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# The urokinase-system – role of cell proliferation and apoptosis

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**Summary.** The serine protease urokinase-type plasminogen activator (uPA) and its receptor (uPAR) are involved in the control of extracellular matrix turnover, cell migration, invasion and cell signalling leading to a variety of different responses, under both physiological and pathological conditions. The urokinase receptor, binding to the growth factor-like domain of uPA, directs membrane-associated extracellular proteolysis and signals through transmembrane proteins, thus regulating tissue regeneration, angiogenesis, cancer growth and metastasis. Since these physiological and pathophysiological processes of the uPA-system are known, less informations concerning uPA-induced cell proliferation and anti-apoptotic effects of the uPA-system are available. Recent studies show a close relationship of the uPA-system and cell proliferation/apoptosis. uPA is responsible for the activation and release of different growth factors and modulates the cell proliferation/apoptosis ratio through the dynamic control of cell-matrix interactions. This article focuses on the important role of the uPA/uPAR-system for cell proliferation and apoptosis.

**Key words:** Urokinase, Urokinase receptor, Cell proliferation, Apoptosis

## Introduction

Metastasis and invasion of malignant tumors are accompanied by the degradation of extracellular matrix elements like type IV collagen, laminin, or vitronectin. This is accomplished by a series of "tumor-associated proteases" including the uPA-system (Schmitt et al., 1992). These proteolytic activities enable several different steps of the metastatic cascade such as local invasion, intravasation, access to the systemic circulation, or establishment of tumor cells at the metastatic site. According to the catalytically active site of these proteases they are classified into serine, aspartic, cysteine, threonine, and metalloproteinases. The proteases are either secreted by the tumor cells or by adjacent stromal cells of the surrounding tissue compartment recruited by the tumor cells. Physiologically the uPA-system is expressed by leukocytes, monocytes/macrophages, endothelial cells, fibroblasts and epithelial cells. We have demonstrated that blood monocytes/macrophages that possess elevated uPAR-levels are selectively recruited from the blood stream to inflammatory sites close to carcinoma cells. These tumor-associated macrophages (TAM) secrete a lot of uPA induced by paracrine interactions. In this context TAM may support tumor progression (Hildenbrand et al., 1999).

The various enzymes do not differ from the enzymes which are involved in several tissue remodeling processes such as wound healing, fibrinolysis, inflammation, embryogenesis and angiogenesis (Dano et al., 1985; Blasi, 1988; Liotta et al., 1991; Andreasen et al., 2000). In fact, the quantitiy rather than the quality of their expression contributes to the invasive phenotype of malignant cells (Ludwig, 2005).

## The uPA-system

Urokinase-type plasminogen activator (uPA), its receptor and inhibitor, uPAR and PAI-I, play a key role in tumor invasion and metastasis. uPA is a serine protease with a molecular mass of 55 kDa. It is an important component in the fibrinolytic system converting plasminogen to an active enzyme, plasmin. Enhanced activity of plasmin promotes the degradation of extracellular matrix components including fibrin, fibronectin, proteoglycans and, as the main molecules in basement membrane, laminin and collagen IV (Andreasen et al., 2000). uPA is produced as an inactive single-chain protein secreted from cells of the urogenital system, leukocytes, fibroblasts, macrophages,

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endothelial cells and also tumor cells including breast, colon, pancreas, prostate, ovary, gastric, cervix, endometrium, bladder, kidney and brain tumors tissues (Andreasen et al., 2000). It is proteolytically activated either in the extracellular space or bound to the urokinase-receptor (uPAR) (Gunzler et al., 1982; Wun et al., 1982; Vassalli et al., 1984). uPA and PAI-1 were the first novel tumor biological factors to be validated at the highest level of evidence (LOE I) regarding their clinical utility in breast cancer (Harbeck et al., 2004).

The 55-60 kDa heavily glycosylated disulfide-linked cell surface receptor (uPAR, CD87) binds both single-chain pro-uPA and active two-chain uPA (Cubellis et al., 1986; Stoppelli et al., 1986). Binding of the A-chain of active two-chain form of uPA, allowing activation of ubiquitously available plasminogen and initiation of the proteolytic cascade via the B-chain (Stoppelli et al., 1985). The biochemical properties of uPAR have been extensively characterized (Ploug, 2003). Human uPAR is a glycolipid-anchored (Ploug et al., 1991) protein having a single-chain polypeptide (283 amino acids) organized into three extracellular domains (Roldan et al., 1990; Ploug and Ellis, 1994), that are all are members of the Ly6/uPAR/α-neurotoxin protein domain family. Each of these comprises approximately 90 residues with four to five disulphide bonds. The GPI-anchor is hypothesized to enable a high intramembrane mobility (Stoppelli et al., 1986). Furthermore, the uPAR is glycosylated at N-residues of glucosamine and sialic acid within the binding site, thereby regulating its affinity (KD of 0.1-1.0 nM) for uPA (Ploug et al., 2002). Receptor-bound uPA, as compared to the fluid phase enzyme, activates plasminogen much more efficiently, this being reflected by a 40-fold decrease in Km of urokinase for its substrate (Ploug et al., 2002). Recently, the crystal structure of a soluble form of human uPAR was reported. Based on this crystal stucture of the uPAR-peptide complex, a model of the human uPA-uPAR complex was constructed, revealing for the first time, how the molecular structure of uPAR may guide uPA-focused proteolysis on the cell surface, and control cell adhesion and migration (Llinas et al., 2005).

