The urokinase receptor as an entertainer of signal transduction

Silvia D'Alessio[1], Francesco Blasi[1,2]

[1] Department of Molecular Biology and Functional Genomics, DIBIT, Universita Vita-Salute San Raffaele, Via Olgettina 58, 20132 Milan, Italy, [2] Fondazione Istituto Firc di Oncologia Molecolare, Via Adamello 16, 20139 Milan, Italy

TABLE OF CONTENTS

1. ABSTRACT

The serine-protease urokinase (uPA) and its specific membrane receptor uPAR controls matrix degradation through the conversion of plasminogen into plasmin and play a crucial role in a number of biological processes including local fibrinolysis, inflammation, angiogenesis, matrix remodelling during wound healing, tumor invasion and metastasis. Most of the cellular responses modulated by the uPA/uPAR system, including migration, cellular adhesion, differentiation, proliferation and apoptosis require transmembrane signaling, which is mediated by direct contacts of uPAR with a variety of extracellular proteins and membrane receptors, such as integrins, EGF receptor, high molecular weight kininogen, caveolin and the G-protein-coupled receptor FPRL1. As a result of these interactions, uPAR activates intracellular signalling molecules such as tyrosine- and serine-protein kinases, Src, focal adhesion kinase (FAK), Rac, extracellular-signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) and JAK/STAT, being part of a large "signalosome" interacting with several molecules on both the outside and inside of the cell. This review is focused on the biochemistry of the pathways affected by uPAR and its partners.

2. INTRODUCTION

Urokinase plasminogen activator (uPA) is a serine protease whose major substrate is the zymogen plasminogen which is cleaved and activated to form plasmin. By activating plasminogen, uPA is at the top of a proteolytic cascade that ends up in the cleavage, degradation, sometimes activation of a myriad of proteins, including other proteases. Unlike other proteases uPA has a specific, high affinity receptor, uPAR that allows its localization (and the localization of its proteolytic activity) at the cell surface. Through their extracellular proteolysis activity, uPA and uPAR are regulators of many cell functions like adhesion, proliferation, chemotaxis, neutrophil priming for oxidant production and cytokine release, functions which can contribute to the development, implantation, angiogenesis, inflammation and metastasis of tumors (1). Levels of components of the uPA/uPAR system correlates with metastatic potential of cell lines *in vitro* and with tumor progression and patient survival *in vivo*; indeed, overexpression of uPA/uPAR was found to be significant in several human tumors including leukemias, tumors of the breast, lung, bladder, colon, liver, pleura, pancreas and brain (2-12). For this reason, inhibition of one or more of the components of this system

is an attractive target for anti-cancer therapy (13). Specific antagonists that suppress binding of uPA to uPAR have also been shown to inhibit cell-surface plasminogen activation, tumor growth and angiogenesis both *in vitro* and *in vivo* models (14-17).

The uPA/uPAR system is made up of the serine protease uPA, its cell membrane-associated receptor (uPAR), a substrate (plasminogen) and the plasminogen activator inhibitors (PAI-1 and PAI-2) (18, 19). Human uPAR is a 335 aminoacids-long polypeptide, which during the cell surface sorting is post-translationally modified, losing an aminoterminal signal peptide and a carboxyterminal GPI-anchor peptide and being processed for GPI anchoring. In addition, the protein is extensively glycosylated. The mature uPAR protein consists of three homologous cysteine-rich repeats of about 90 amino acids each (Domain I, II and III). Cell surface uPAR is found both as full length (DIDIIDIII) as well as two domains derivative (DIIDIII) having lost the amino-terminal domain. Both forms can also be found in the serum, urine and other body liquids. Their function is largely unknown. The formation of a full length soluble suPAR (sDIDIIDIII) is caused either by a proteolytic cleavage close to the GPI anchor or to the hydrolysis of the GPI-anchor by a phospholipase (20, 21). This process is referred to as uPAR *shedding*. The second type is a proteolytic cleavage in the linker region connecting DI and DII and results in the release of the D1 fragment from the rest of the receptor. These cleavages change the biochemical properties of uPAR completely, probably facilitating appearance of previously hidden epitopes on the surface of the molecule further broadening the spectrum of uPAR interactions. In fact, different conformations of uPAR are able to interact with different proteins, e.g. the interaction with FPR receptors appears to require cleavage of uPAR between DI and DII at position 84 (22). Uncleavable uPAR mutants still binding uPA can differentially interact with different trans-membrane proteins, such as integrins and the EGFR (23). Thus, cleavage of uPAR appear to be an important physiologic/pathologic event.

