients' OS, as shown by uni- and multivariate analyses.[102] Low level suPAR patients responded better to conventional chemotherapy than the ones with high suPAR concentrations. A research of Bujanda et al[103] also showed that uPAR was a valuable CRC prognostic factor since its mRNA levels in blood correlated with and accurately diagnosed early-stage CRC. In another study, uni- and multivariate analyses showed that the number of high expressing uPAR macrophages, found in tumour centre, was significantly associated with poor OS of CRC patients.[104] Expression of uPAR in epithelium (uPAR^E) and stroma-related (uPAR^S) rectal cancer cells was reported to be dissimilar suggesting different roles of uPAR in those two cell types.[105] The authors discovered, by using multivariable model, significant and independent correlation between elevated uPAR^E and poor OS in patients with non-metastatic rectal cancer. Contrastingly, uPAR^S was prognostically associated with better OS in nodal-metastatic rectal cancer patients.

An in vitro study discovered that the transcription factor GATA6 induced CRC cell migration and invasion by enhancing uPA expression through Sp1-associated activation of uPA promoter.[34] Similarly, it was reported that stromal cell-derived factor-1 induced uPA expression and secretion by stimulating Sp1 and AP-1 binding on uPA promoter in DLD-1, SW48 and COLO205 CRC cell lines.[106] Moreover, observed uPA up-regulation was connected to the activation of p38-MAPK and phosphatidyl-inositol-3 kinase (PI3K)/Akt pathways. Another study has reported that uPAR inactivation altered the expression of Bcl-2, Bax, Bid and p53 which promoted tumour necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in HCT116 colon cancer cells.[107] A recent study by Jin et al[108] observed a decline in CRC cell invasion and migration, a consequence of uPA and MMP-9 inhibition caused by 3,3′-diindolylmethane.

In another study, treatment with uPAR monoclonal antibody ATN-658 suppressed CRC cell migration and invasion in vitro and caused a significant inhibition of tumour growth in the liver of CRC cell-xenograft model.[109] Bacterial endotoxin lipopolysaccharide has been shown to induce CRC cell invasion by stimulating uPA and uPAR expression in vitro.[32] Observed uPAS activation and subsequent increase in cell migration was attributed to lipopolysaccharide-mediated up-regulation of Toll-like receptor-4 and NF-κB. Tumour budding is a process occurring in some colorectal tumours when tumour cells disengage from the invasive tumour front as single cells or small clusters (up to five cells), to infiltrate blood vessels and establish metastases.[110] A study by Märkl et al[111] observed a significant association between uPA expression and tumour budding in colon cancer. In a recent study, the same research group provided a follow-up of colon cancer patients who were analysed in previous study.[112] Their findings confirmed that uPA and PAI-1 were adverse predictive factors in colon cancer. Contrastingly to previous studies, they observed uPA to have lesser prognostic value than PAI-1, which, according to uni- and multivariate analyses, in tandem with tumour budding correlated independently with distant metastases in patients.

Gastric Cancer

Previous studies have shown an association between uPAS components over-expression and gastric cancer invasion (for an overview of earlier studies see Schmitt et al[40]). A study by Kim et al[113] reported that elevated uPAR expression by lysophosphatidic acid treatment induced invasive capacity of AGS gastric cancer cells. uPAR levels were amplified through NF-κB and AP-1 signalling. Similarly, the same group reported that cadmium had up-regulated uPAR expression by activating AP-1, NF-κB and ERK-1/2 pathways and by doing so induced migration and invasion of AGS cells.[114] Furthermore, gastric cancer cells exposed to prostaglandin E$_2$ exhibited increased invasion due to the up-regulation of uPAR, mediated through EGFR–MAPK–NF-κB and EGFR–MAPK–AP-1 pathways.[115] In another study, over-expression of semaphorin 5A enhanced AGS cells invasiveness by activating PI3K/Akt signalling and inducing uPA expression.[116]