The two known physiological inhibitors for uPA are the glycoproteins plasminogen activator inhibitor-1 and -2 (PAI-1 and PAI-2, respectively). They belong to the gene family of serine protease inhibitors called serpins (Ny et al., 1986; Antalis et al., 1988; Ye et al., 1989). PAI-1 forms a complex with two-chain uPA (Andreasen et al., 1986), and it has also been shown to form a reversible complex with single chain uPA (Manchanda and Schwartz, 1995). The receptor-bound uPA is inactivated by PAI-1 (-2). The trimeric complex uPAR/uPA/PAI is internalized into the cell together with alpha-2 macroglobulin receptor and its ligand (Cubellis et al., 1990; Olson et al., 1992; Andreasen et al., 1997), the free uPAR is recycled to the cell surface, and binding and activation of a second uPA molecule can occur (Laufs et al., 2006).

In addition to a role in localising uPA proteolytic activity to the cell surface, the uPAR also interacts with integrins such as vitronectin (Vn) and thus facilitates cell-matrix interactions (Wei et al., 1994). It was demonstrated by Wei and coworkers (Wei et al., 1996) that the capacity of uPAR to act as an adhesion receptor depends on both a functional and physical association with integrins. uPAR forms complexes with activated integrins, presumably utilizing integrin connections to the cytoskeleton to promote stable adhesion to vitronectin mediated by a distinct binding site on uPAR. uPAR/Vn can be enhanced by uPA and attenuated by PAI-1, which binds to the somatomedin B domain of Vn (Deng et al., 1996). In addition, uPAR is capable of modulating cell adhesion by activating cells directly via G-protein-coupled receptor (Liu et al., 2002), by sequestering caveolin (Wei et al., 1999), and by affecting intracellular signalling events (Nguyen et al., 1999). Thus uPAR is an important regulator of the adhesive behaviour of cells. We have demonstrated that PAI-1 may be an important component in the detachment of breast cancer cells and myoepithelial cells in ductal carcinoma in situ of the breast (DCIS), since PAI-1 is able to attenuate the cell matrix interaction by resolving the uPAR/Vn binding. The loss of the anti-invasive myoepithelial layer in DCIS may be triggered by PAI-1 and could be an early sign of subsequent tumor cell infiltration (Hildenbrand and Arens, 2004).

### The uPA-system and cell proliferation

The serine protease urokinase-type plasminogen activator is synthesized and secreted by stromal cells, malignant epithelial cells and other tumor cells including tumor cells of cancer precursor lesions and is implicated in a variety of physiological and pathophysiological processes. (Hildenbrand et al., 1999; Harbeck et al., 2004). The protease uPA also exerts biological effects characteristic of molecules with signal transducing properties including chemotaxis, migration, adhesion, and mitogenesis (Reuning et al., 1998). Mitogenic activity of uPA has been observed for several cell types in a cell-type-specific manner with still inconclusive reports on the structural and molecular requirements. In human epidermal cells, normal and malignant renal cells and in melanoma cells it was evident that uPA stimulates growth (Kirchheimer et al., 1988, 1989). Among the distinct components of uPA, aminoterminal fragment (ATF) was identified as mitogenic factor isolated from osteoblast-like cells (Rabbani et al., 1990). In these reports, an uPA active site was required to elicit the observed mitogenic effects. Furthermore, it was reported by Fischer and coworkers (1998) that binding of uPA or ATF to uPAR leads to increased mitogenic response in the human ovarian cancer cell line OV-MZ-6, which can be inhibited by the monoclonal antibody IIIF10 blocking uPA/uPAR interaction and by soluble uPAR (Fischer et al., 1998).

The binding of uPA to uPAR and its inhibitor, plasminogen activator inhibitor type 1 (PAI-1), induces internalization of the complex, thereby facilitates extracellular proteolysis and growth factor activation.