In addition to directing extracellular proteolysis, uPAR is a genuine signalling receptor. Indeed, uPAR knock-out mice while not showing any evidence of deficient fibrinolysis, are deficient in a series of signalling pathways. Most of the cellular responses modulated by the uPA/uPAR system, including migration, cellular adhesion, differentiation and proliferation require transmembrane signaling, which has been reported to be mediated by direct contacts of uPAR with a variety of extracellular proteins and membrane receptors, such as integrins, EGF receptor, high molecular weight kininogen, caveolin and the G-protein-coupled receptor FPRL1 (24, 25). Besides, the results of uPAR structure analysis strongly support the hypothesis that uPAR is a molecule capable of establishing multiple contacts, since it shows a large outer surface necessary for various binding sites (26-29). As a result, uPAR activates intracellular signalling molecules such as tyrosine- and serine-protein kinases, Src, focal adhesion kinase (FAK), Rac, extracellular-signal-regulated kinase (ERK)/mitogen- activated protein kinase (MAPK) and

JAK/STAT, leading to a predominantly migratory and adhesive, but also, in various cells, proliferative and more recently apoptotic response. In this review, we will confine ourselves to the biochemistry of the pathways affected by uPAR and its partners.

3. MAP KINASE PATHWAY

MAPKs are known to be key elements of signal transduction chains leading to the activation of early immediate genes (30-32). In many cancers, as breast cancer, the mitogen-activated protein kinases, extracellular signal-regulated kinase ERK-1 and ERK-2, are frequently hyperexpressed and exhibit increased activity (33). This is important because activated ERKs control many processes that are central to cancer progression, including cell growth, apoptosis and cell migration (34). Activated ERK may also promote cancer cell invasion by upregulating expression of proteinases and associated receptors that are involved in this process, including uPA and its cell-surface receptor uPAR (35-37). Most of the cellular responses modulated by the uPA/uPAR system, including migration, cellular adhesion, differentiation, proliferation and apoptosis (24) require in fact the activation of ERK1/2. In the context of tumor proliferation, the best-characterized pathway has been described by Aguirre-Ghiso and colleagues (38). They describe a uPAR-dependent mechanism by which the majority of tumor cells modulate the activity ratio between the proliferation inducer ERK (39) and the negative growh regulator p38 (40). Based on the study of 10 different cell lines, their results show how uPAR and α5β1 activate the EGFR in a EGF-independent but FAK-dependent manner (41), generate high ERK and low p38 activity necessary for the *in vivo* growth of cancer cells. A positive loop is activated in which ERK activity transactivates uPAR and uPA expression (35, 41-43). Besides, high uPAR level, by activating α5β1 maintains high ERK activity (41, 44). Sustained ERK phosphorylation allows then for nuclear localization and subsequent stabilization of c-Fos and other immediate early genes, which are necessary for S-phase entry (45-47).

The EGFR has been implicated in signalling from uPAR to ERK, as well; however, previous studies suggest that the EGFR is not essential (23, 48). In fact, in its absence, alternative co-receptors function to activate the Ras-ERK pathway (23) but when it is expressed, the EGFR assumes a dominant role and becomes essential for uPA-initiated signalling to ERK, without influencing the kinetics of ERK activation, thus promoting cell proliferation (48). Because uPAR is linked to the cell surface by a GPI anchor, it is generally assumed that uPAR signals as part of a multiprotein signalling-receptor complex (MSRC). In support of this hypothesis, soluble human uPAR (SuPAR) has been shown to activate cell signalling similarly to uPA (49-52). Jo *et al.* (52) demonstrated, for example, that human SuPAR may activate or inhibit ERK phosphorylation, depending on the state of the autocrine uPA-uPAR signalling system. They demonstrated for the first time that SuPAR may antagonize cancer progression by direct, uPA-independent effects on cell signalling. These data support a model in which uncleaved SuPAR functions

as a partial agonist that triggers cell signalling but not as effectively as membrane-anchored uPAR-uPA complex. In A1 MEFs and HEK293 cells, which lack uPAR, SuPAR find no competition and thus activates ERK. By contrast, in cells that have a highly activated autocrine signalling system, such as MDA-MB 231 breast cancer cells or MEFs2, SuPAR inhibits ERK activation and consequently, cell growth and Matrigel invasion. Since murine uPA does not bind human uPAR or suPAR (53), this precludes the alternative model in which SuPAR inhibits ERK activation by binding uPA produced endogenously by the MEFs2 (54).

These results were confirmed in xenograft animal model experiments, where SuPAR reduces the growth and the metastasis of MDA-MB 231 and OV-MZ-6#8 ovarian cancer cells (55, 56).