A study designed to evaluate the prognostic significance of uPAR in gastric cancer found that the presence of invasive gastric cancer cells over-expressing uPAR was a prognostic factor for poor OS, independent of usual prognostic parameters, as shown by uni- and multivariate analysis.[117] In another study, expression of uPAS components was shown to be associated with peritoneal metastasis in gastric cancer patients.[118] Using peritoneal implantation rat models and gastric cancer cell lines, the same authors have recently confirmed the connection between uPAS and gastric cancer metastasis.[119] High uPA expression was found to correlate with high VEGF expression in gastric cancer tissue suggesting that the combination of these two factors could be used as a prognostic tool for invasive gastric cancer.[120] Furthermore, analysis of peripheral blood from gastric adenocarcinoma patients revealed that elevated uPA expression was significantly linked to cancer stage and was negatively correlated with OS in univariate survival analysis.[121] A recent study by Tian et al[122] discovered elevated uPA expression in cancer-associated fibroblasts within the oesophageal squamous cell carcinoma tumours. Their findings showed that uPA concentration was inversely associated with patients' OS and was able to stimulate cancer cells migration and invasion in vitro. Also, quercetin has been recently shown to suppress the migratory ability of BGC823 and AGS gastric cancer cells by decreasing uPA and uPAR expression.[123]

Ovarian Cancer

High uPA and PAI-1 expression was found not only in ovarian cancer tissue, but also in adjacent cyst and ascitic fluids.[40] An in vitro study by Li and Jiang[124] demonstrated that basic fibroblast growth factor stimulates the invasion of SKOV3 ovarian cancer cells by increasing uPA expression through transcription factor Ets-1. Administration of ATN-658 uPAR antibody supressed the invasion and migration in SKOV3ip1, HeyA8 and CaOV3 ovarian cancer cell lines and attenuated the development of xenograft tumours by inducing apoptosis and disrupting the uPAR–integrin interactions.[125] A study by Zhang et al[126] showed that the expression of uPA and PAI-1 in malignant and non-differentiated ovarian cancers was much higher than in normal or benign ovarian tissue, with uPA expression being an independent prognostic factor as demonstrated by multivariate analysis. In the same study, over-expression of uPA increased the adhesion and metastatic capacity of SKOV3 cells. Also, it has been reported recently that uPA contributed to leptin-associated induction of ovarian cancer cell migration and invasion.[127] Leptin-stimulated uPA over-expression in OVCAR3 and SKOV3 cells was regulated through Rho/Rho-associated protein kinase, Janus kinase/Signal transducer and activator of transcription-3, PI3K/Akt and NF-κB signalling pathways.

Furthermore, a clinical study by Henic et al[128] showed that cleaved uPAR forms possessed significant prognostic value in ovarian cancer patients, as demonstrated by univariate analysis. In a multivariate setting, high uPAR(I) concentration was an independent indicator of poor OS, while the combination of suPAR(I-III) + suPAR(II-III) with conventional ovarian cancer marker, cancer antigen-125, differentiated benign from invasive tumours. In contrast to their prognostic relevance in breast cancer,[38,42,63] mRNA expression levels of wild-type uPAR, uPAR-del4/5 and rab31, albeit correlated among themselves, did not have any significant prognostic effect on PFS and OS of ovarian cancer patients.[129] In another study, eight different ovarian cancer cell lines exhibited over-expressed uPA and uPAR.[130] Similar over-expression was found in ovarian cancer samples and corresponding metastatic lesions, where it correlated with tumour grade, clinical stage, relapse time and PFS of patients. In the next study, same authors reported of co-expression of uPA with multiple drug-resistance-1 (MDR-1) marker in primary ovarian tumours and ovarian cancer cell lines.[131] Observed up-regulation of uPA and MDR-1 correlated with the expansion of ovarian cancer, suggesting that uPA interaction with drug resistance could be targeted to tackle resistant forms of metastatic ovarian cancer. Contrary to the findings in uPAS research, univariate model by Zhao et al[12] recently demonstrated that high expression of plasminogen was associated with extended OS. A multivariable analysis confirmed that plasminogen was a statistically independent prognostic factor for OS in advanced ovarian cancer.

Lung Cancer

Interestingly, some of the previous studies in lung cancer have revealed that not uPA, but rather PAI-1 expression was related to patient's prognosis.[40] Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, characterized by insensitivity to chemotherapy and unfavourable prognosis. A clinical study by Almasi et al[132] reported of the link between poor OS and intact and cleaved forms of uPAR from pre-operatively sampled blood of 32 NSCLC patients. Next study, including more NSCLC patients, confirmed, in a univariate setting, that all uPAR forms were predictors of poor OS.[133] In particular, multivariate OS model identified serum uPAR(I–III) and uPAR(I) as lung cancer prognostic factors, independent of the general parameters. Similarly, the authors have analysed soluble uPAR levels in small cell lung cancer patients.[134] Their uni- and multivariate regression analyses showed that serum uPAR(I) concentration significantly and independently correlated with short patients' OS.