Beyond these functions uPA is reported to generate intracellular signals via both high-affinity receptor-dependent and independent mechanisms. Experimental evidence on various cell types of immune system indicates that gycosyl-phospahtidyl-inositol (GPI) - anchored receptors which are devoid of cytoplasmic domains are nevertheless able to relay signals by an association with src-like protein tyrosine kinase (PTKs) (Shenoy-Scaria et al., 1992; Stefanova et al., 1993). Likewise, uPAR has recently been shown to associate with PTKs of the src-family in human monocytes and the myeloid cell line THP1 (Bohusalv et al., 1995). These kinases have been assumed to provide a gate-keeper function to the signalling cascade. Indeed, the earliest event in uPAR-mediated signal transduction is the induction of tyrosine phosphorylation (Dumler et al., 1993; Bohuslav et al., 1995). In another study performed on human epithelial cells, uPAR has been found to form a complex with two serine-phosphorylated proteins identified as cytokeratins 18 and 8 (Busso et al., 1994).

Although uPAR lacks cytoplasmic and transmembrane domains, binding of uPA and uPAR is known to activate several signaling intermediates. These include tyrosine phosphorylation of a 38-kDa protein in U-937 cells (Dumler et al., 1993), activation of protein kinase C-e in WISH cells (Busso et al.,1994), diacylglycerol formation in epidermal cells (Del Rosso et al., 1993), activation of Janus kinase-signal transducers and activators of transcription (JAK-STAT) signaling in the epithelial cells TCL-598 (Koshelnick et al., 1997) and vascular smooth muscle cells (Dumler et al., 1998). These signaling molecules may contribute to uPA-mediated cellular responses, including cellular proliferation.

uPAR has been shown to colocalize with integrins. In human monocytes, it was demonstrated by Sitrin and coworkers that integrins and uPAR associate via carbohydrate-lectin interaction and that this physical association is necessary for collaboration (Sitrin et al., 2001). Previously we demonstrated this integrin-uPAR interaction in tumor-associated macrophages of breast cancer (Hildenbrand et al., 1999).

Ligation of $\alpha_v$ integrins with their ligands has been reported to regulate the expression of several metalloproteinases (MMPs) and uPA/uPAR in various cancer cell types (Nip et al., 1997). Furthermore, it was demonstrated that integrins ($\alpha_3ß_1$) are associated with uPAR in MDA-MB-231 breast cancer cells (Wei et al., 2001; Gandhari et al., 2006). In this cell line uPAR preferentially complexes with α3ß1, promotes direct (Vitronectin) and indirect (Fibronectin) pathways of cell adhesion.

Since uPAR lacks transmembrane and intracytoplamsic domains, uPAR-integrin collaboration is necessary for uPA-induced signal transduction. It has been observed that high level expression of recombinant uPAR or soluble uPAR impairs ligand binding by integrins and their adhesive functions (Wei et al., 1994, 1996). However, in most cells bearing endogenously expressed uPAR, uPAR, like other integrin-associated proteins, promotes integrin function. It was evident that signaling through the fibronectin receptor α5ß1 was promoted by the association of this integrin with uPAR (Aguirre-Ghiso et al., 1999; Wei et al., 1999). Furthermore, it was demonstrated that endogenous p38 MAPK/ERK (mitogen-activated protein kinase/Ras-extracellular signal-regulated kinase) activity is elevated in cultured invasive breast cancer cells and that the high p38 MAPK activity is important for breast cancer invasiveness by stabilising uPA mRNA (Chen et al., 2001). Recent studies have also shown that p38 MAPK modulates proliferation of cancer cells. The tumor cell proliferation of Hep3 human carcinoma cells depends on the interaction of uPA/uPAR complex with integrins which has a direct link to the p38 MAPK activity (Aguirre-Ghiso et al., 2001).

The biological functions of the uPA-system are not just related to cell adhesion, migration, chemotaxis and cytoskeleton dynamics. In the human melanoma cell line GUBSB, inhibition of receptor-bound uPA by specific anti-uPA antibodies reduces cell proliferation, suggesting that cell growth is constantly stimulated by uPAR engagement in an autocrine way (Krichheimer et al., 1989). Vascular smooth muscle cell proliferation is provoked by uPA through uPA/uPAR-interaction, suggesting the involvement of still unknown factors (Kanse et al., 1997). Further information is provided by another study demonstrating that proteolytically inactive uPA is a mitogen for dorming melanoma cells by an uPAR-independent effect that involves the N-terminal region through a yet unindentified membrane-associated mediator (Koopman et al., 1998). Additionally, proliferating effects were observed following exposure of the human osteosarcoma cell line SaOs-2 to amino-termial fragment (ATF) of uPA. In this study it was shown, that fucosylation of $Thr^{18}$ within the EGF-like domain is required for eliciting this catalytical independent response (Rabbani et al., 1992).