Cleavage of SuPAR by proteinases increases its signalling agonist activity and reverses its inhibitory effects on growth and invasion. Thus, proteolytic cleavage represents a molecular switch that neutralizes the anticancer activity of SuPAR.

In terms of cell migration and invasion, Mirshahi *et al.* many years ago demonstrated that uPA/uPAR-stimulated ovarian cell motility depends on tyrosine kinase activation (57). In 1999, using a transfection strategy that included dominant-negative and costitutively active Ras and MEK mutants, Nguyen *et al.* showed that uPA promotes cell migration, in an integrin-selective manner, by initiating a uPAR-dependent signalling cascade in which Ras, MEK, ERK and Myosin light chain kinase (MLCK) serve as essential downstream effectors (58). In these cells uPA-induced ERK activation is highly transient; however, the effects of uPA on cellular migration are sustained (59). Degryse *et al.* (2001) also showed that pro-uPA might promote a direct interaction between uPAR and integrins, modulating their function and this stimulates phosphorylation and nuclear translocation of ERK inducing rat smooth muscle cell (RSMC) migration, a pathway which differs from the one induced by Vitronectin (VN) (60).

Other reports have shown that uPAR might underlie a MEK/ERK-dependent signalling mechanism in cancers (i.e. ovarian, breast, melanoma, hepatocarcinoma) (58, 61-63) and an ERK-dependent signalling event via uPAR drove motility through polarized lamellipodia extension in colon cancer cells (64).

Since it is well known that at least two forms of uPAR are present on the cell surface (full length and cleaved uPAR), each specifically interacting with one or more transmembrane proteins, Mazzieri *et al.* (2006) exploited an uPAR mutant (*hcr*, human cleavage resistant) to dissect the pathways involved in uPA-induced cell migration. Both wild-type and *hcr*-uPAR are able to mediate uPA-induced migration, are costitutively associated with the EGFR, and associate with α3β1 integrin upon uPA binding. However they engage different pathways in response to uPA. Wt-uPAR requires both

integrins and FPRL1 to mediate uPA-induced migration, and association of wt-uPAR to α3β1 results in uPAR cleavage and ERK activation. On the contrary, *hcr*-uPAR doesn't activate ERK, but it activates an alternative pathway engaging different trans-membrane receptors. uPAR can thus signal through several types of trans-membrane receptors upon "activation" by several ligands and/or upon cleavage by different proteases.

A recent evidence shows a close relationship between the uPA/uPAR system and cell sensitivity to programmed cell death (65). In this context the anti-apoptotic ability of uPAR may be due, at least in part, to its ability to activate the Ras-ERK signalling pathway in many different cell types. In MDA-MB-231 breast cancer cells cultured in the presence of anti-uPA antibodies that block the binding of uPA to uPAR, the level of phosphorylated ERK decreases substantially and apoptosis is promoted, showing that endogenous uPA is a major determinant of ERK activation and protection from apoptosis (66). Activated ERK was also necessary to maintain uPA and uPAR expression. This positive-feedback loop may in fact be critical in determining the aggressive nature of MDA-MB-231 cells. The ability of uPAR and ERK to function in a positive feedback loop and to suppress apoptosis represents a novel mechanism whereby the uPA-uPAR system may promote cancer progression.

4. FOCAL ADHESION KINASE SIGNALLING

FAK is a cytoplasmic tyrosine kinase involved in the transduction of signals generated by cell matrix contacts and is overexpressed in several human cancers. It localizes to focal adhesions and becomes tyrosine phosphorylated in response to integrin-derived signals for motility, survival and proliferation (67, 68). Yebra *et al.* (69) demonstrated an association between uPAR and β1 integrin in the cytoskeletal fraction of a LNCaP human prostate carcinoma cell line, that depends on the presence of uPA. This findings suggests that uPA binding to uPAR induces either a conformational change or a change in the lateral mobility of uPAR so it can physically associate with α5β1, leading to enhanced FAK and p130^{Cas} tyrosine phosphorylation and enhanced cell migration by increasing turnover of focal adhesion contacts (69). Nguyen *et al.* in 2000 also showed that binding of uPA to uPAR can stimulate the Ras/ERK signalling pathway and migration of MCF-7 breast cancer cells by a mechanism that requires FAK, Src and Shc (70). Most importantly Aguirre Ghiso in 2002 showed one of the first attempts of testing the role of FAK in signal transduction induced by activating association of uPAR with integrin and its effect on epithelial tumor growth *in vivo*. He explored the role of FAK in regulating tumorigenicity of human carcinoma cells, HEp3, which is dependent on uPAR- α5β1-integrin association (41, 71). Active FAK is an important mediator of uPAR-regulated tumorigenicity of HEp3 cells and interruption of FAK mitogenic signalling either through down-regulation of uPAR or by expression of a FAK related non-kinase (FRNK), known to have a dominant negative function, can force human carcinoma cells into dormancy (72). These results lend functional

significance to the finding of frequent overexpression of FAK and uPAR in tumors from different origin, indicating that uPAR-mediated active FAK may enable tumor cells to activate more efficiently survival and mitogenic signals derived from the ECM, providing a growth advantage at the primary or metastatic growth site.