An in vitro study demonstrated that elevated uPA expression was crucial for invasion promotion in A549 lung cancer cells by peroxiredoxin-6, an effect which was mediated through PI3K/Akt signalling pathway.[135] Lung ECM is rich in elastin which, upon degradation, forms peptides with biological activities. One of such elastin-derived peptides is kappa-elastin, capable of augmenting the invasiveness of lung cancer cells. The findings of Toupance et al[136] revealed that the mechanism behind kappa-elastin-mediated induction of invasion was the stimulation of uPA secretion, observed only in invasive lung cancer cell lines.

In another study, Provost et al[137] demonstrated that the activity of sodium hydrogen exchanger isoform-1 played a crucial role in uPA/uPAR-mediated metastasis of NSCLC cells, both in vitro and in vivo, indicating that pH alternation could impact cancer cell growth. Interestingly, a study by Shih et al[138] showed that single-nucleotide polymorphisms in uPA and uPAR gene have a prognostic value when it comes to the predisposition and stage of NSCLC. The authors have associated uPA rs4065 TT genotype with higher susceptibility to lung cancer, while the patients with uPAR-344781 CC genotype were less probable to be diagnosed with lung cancer.

Pancreatic Cancer

Studies have shown that uPA expression is elevated in pancreatic cancer. It was found to be associated with survival and able to predict metastasis in pancreatic cancer patients.[40] In one of the previous studies, authors have shown that hypoxic conditions and resulting activation of hypoxia-inducible factor induce uPAR expression, enabling in that way tumour angiogenesis and invasion of pancreatic cancer cells (PaCC).[139] A study by Gorantla et al[140] reported that suppression of uPA and uPAR strongly weakened metastatic capacity of MIA PaCa-2 and PANC-1 PaCC. This uPAS down-regulation also triggered cell cycle arrest, activated apoptosis and inhibited angiogenic factors in affected cancer cells. Additional study by the same group analysed the correlation between uPA and formation of CSC in pancreatic cancer.[141] Their findings showed that uPA induced stem-like characteristics to MIA PaCa-2 and PANC-1 PaCC by interacting with specific homeobox transcription factors LIM/homeobox-2 and homeobox-A5. By inhibiting uPA expression, the authors were able to decrease tumorigenicity and chemoresistance of CSC-like PaCC. Furthermore, uPA suppression impaired the development of pancreatic tumours in vivo and diminished the expression of p53. Another

in vitro study reported that serine protease polyserase-1 enhanced invasive and migratory capacity of PANC-1 PaCC by stimulating the conversion of pro-uPA to active uPA.[142]

Parkinson's disease-associated protein-7 (PARK-7) has been shown to control PaCC invasiveness through uPAS.[143] The authors have suppressed PARK-7 expression in BxPC-3 and SW1990 PaCC and observed concomitant inhibition of cell invasion and metastasis, both in vitro and in vivo. These effects were related to reduced uPA activity which was also a result of PARK-7 knockdown. Recent immunohistochemical evaluation of 122 pancreatic adenocarcinoma patients discovered that more than 60% of patients expressed uPAR not only in tumour cells, but in surrounding stromal cells as well.[144] Nevertheless, only tumour cell uPAR expression was significantly (reversely) correlated with patient's DFS and OS in multivariate model, implicating its independent clinical significance in discriminating patients with higher risk.

Conclusion and Future Perspectives

In this review, a summary of studies reporting on the roles and activities of uPAS components in various types of human cancers is presented. uPAS, consisting of uPA, PAIs and uPAR, regulates ECM remodelling by activating ubiquitous protease plasmin in many important physiological processes. Because of their significance, expression of uPAS components is controlled on several levels. Furthermore, they participate in pathological processes as well, where the de-regulation of their activity and expression promotes tumour development and metastasis. Even though PAI-1 is the main inhibitor of uPA's proteolytic activity, several studies demonstrate that this over-expression stimulates cell migration and tumour invasion. A reason for this contradiction is the fact that PAI-1 mediates signalling pathways independent of uPA inhibition. High PAI-1 expression has been reported to cause tumour cell detachment, which enables metastasis and invasion, to inhibit apoptosis and stimulate tumour cell proliferation.