Shapiro and coworkers studied the contribution of the uPA to the malignant phenotype through the chemical induction of melanocytic neoplasms in uPA-deficient mice. In this study primary tumors were induced and promoted concurrently in 35 uPA-/- deficient and 35 uPA+/+ wild-type mice using a single application of 7,12-dimethylbenz(a)anthracene followed by repetitive applications of croton oil. Unlike the wild type mice, in which chemically induced melanocytic tumors progress to malignant melanoma, the uPA-/-mice were not susceptible to melanoma induction. This resistance of the uPA -/- strain to melanoma induction suggests that uPA contributes to malignant progression, possibly by decreasing the release and availability of growth factors such as bFGF (Shapiro et al., 1996). In studies from Gyetko and coworkers (2002) it was demonstrated that a causal relationship between T cell proliferation and uPA exsits. uPA-deficient mice exhibit an impaired T cell proliferation due to a change in cytokine expression. In absence of uPA, the cytokine profile of regional lymph nodes shifted from a T1 pattern characterized by IFN-gamma and IL-2 to a weak,

nonpolarized response. In absence of uPA, lymphocyte proliferative responses are diminished, and mice fail to generate protective T1 cytokines, resulting in impaired antimicrobial activity. This study provides evidence that uPA is a critical modulator of immune responses and of immune cell effector functions in vivo (Gyetko et al., 2002).

The mitogenic and chemotactic responses to bFGF are specifically inhibited in uPAR-deficient or in wild-type smooth muscle cells, cultured in the presence of antibodies to uPAR. Additionally, smooth muscle cells isolated from uPA-deficient mice are less sensitive to bFGF than the cells isolated from wild type animals (Herbert et al., 1997).

Suelves and coworkers (2002) reported that inhibition of uPA proteolytic activity with anti-uPA antibodies abrogated migration, fusion and differentiation of murine myoblasts in vitro. Moreover they demonstrated that uPA activity is required for efficient skeletal muscle regeneration in vivo (Suelves et al., 2002). In another study a relevant role for uPA in liver regeneration in vivo is supported by the impaired proliferation following partial hepatectomy in uPA-deficient mice (Rosselli et al., 1998).

Although the exact mechanisms are unclear it is probable that uPA can support cell proliferation. Thereby integrins situated adjacent to uPAR carry the signals into the cell.

Previously we have determined a strong correlation (Fig. 1) between uPA levels in tumor tissue extracts and Ki67-associated proliferation rate in breast cancer (Hildenbrand et al., 1995). This prompted us to investigate the mitogenic activity of uPA in the breast cancer cell line MDA-MB-231 in detail. The MDA-MB-231 cell line is highly invasive and expresses high levels of uPA. In our experiments, uPA inhibition was done by two methodologies, stable transfection with an antisense uPA vector and by transfection with siRNA molecules (small interfering RNA). After transfection, proliferation was determined by Ki67 staining, proliferation assay, cell counting, and S-phase analysis. In these experiments we have demonstrated that uPA plays a major role in cell proliferation by acting as mitogenic factor for breast cancer cells (Arens et al., 2005; Gandhari et al., 2006; Fig. 2). Furthermore, mitogenic activity of uPA was tested by adding uPA (HMW-uPA) or incubation with the amino terminal fragment (ATF), both resulting in increasing cell proliferation rates. After blocking the uPA/uPAR interaction by adding monoclonal anti-uPAR IIIF10 antibody (blocking uPA binding site), or soluble uPAR (scavenger effect), or phosphatidyl-inositol specific phopholipase-C (PI-PLC, degrading uPAR) proliferation rates strongly decreased. The results of these experiments revealed that the uPA-uPAR interaction is necessary for uPA-induced cell proliferation. We have demonstrated by immuno-histochemistry that integrin and uPAR are colocalized in native MDA-MB-231 cells (Gandhari et al., 2006). Since uPAR lacks transmembrane and intracytoplasmic domains for uPA-induced signal transduction, uPAR-

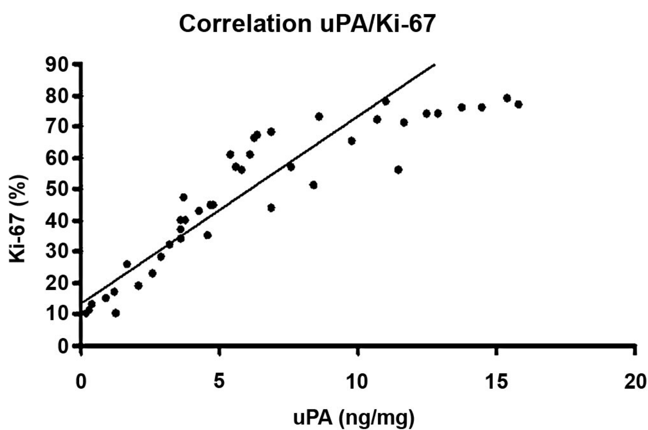


**Fig. 1.** Correlation of uPA in breast cancer tissues extracts determined by ELISA and Ki67 associated proliferation rates of the same cases (n=42). 95% Confidence Interval (CI): 0.84<r<0.95; correlation coefficent (pearson): r=0.91; p<0.001.