A role for caveolin, a non-integrin membrane protein, and uPAR in integrin-mediated adhesion and signalling has also been shown (73-75). Many data indicate that uPAR is localized in caveolae and forms a stable complex with caveolin (76-79). Caveolin and uPAR may in fact operate within adhesion sites to organize kinase-rich lipid domains in proximity to integrins, promoting efficient signal transduction. Lipid rafts seem to be important for src-kinase signalling and for the GP130 mediated pathway: upon clustering of uPAR, activation of JAK occurred, followed by STAT phosphorylation and redistribution from the caveolae to the nucleus (80).

5. JAK/STAT- AND Src-FAMILY SIGNALLING PATHWAY

Kinases of the Janus kinase (JAK)-family were found to be associated with uPAR, in several cell types. One example is the kidney tumor epithelial cell line TCL-598 in which uPAR was found to be associated with JAK1 and STAT proteins in detergent-insoluble membrane fractions, as revealed by coimmunoprecipitation (80). Upon clustering of uPA/uPAR complex by a monoclonal antibody, JAK1 associates with uPAR, which in turn leads to STAT1 phosphorylation, dimerization, nuclear translocation, specific binding to the DNA interferon-gamma activation site (81) or interferon-stimulated response elements (ISREs), and gene activation (80). Similar findings were reported by Dumler *et al.* showing that uPA binding to its receptor induces the JAK/STAT pathway, thereby regulating migration of smooth muscle cells (82, 83). In this case uPAR was found to co-localize with JAK1 and Tyk2 in the leading edge of the migrating human aortic smooth muscle cells, while JAK2, JAK3 and the Src-PTKs remained mobile in the plane of the plasma membrane. This result links uPAR to a known signalling pathway mainly utilized by cytokines. Thereby, most likely the glycoprotein (GP) gp130 might be the transmembrane adapter for this signal transduction pathway.

More recent data show for the first time that uPA leads to activation of STAT3, independent of its catalytic activity but dependent on its interaction with uPAR, leading to DNA synthesis in lung epithelial cells (84). Jo *et al.* instead demonstrate that in Chinese hamster ovary (CHO)-K1 cells, EGFR supports uPA mitogenic activity by recruiting and activating STAT5b downstream of uPAR. They support a model in which STAT5b and ERK function independently, but in a complementary manner, to promote cell growth and that the composition of the uPAR multiprotein signalling-receptor complex (MSRC) is critical in determining cellular response to uPA (85).

Concerning regulation of transcriptional activity, induction of the c-fos gene has also been shown as a consequence of uPAR activation (86, 87), also indicating involvement of STAT1 in signal transduction.

Besides the JAK-family, src-kinases can also associate with uPAR in several cell types (81, 88, 89). c-Src, normally localized to endosomal membranes, is redistributed to focal adhesions upon cell activation, where it regulates cell adhesion and migration (90-94). Fazioli *et al.* demonstrated that peptides derived from the linker region between the first and the second domains of uPAR, which contain the sequence SRSRY, activate the Src-family tyrosine kinase, p56/p59^{hck}, and demonstrate chemotactic activity, similarly to uPA (81). In smooth muscle cells, uPA induces the sub-cellular relocalization of c-Src to the plasma membrane, preferentially toward the leading-edge of migration (95). uPA also causes cytoskeletal reorganization in c-Src$^{+/+}$ but not in c-Src$^{-/-}$ fibroblasts (95). However, in endothelial cells, uPA activates ERK by a pathway that is not affected by a general antagonist of Src family kinases (96). Nguyen *et al.*, on the contrary, support a model in which c-Src is necessary for uPA-induced ERK activation and MCF-7 cell migration, together with FAK, Shc and Ras, which demonstrates for the first time a link between uPAR and Ras (70). Although they cannot precisely define the relationship of these factors in activating the Ras-ERK pathway, it is significant that the uPAR-initiated pathway shares many similarities with pathways that link integrins to ERK (97-101).