uPAR is an integral component of uPAS which not only concentrates uPA's proteolytic activity at cell surface, but also acts as a receptor in signalling pathways which are not associated with uPA proteolysis. Elevated uPAR expression also correlates with aggressive cancer phenotype and precedes invasion and metastasis in almost all types of human cancers. Therefore, future efforts should focus on developing cancer therapies which target and/or inhibit uPAR and its interaction partners. Seeing as high uPAR expression is feature characteristic to cancer cells, such therapeutic targeting would have little or no effect on normal cells. Beside uPA, many other uPAR-binding ligands have been discovered suggesting alternative uPAR-targeting therapies in future studies. Given that most of the cell uPAR is bound to uPA, a relevant subject for future research would be the development of therapeutic drugs capable of targeting bound uPAR such as monoclonal anti-uPAR antibody ATN-658.

Despite numerous studies on the uPAS' role in tumour development, a few unanswered questions still remain. Tumour microenvironment with nearby stroma cells has been shown to play a role in tumour development. Studies reviewed here report of high uPAS components' expression in tumour stroma, which suggests possible inter-play between stroma and tumour cells, and even regulation of tumour progression by paracrine stroma signalling. Consequently, a better clarification of stroma cell's role in tumour development is needed to better understand their complex interaction. Moreover, modulation of uPAS expression might be used to control that interaction in new therapeutic approaches targeting the tumour stroma. At last, what should be also evaluated in the future is which uPA contributes more to tumour growth; host-derived or tumour-derived? What complicates this discernment is that uPA and PAI-1, are also secreted by normal cells in physiological processes such as wound healing, developmental tissue remodelling, vessel growth and post-lactational mammary gland involution. Therefore, more specific drugs, targeting only tumour-associated uPAS components, are needed for more efficient therapies. One of the knowledge gaps in this field of research is the thorough identification and description of uPAS cellular interactions. It is necessary to elucidate the interaction of uPAS with other ECM-degrading proteases active in carcinogenesis, such as MMPs. Previous studies have observed that uPA and uPAR influence tumour development by interacting with integrins and growth factors. Even more, some authors suggest that uPAR–Vn interaction could be an attractive target for uPAS modulation in tumour progression and metastasis. CSCs, cancer cells with stem-like properties, have been found to influence metastasis and relapse of various types of both haematopoietic and solid tumours. Studies reviewed here show that uPA, PAI-1 and uPAR participate in the emergence of CSCs as well as in the induction of EMT, two processes which are associated with drug resistance and apoptosis evasion in cancer. However, further studies are needed to elucidate the exact contribution of uPAS components in maintaining cancer cell stemness and enabling chemoresistance. Additionally, in the future studies, an emphasis should be placed on in vivo models because tumour cell xenografts are not fully suitable due to incompatibility between human uPA and murine uPAR (or vice versa), which excludes any tumour–stroma interactions in uPAS.

An early diagnosis, in most types of cancer, is the only thing that might improve prognosis and positively affect OS in cancer patients. Sometimes, an absence of any strong symptoms conceals the disease until the patients are already in the advance stage, which complicates any further effective treatment. The clinical studies that are reviewed here have evaluated and confirmed the clinical utility of uPA and PAI-1, especially their role as reliable prognostic and predictive cancer markers. This is supported by the fact that uPA and PAI-1 have been declared as LOE-1 in breast cancer. Furthermore, both uPAR and its soluble forms have been shown to be efficient prognostic markers in distinguishing poor prognosis and predicting therapy response in cancer patients. Therefore, the determination of uPAS components could help with the pre-treatment screening of patients and their subsequent stratification in low- or high-risk group. That knowledge could help advance the individualization of oncotherapy, particularly in the terms of selecting the appropriate therapy and predicting its specific benefit.

In summary, the assessment of the existing literature indicates that uPAS plays a clinically relevant role as a prognostic and predictive factor in several human malignancies. Over-expression of uPA, PAI-1 and uPAR not only enhances tumour cell invasion capacity and metastasis, but also corresponds to a higher risk of disease and correlates with poor prognosis. Moreover, uPAS expression has been reported to be associated with common clinicopathological features such as pT stage, Gleason grading, lymph node metastasis, lymphovascular invasion and tumour size. It is important to note that, even though uPAS participates in a variety of physiological and pathological processes, it was not reported to be vital for cell proliferation and/or survival under normal physiological circumstances. Correspondingly, uPAS is emerging as an attractive target in novel therapeutic approaches for cancer treatment, but it is necessary to address all knowledge uncertainties before bridging the gap between laboratory findings to clinic use.

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