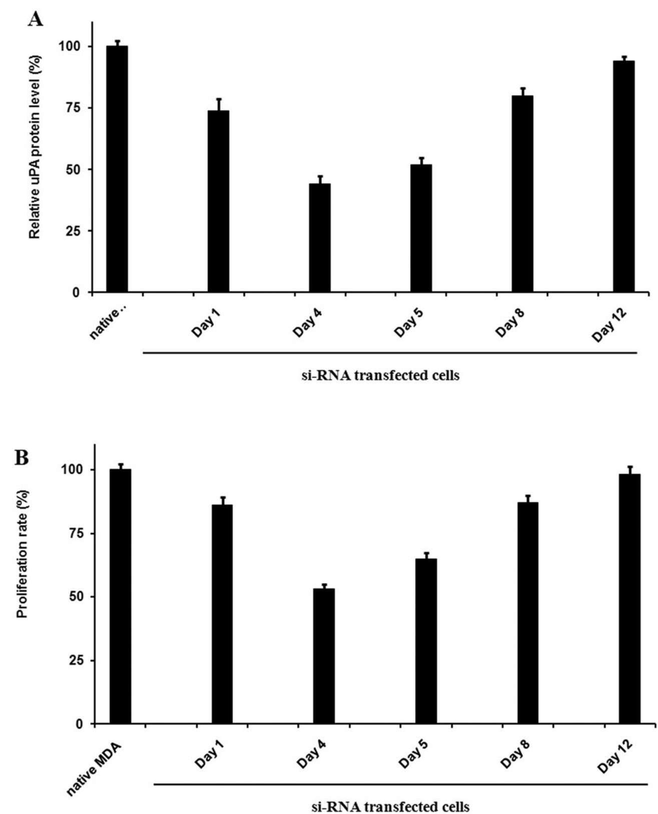


**Fig. 2.** Anti-sense uPA siRNA transfection of MDA-MB-231 breast cancer cell line. **A.** After antisense uPA-siRNA-transfection uPA-mRNA- and subsequently uPA-protein-levels decreased. The strongest suppression was obtained 4 days after siRNA transfection (50%). Thereafter the suppression effect diminished and the uPA-protein-levels increased at normal levels. **B.** The proliferation rates show similar effects (mean +/- SEM).

integrin interaction is necessary. To test this assumption we have used a monoclonal anti-α3ß1 antibody (P1B5) to block uPAR-integrin function in MDA-MB-231 cells. The proliferation rate was significantly reduced when this antibody was added prior to the addition of HMW-uPA. Considering our results we conculde that the mitogenic activity of uPA is mediated via integrins adjacent to uPAR (Gandhari et al., 2006).

It was reported by Sitrin and coworkers that integrins and uPAR are associated via carbohydrate-lectin interactions and that this physical association is necessary for collaboration (Sitrin et al., 2001). Recent studies have shown that integrins can activate the p38 MAPK/ERK pathway and that α-integrin, p38 MAPK and uPA are functionally linked in breast cancer cells (Chen et al., 2001). These earlier observations prompted us to investigate whether p38 MAPK pathway is important for breast cancer cell proliferation. The treatment of invasive breast cancer MDA-MB-231 cells with the p38 MAPK inhibitor SB203580 prior of the addition of HMW-uPA significantly abrogates the proliferation of these cells, thus demonstrating the importance of the p38 MAPK signaling pathway for the proliferation of breast cancer cells (Gandhari et al., 2006). In summary, HMW-uPA and ATF induce proliferation of breast cancer cells by binding to uPAR. Integrins situated adjacent to uPAR carry the signals into the cell, and ultimately stimulate proliferation via the ERK/MAPK pathway (Fig. 3).