In a more recent study, experiments were conducted by Monaghan-Benson *et al.*, to address the mechanism of uPAR regulation of matrix assembly. This group has previously demonstrated that treatment of fibroblasts with the uPAR ligand, P25, results in an increase in the activation of the β1 integrin and a 35-fold increase in fibronectin matrix assembly (102). Lately, they demonstrated that ligation of uPAR with P25 causes a Src-dependent transactivation of the EGFR and promotes the formation of EGFR-β1 integrin complexes. Both Src kinase and EGFR are required for the uPAR-dependent increase in β1 integrin activation and fibronectin matrix assembly (103). These studies suggest that both uPAR and EGFR may represent novel targets for the regulation of fibronectin matrix deposition under conditions where dysregulated fibronectin deposition may contribute to pathological conditions such as tumor survival and tissue fibrosis.

6. INTRACELLULAR CALCIUM MOBILIZATION

As a GPI-anchored protein, uPAR has no direct link to signalling elements inside the cells (104). This could be circumvented by uPAR using other proteins as signal transduction devices, and precisely this mechanism has been demonstrated for uPA-induced Ca^{2+} fluxes in neutrophils, in which CR3 (Mac-1; CD11b/CD18), a β2 integrin, serves as the partner protein (105). However, few years later Sitrin *et al.*, demonstrated that uPAR aggregation of human promyelocytic cell line U937 and human monocytes, initiates phosphoinositide hydrolysis and subsequent Ca^{2+} mobilization by mechanism that are not strictly dependent on associated uPA or CR3 (106). At

the same time Christow *et al.* using patch-clamp techniques showed that uPA binding to uPAR stimulates Ca^{2+}-activated K^+ channels via induction of inositol 1,4,5-triphosphate (Ins (1,4,5)P$_3$) formation and the liberation of Ca^{2+} from internal stores by G-protein- and phospholipase C-dependent mechanism (107). The release of Ca^{2+}, as indicated by FRET analysis, could be linked also to a direct interaction of uPAR with L-selectin (CD62L), an adhesion protein that participates in the initial stages of leukocyte rolling on endothelial cells (106). Essentially all chemokine receptors induce the mobilization of Ca^{2+} from intracellular stores, and FPRL1, which likely mediates uPA signaling, is not an exception (108). However, uPA and uPAR are different, as clustering of uPAR must occur before uPA can induce the mobilization of Ca^{2+} (106).

7. Rho-FAMILY GTPase AND VITRONECTIN (VN)-INDUCED SIGNALLING PATHWAY

An additional complication to the role of uPAR in cell adhesion and motility is its ability to bind VN. Binding of multimeric or surface-absorbed forms of VN to uPAR has been demonstrated both *in vitro* with purified components and *in vivo* where the uPAR-VN interaction mediates cellular adhesion of cytokine-stimulated monocytes as well as uPAR-transfected HEK293 and erythroid progenitor cells (79, 109-112). uPAR-mediated cell adhesion to Vn does not always depend on receptor occupancy as several transfected cell lines, which do not produce uPA, still adhere strongly to VN in a uPAR-dependent manner (79, 113-115). However at physiological expression levels, uPAR-dependent cell adhesion to Vn requires uPA binding (112, 115, 116). Lately Madsen *et al.*, showed that a direct uPAR–Vn interaction is required for ERK1/2 activation, as Vn binding–deficient uPAR mutants displayed levels of active ERK1/2 comparable to those of mock-transfected cells (117). Interestingly, uPA binding to uPAR also leads to ERK1/2 activation in different experimental systems (59, 71), suggesting that both overexpression of the receptor and ligand binding induces the same signal transduction pathway (s), possibly through a common molecular mechanism. However, it appears likely that uPA binding may actually induce "Vn signaling" by stimulating uPAR binding to matrix Vn. In support of this possibility there is a strict correlation between the ability of pro-uPA to promote Vn binding and to induce ERK1/2-activation and changes in cell morphology (117). The interactions of uPAR with components normally associated with cytoskeletal structures such as integrins and extracellular matrix molecules and its co-localization with integrins and cytoskeletal components such as vinculin at sites of cell-matrix contact (118-122) suggest that its role in cell motility may involve regulation of the actin cytoskeleton. It has been demonstrated years ago that the interaction between cell surface uPAR and ECM Vn causes a potent induction of actin cytoskeleton rearrangement and cell motility (60) by a mechanism which requires Rac-activation (123).