The ability of uPA to activate the ERK/MAPK pathway by binding to uPAR was reported previously (Tang et al., 1998; Aguirre-Gisho et al., 1999, 2001). Ma and coworkers (2001) postulated a positive feedback loop in MDA-MB-231 breast cancer cells between uPA/uPAR expression and the ERK/MAPK level. They demonstrated that on the one hand the endogenous uPA/uPAR system plays a critical role in maintaining a high level of activated ERK/MAPK in these cells. On the other hand, they showed that activated ERK/MAPK is necessary to maintain uPA and uPAR expression. When this feedback loop was disrupted with uPA- or uPAR-specific antibodies, uPA mRNA-specific antisense oligodeoxynucleotides or PD098059 (MAPK inhibitor), cell proliferation was inhibited and apoptosis was promoted (Ma et al., 2001). In conclusion, this positive feedback loop might be critical in determining the aggressive nature of MDA-MB-231 cells. One important consequence of ERK/MAPK regulation by the uPA/uPAR-system in MDA-MB-231 breast cancer cells is regulation of cell proliferation and apoptosis. An autocrine signalling pathway, in which endogenously produced uPA binds to uPAR and thereby activates ERK/MAPK, was demonstrated in HT 1080 fibrosarcoma cells and MCF-7 breast cancer cells; in these cells the pathway was observed only when low density lipoprotein receptor-related proteins (LRP) were neutralized (Webb et al., 1999, 2000).

The ability of uPA to signal through its receptor maintains an elevated basal level of activated ERK/MAPK and the resulting increased cell proliferation and reduced apoptosis represent a novel mechanism whereby the uPA/uPAR system may affect breast cancer progression in vivo.

**The uPA-system and apoptosis**

The ability of tumor cells to migrate and invade through the basement membrane into surrounding tissues is one of the essential hallmarks of cancer and a prerequistite for both local tumor progression and

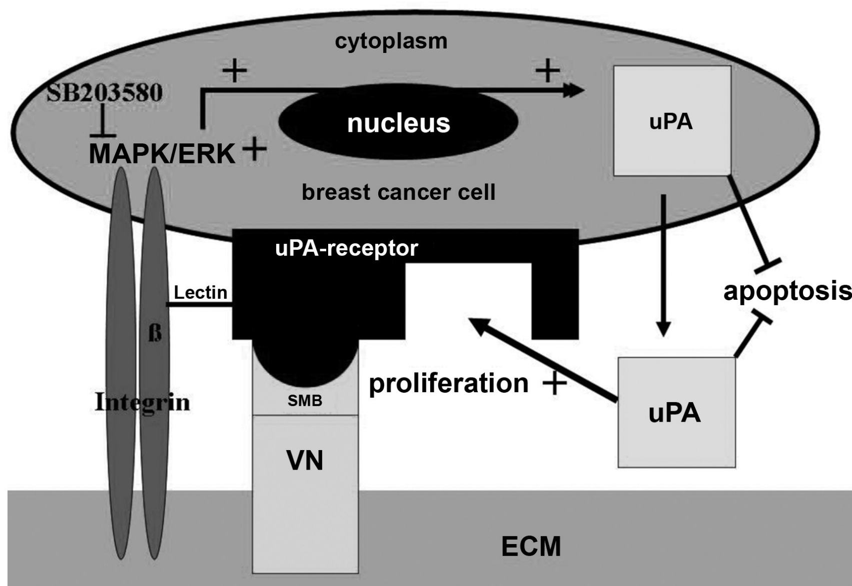


**Fig. 3.** Positive feedback loop between uPA/uPAR and MAPK/ERK. Since uPAR lacks transmembrane and cytoplasmic domain, for uPA induced signal transduction uPAR-intergrin collaboration is necessary. uPA (urokinase plasminogen activator) binding to its receptor (uPAR) activates MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) via integrins adjacent to uPAR. High levels of activated MAPK/ERK induce cell proliferation via stimulating uPA expression. High uPA-levels protect cancer cells from apoptosis. Inhibition of MAPK/ERK activity by SB203580 decrease cell proliferation, uPA-expression and increase apoptosis. The ability of uPA/uPAR and MAPK/ERK to function in a positive feedback loop by stimulating cell proliferation and suppressing apoptosis represents a novel mechanism whereby the uPA-system may promote cancer progression. uPAR forms complexes with vitronectin (VN) by binding to the somatomedin B domain of VN. Thus uPAR is an important regulator of the adhesive behaviour of cells. uPAR/VN can be enhanced by uPA binding and attenuated by PAI-1, which binds to the somatomedin B domain of vitronectin.

metastasis, but it can also lead to apoptosis if not counterbalanced by survival signals. In particular, apoptosis induced by loss of anchorage, a phenomenon also known as anoikis, has been shown to limit the spread of epithelial cells outside the tissue environment. Therefore, successful cellular transformation, migration and invasion of tumor cells requires their resistance to the endogeneous death program once the cell has detached from the primary tumor tissue. Many cell types derived from human malignancies, such as gastric cancer, breast cancer, colon cancer, osteosarcomas, and lung cancer, are resistant to anoikis (Khwaja et al., 1997).