In the regulation of the actin cytoskeleton, small GTPases of the Rho-family play a pivotal role and the best

characterized members of this family are Rho, Rac and Cdc42. Kjoller *et al.* showed that upon uPAR binding to VN, RhoA and Cdc42 are not involved in actin reorganization, while this process resulted to be Rac-dependent and accompanied by an increase in Rac-mediated Swiss 3T3 cell motility (113). However, independently from VN, uPAR was found to activate Rac and regulate lamella/ruffling activity in Hct-116 and BE colon carcinoma cells in an ERK-dependent manner (64). Besides, other studies strongly suggests that the small GTPases RhoA and Rac1 may be important downstream mediators of the uPAR/Tyk2/PI3-K signaling pathway in human vascular smooth muscle cells (124).

8. OTHER SIGNALLING MOLECULES

Several signaling molecules were found to cooperate with uPAR, including vinculin, alpha-actinin, actin (125), PKC (126) and PI3-K (124, 127, 128). In the last case, the positive correlation between uPAR expression level and activation of the PI3K-Akt-dependent anti-apoptotic pathway is suggested by the finding that glioblastoma cells bearing an antisense to uPA exhibit a reduced level of phosphorylated PI3K and Akt as well as impaired migration and survival (124, 127, 128). It has been shown that uPAR itself, other than concentrating uPA proteolytic activity on cell surface and being a mediator of most ligand-dependent effects on growth, motility and apoptosis, could be an anti-apoptotic factor (66). The reduced levels of active PI3K/Akt and ERK1/s in uPAR-deficient cells indicate that uPAR may modulate the survival/apoptosis ratio through the control of crucial signaling cascades.

Several years ago two groups demonstrated that the DII-DIII fragment of uPAR was involved in binding to the cation-independent, mannose 6-phosphate/insulin-like growth factor-II (M6P/IGF-II) receptor (129, 130). This binding is not affected by uPA or mannose-6-phosphate and leads to internalization of uPAR in lysosomes (130). Whether association of uPAR with M6P/IGF-II receptor has only clearing function or might contribute to signal transduction is not yet understood. The M6P/IGF-II receptor interaction with uPAR, however, seems to be involved in the plasmin-dependent generation of TGF-β and, thereby, indirectly in signal transduction via uPA/uPAR (129).

9. THE uPAR-INDUCED SIGNALLING PATHWAYS AS TARGET FOR ANTI-CANCER THERAPY

Researchers that have used either antisense or siRNA technologies for the successful *in vivo* downregulation of uPAR in various cancers have concurrently tested these same technologies in *in vitro* biological assays. Evaluation of the results of these *in vitro* assays reveals that downregulation of uPAR has lead, in most cases, to inhibition of invasion (14, 131-135), migration (131, 133), adhesion (131) and proliferation (14, 132, 134). In addition, reduced uPAR levels lead to inhibition of tumor-induced angiogenesis (132) and ECM degradation (136, 137).

As stated earlier, some of the biological functions of uPAR, such as proliferation, are facilitated by the regulation of several different signaling molecules. In an attempt to understand and/or elucidate the involvement of uPAR in downstream signaling pathways, studies have investigated the effect of uPAR downregulation on components of the relevant signaling pathways. D'Alessio *et al.* (14) reported that melanoma cells exhibited a strong decrease in ERK1/2 activation when an 18mer asODN was used to downregulate uPAR. Using this same asODN for the downregulation of uPAR in prostate cancer cells, Margheri *et al.* (134) reported a strong decrease of FAK/JNK/Jun phosphorylation (thereby causing a decrease in the activation of the FAK/JNK/Jun pathway). At the same time, the synthesis of cyclins A, B, D1 and D3 was inhibited, and these prostate cancer cells accumulated in the G2 phase of the cell cycle. The downregulation of uPAR by a plasmid construct expressing shRNA for uPAR resulted in significantly reduced levels of the phosphorylated forms of MAPK, ERK and AKT signaling pathway molecules (132). However, the majority of studies applying uPAR downregulation for cancer *in vivo* failed to identify the perturbed signaling pathways. In any case, different laboratories chose to elucidate effects on different pathways and, although there is an abundance of literature looking at individual pathways *in vitro,* it is difficult to compare results from separate studies because various parameters, including cell line, passage number, minor technical differences, the antisense sequence, the concentration of constructs, the time-points evaluated, and the way the data are reported, often prevent such comparisons.

10. CONCLUSION: THE uPAR SIGNALOSOME

It is now well established that the uPA/uPAR system is connected to the malignant process of tumor growth and invasion and it is clear that uPAR is part of a large "signalosome" associated and interacting with several proteins on both the outside and inside of the cell. Most of the cellular responses modulated by the uPA/uPAR system, including migration, cellular adhesion, differentiation, proliferation and apoptosis require transmembrane signaling, which cannot be mediated directly by a GPI-anchored protein such as uPAR. For this reason, besides the well-established interactions with uPA and Vn, uPAR has been reported to entertain direct contacts with a variety of extracellular proteins and membrane receptors, such as integrins, EGF receptor, high molecular weight kininogen, caveolin and the G-protein-coupled receptor fMLP-receptors. Besides, the results of uPAR structure analysis (29), strongly support the hypothesis that uPAR is a molecule capable of establishing multiple contacts, since it shows a large outer surface necessary for various binding sites. As a result, uPAR activates intracellular signalling molecules such as tyrosine-, serine-protein kinases and small G proteins, Src, focal adhesion kinase (FAK), Rac, extracellular-signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) and JAK/STAT, leading to a predominantly migratory and adhesive, but also, in various cells, proliferative and more recently apoptotic response. In an attempt to understand and/or elucidate the involvement of uPAR in downstream signaling pathways, studies have investigated the effect of uPAR downregulation on components of the relevant signaling pathways, validating uPAR as an anti-tumor therapeutic target and many studies are still in progress. However, the existence of many redundant cellular processes in nature would suggest that because the uPA/uPAR system is inhibited, other similar systems might become biologically more active and ultimately overrule the inhibition of the uPA/uPAR system. Therefore, combination therapy that includes the uPA/uPAR system among others, and that targets several processes at a time may have a greater chance of success at producing tumor killing while reducing the development of resistance.

11. REFERENCES

1. Y. Ge and M. T. Elghetany: Urokinase plasminogen activator receptor (CD87): something old, something new. *Lab Hematol* 9, 67-71 (2003)

2. M. V. Carriero, S. Del Vecchio, P. Franco, M. I. Potena, F. Chiaradonna, G. Botti, M. P. Stoppelli and M. Salvatore: Vitronectin binding to urokinase receptor in human breast cancer. *Clin Cancer Res* 3, 1299-308 (1997)

3. G. De Petro, D. Tavian, A. Copeta, N. Portolani, S. M. Giulini and S. Barlati: Expression of urokinase-type plasminogen activator (u-PA), u-PA receptor, and tissue-type PA messenger RNAs in human hepatocellular carcinoma. *Cancer Res* 58, 2234-9 (1998)

4. F. Lanza, G. L. Castoldi, B. Castagnari, R. F. Todd, 3rd, S. Moretti, S. Spisani, A. Latorraca, E. Focarile, M. G. Roberti and S. Traniello: Expression and functional role of urokinase-type plasminogen activator receptor in normal and acute leukaemic cells. *Br J Haematol* 103, 110-23 (1998)

5. S. Morita, A. Sato, H. Hayakawa, H. Ihara, T. Urano, Y. Takada and A. Takada: Cancer cells overexpress mRNA of urokinase-type plasminogen activator, its receptor and inhibitors in human non-small-cell lung cancer tissue: analysis by Northern blotting and *in situ* hybridization. *Int J Cancer* 78, 286-92 (1998)

6. S. Mustjoki, R. Alitalo, R. W. Stephens and A. Vaheri: Blast cell-surface and plasma soluble urokinase receptor in acute leukemia patients: relationship to classification and response to therapy. *Thromb Haemost* 81, 705-10 (1999)

7. S. Mustjoki, R. Alitalo, R. W. Stephens and A. Vaheri: Plasminogen activation in human leukemia and in normal hematopoietic cells. *Apmis* 107, 144-9 (1999)

8. T. Plesner, E. Ralfkiaer, M. Wittrup, H. Johnsen, C. Pyke, T. L. Pedersen, N. E. Hansen and K. Dano: Expression of the receptor for urokinase-type plasminogen activator in normal and neoplastic blood

cells and hematopoietic tissue. *Am J Clin Pathol* 102, 835-41 (1994)

9. C. Pyke, P. Kristensen, E. Ralfkiaer, J. Grondahl-Hansen, J. Eriksen, F. Blasi and K. Dano: Urokinase-type plasminogen activator is expressed in stromal cells and its receptor in cancer cells at invasive foci in human colon adenocarcinomas. *Am J Pathol* 138, 1059-67 (1991)

10. S. Shetty and S. Idell: A urokinase receptor mRNA binding protein-mRNA interaction regulates receptor expression and function in human pleural mesothelioma cells. *Arch Biochem Biophys* 356, 265-79 (1998)