Recent studies demonstrate a close correlation between the uPA/uPAR-system and cell sensitivity to programmed cell death. Gutierrez and coworkers (2000) implanted T241 fibrosarcoma cells in uPA-/- mice which exhibit decreased cell proliferation and increased apoptotic indices, suggesting that alterations in host uPA expression may effect the balance between apoptosis and proliferation (Gutierrez et al., 2000). They demonstrated a decrease and delay of metastasis of fibrosarcoma in uPA- and PAI-1-deficient mice.

Yanamandra and coworkers reported that SNB19 glioblastoma cells expressing antisense uPAR constructs are less invasive than parental cells when injected in vivo (Yanamandra et al., 2001). They found that in vivo inhibition of SNB19 tumor growth is associated with a loss of mitochondrial transmembrane potential, release of cytochrome C from mitochondria, subsequent activation of caspase-9 and apoptosis compared to parental cells and vector controls. Their results indicate that suppression of uPAR results in apoptosis and suggest that caspase-9 dependent apoptosis plays an important role in SNB19 AS-uPAR-induced apoptosis. Additionally, glioma cells deficient in uPAR-expression were more susceptible to tumor necrosis factor-alpha-related apoptosis-inducing ligand (TRAIL) induced apoptosis compared to parental cells. The findings that uPAR-deficient clones had higher levels of the TRAIL receptors DR4 and DR5 mRNAs than parental cells may at least in part account for their different susceptibility to apoptosis (Krishnamoorthy et al., 2001). The anti-apoptotic ability of uPAR is also due to its ability to activate the Ras-ERK signaling pathway in many different cell types. Our group has demonstrated that the proliferation rate of MDA-MB-231 breast cancer cells decreases and apoptosis increases after reducing endogenously produced uPA by transfection with antisense-uPA siRNA or conventional antisense-uPA vector transfection (Arens et al., 2005). We have shown that ERK/MAP kinase (Ras-extracellular signal-regulated kinase, ERK; mitogen-activated protein kinase, MAPK) is activated by incubation of MDA-MB-231 breast cancer cells with exogenously added uPA resulting in increased phosphorylated ERK/MAP kinase-levels and in increasing proliferation rates. Ma and coworkers reported that MDA-MB-231 breast cancer cells cultured in the presence of anti-uPA antibodies (which block uPA/uPAR binding) had decreased levels of phosphorylated ERK/MAP kinases and increased apoptosis, showing that endogenous uPA is a major activator of ERK/MAPK and protects from apoptosis (Ma et al., 2001). They provide evidence that PD098059 (ERK/MAPK inhibitor), uPA-specific antibody, uPAR-specific antibody and uPA-specific antisense oligodeoxynucleotides increase apoptosis in MDA-MB-231 breast cancer cells. The role of activated ERK/MAPK in preventing apoptosis has been observed previously. Activated MAPK protects PC-12 cells from apoptosis after withdrawal of nerve growth factor-beta (Xia et al.,1995) and plays a pivotal role as a downstream mediator of the anti-apoptotic activity of acid fibroblast growth factor (Guillonneau et al.,1998). Terada and coworkers demonstrated in BaF3 hematopoietic cell line that the activation of caspase-3 upon IL-3 removal is suppressed by expression of activated ERK (extracellular signal-regulated kinase), which prevents cell death. After IL-3 withdrawal activation of both ERK and phosphatidyl-inositol 3-kinase is required to suppress caspase-3 activation (Terada et al., 2000). The anti-apoptotic activity of ERK/MAPK may reflect its ability to activate kinases of the Rsk family, which consecutively phosphorylate the pro-apoptotic protein BAD and the pro-survival transcription factor CREB (cAMP response element-binding protein) (Bonni et al., 1999). Both Rsk-catalyzed mechanisms prevent apoptosis.

The hypothesis that the uPA-system and MAPK/ERK activity are regulated in one pathway to prevent apoptosis is reported by Ma and coworkers. They demonstrate studies in which PD098059 and uPA-specific or uPAR-specific antibody increased cell death to the same extent as if these substances were added separately. Furthermore, they showed that activated MAPK/ERK increases the expression of uPA and uPAR. Because of this positive feedback loop they could not exclude the possibility that other activities of the uPA-system are responsible for the apoptosis observed with uPA-specific antibody or PD098059. Possible alternative mechanisms whereby uPA and uPAR may oppose apoptosis include signal transduction through pathways other than the MAPK/ERK pathway, modulation of cell adhesion and regulation of cell surface proteolysis.

The positive correlation between uPAR expression levels and activation of the phosphatidylinositol 3-kinase (PI3k) and Akt signaling anti-apoptotic pathway is supported by the finding that uPA-deficient glioblastoma cells exhibit a reduced level of posphorylated PI3k and Akt as well as impaired migration and survival. Decreased activation of PI3k and the anti-apoptotic factor Akt was not sufficient to induce apoptosis in the uPA-deficient glioblastoma cells, but staurosporine sensitized them to apoptosis to a greater extent than control cells. Furthermore these results indicate that PI3k/Akt pathway is involved in the signaling cascade required to induce cell migration and that uPA has a direct role in regulating migration (Chandrasekar et al., 2003).

Members of the Bcl-2 protein family have been

shown to play a key role in regulating apoptosis by acting as general guardians of mitochondrial integrity. The Bcl-2 protein family consists of proteins with both anti-apoptotic and pro-apoptotic functions (Cheng et al., 2001; Wei et al., 2001). Bcl-2, Bcl-XL and possibly further anti-apoptotic proteins of this family have been proposed to exert their anti-apoptotic effect by antagonizing the action of the pro-apoptotic proteins of the same family. Since the discovery of Bcl-2 and Bcl-XL, their role in cancer progression and apoptosis has been well documented. Both proteins are highly overexpressed in different cancer cell lines and tumor entities. Only a few reports document the interaction between the uPA/uPAR-system and the Bcl-2 protein family. Wick and coworkers (1998) reported that the level of Bcl-2 in malignant glioma cell lines positively correlates with the expression of metalloproteinases and cell surface-uPA. They postulate a novel function for Bcl-2 in malignant glioma cells by enhancing migration and invasion through alterations in uPA- and metalloproteinase-expression (Wick et al., 1998). Bcl-2 overexpression of breast cancer cells and melanoma cells in hypoxia increases SP1 transcription factor expression and activity with the result of enhanced uPAR transcription and expression. MAPK/ERK may function as an important mediator in this regulatory circuitry. A decrease of uPAR protein expression is induced by treatment of cells with specific bcl-2-antisense oligonucleotides (Trisciuoglio et al., 2004). An important factor mediating apoptosis is the death receptor Fas (CD95), which belongs to the tumor necrosis factor (TNF) family. CD95 exhibits an extracellular binding region for Fas ligand (FasL or CD95L) and an intracellular region that is critical for transduction of apoptotic signals. Upon ligation of Fas and FasL, an adaptor protein, Fas-associated death domain (FADD) is recruited and pro-caspase-8 activation occurs. Further activation of the effector-caspase-3 is then followed by amplification through mitochondria. Recent studies demonstrate that Fas, which is overexpressed in a lot of human cancer cells, when incubated with FasL results in an increase of tumor cell motility and invasiveness. Under these circumstances CD95L may induce NF-κB, ERK/MAPK, and uPA gene transcription. Therefore uPA is an excellent candidate gene for the general regulation of CD95-induced invasiveness. The blocking effect of anti-uPA antibodies on Fas-induced motility and invasiveness suggests a functional link between uPA and CD95-mediated signaling (Barnhart et al., 2004).

Recently Alfano and coworkers provided a causal link between uPAR signaling and protection from apoptosis, by demonstrating that ligand engagement of uPAR counteracts the pro-apoptotic effect triggered by cisplatin, UV-light and forced detachment from the culture dish. In their study the expression level of uPAR positively correlated with resistance to anoikis in retinal pigment epithelial- and embryonic kidney epithelial-cell lines. They furthermore reported that the uPA/uPAR interaction results in a marked upregulation of the anti-apoptotic factor Bcl-xL, which is required for the uPA-dependent anti-apoptotic activity (Alfano et al., 2005, 2006).

Nadia Harbeck and coworkers reported that breast cancer patients with high uPA/PAI-1 levels benefit more strongly from adjuvant chemotherapy and tamoxifen therapy than those with low levels. This superior treatment response may be due to interaction of the applied substances to upregulated uPA-associated pathways concerning cell proliferation and apoptosis (Harbeck et al., 2002; Meijer-van Gelder et al., 2004).

## Conclusion

In different cancer types elevated levels of uPA and uPAR are associated with a poor prognosis. In order to better understand the role of the uPA-system in the development and progression of cancer, it will be important to further elucidate the diverse properties of this system. The ability of uPA/uPAR and MAPK/ERK to function in a positive feedback loop by stimulating cell proliferation and supressing apoptosis represents a novel mechanism whereby the uPA-system may promote cancer progression. Given its ability to promote invasion, motility and proliferation and to protect cancer cells from programmed cell death, the uPA/uPAR system is a highly promising target for therapeutic interventions. These strategies should focus on both the proteolytic cascades initiated by uPA and on the various signalling pathways mediated by uPAR (Romer et al., 2004). An increasing number of substances targeting the uPA-system is becoming available. Several strategies involving direct targeting of the uPA-system with other components such as intergrins or fibronectin, and also an inhibition of molecular/transcriptional regulators of gene expression, should be anticipated as promising strategies to improve prognosis of cancer patients.

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