11. T. Taniguchi, A. K. Kakkar, E. G. Tuddenham, R. C. Williamson and N. R. Lemoine: Enhanced expression of urokinase receptor induced through the tissue factor-factor VIIa pathway in human pancreatic cancer. *Cancer Res* 58, 4461-7 (1998)

12. M. Yamamoto, R. Sawaya, S. Mohanam, A. K. Bindal, J. M. Bruner, K. Oka, V. H. Rao, M. Tomonaga, G. L. Nicolson and J. S. Rao: Expression and localization of urokinase-type plasminogen activator in human astrocytomas *in vivo*. *Cancer Res* 54, 3656-61 (1994)

13. C. W. Crowley, R. L. Cohen, B. K. Lucas, G. Liu, M. A. Shuman and A. D. Levinson: Prevention of metastasis by inhibition of the urokinase receptor. *Proc Natl Acad Sci U S A* 90, 5021-5 (1993)

14. S. D'Alessio, F. Margheri, M. Pucci, A. Del Rosso, B. P. Monia, M. Bologna, C. Leonetti, M. Scarsella, G. Zupi, G. Fibbi and M. Del Rosso: Antisense oligodeoxynucleotides for urokinase-plasminogen activator receptor have anti-invasive and anti-proliferative effects *in vitro* and inhibit spontaneous metastases of human melanoma in mice. *Int J Cancer* 110, 125-33 (2004)

15. H. Li, H. Lu, F. Griscelli, P. Opolon, L. Q. Sun, T. Ragot, Y. Legrand, D. Belin, J. Soria, C. Soria, M. Perricaudet and P. Yeh: Adenovirus-mediated delivery of a uPA/uPAR antagonist suppresses angiogenesis-dependent tumor growth and dissemination in mice. *Gene Ther* 5, 1105-13 (1998)

16. H. Y. Min, L. V. Doyle, C. R. Vitt, C. L. Zandonella, J. R. Stratton-Thomas, M. A. Shuman and S. Rosenberg: Urokinase receptor antagonists inhibit angiogenesis and primary tumor growth in syngeneic mice. *Cancer Res* 56, 2428-33 (1996)

17. V. Pillay, C. R. Dass and P. F. M. Choong: The urokinase plasminogen activator receptor as a gene therapy target for cancer. *Trends in Biotechnology* 25, 33-39 (2007)

18. A. Mazar, J. Henkin and R. Goldfarb: The urokinase plasminogen activator system in cancer: Implications for

tumor angiogenesis and metastasis. *Angiogenesis* 3, 15-32 (1999)

19. Y. Wang: The role and regulation of urokinase-type plasminogen activator receptor gene expression in cancer invasion and metastasis. *Med Res Rev* 21, 146-70 (2001)

20. N. Pedersen, M. Schmitt, E. Ronne, M. I. Nicoletti, G. Hoyer-Hansen, M. Conese, R. Giavazzi, K. Dano, W. Kuhn, F. Janicke and *et al.*: A ligand-free, soluble urokinase receptor is present in the ascitic fluid from patients with ovarian cancer. *J Clin Invest* 92, 2160-7 (1993)

21. C. F. Sier, R. Stephens, J. Bizik, A. Mariani, M. Bassan, N. Pedersen, L. Frigerio, A. Ferrari, K. Dano, N. Brunner and F. Blasi: The level of urokinase-type plasminogen activator receptor is increased in serum of ovarian cancer patients. *Cancer Res* 58, 1843-9 (1998)

22. M. Resnati, I. Pallavicini, J. M. Wang, J. Oppenheim, C. N. Serhan, M. Romano and F. Blasi: The fibrinolytic receptor for urokinase activates the G protein-coupled chemotactic receptor FPRL1/LXA4R. *Proc Natl Acad Sci U S A* 99, 1359-64 (2002)

23. R. Mazzieri, S. D'Alessio, R. K. Kenmoe, L. Ossowski and F. Blasi: An uncleavable uPAR mutant allows dissection of signaling pathways in uPA-dependent cell migration. *Mol Biol Cell* 17, 367-78 (2006)

24. F. Blasi and P. Carmeliet: uPAR: a versatile signalling orchestrator. *Nat Rev Mol Cell Biol* 3, 932-43 (2002)

25. L. Ossowski and J. A. Aguirre-Ghiso: Urokinase receptor and integrin partnership: coordination of signaling for cell adhesion, migration and growth. *Curr Opin Cell Biol* 12, 613-20 (2000)

26. C. Barinka, G. Parry, J. Callahan, D. E. Shaw, A. Kuo, K. Bdeir, D. B. Cines, A. Mazar and J. Lubkowski: Structural basis of interaction between urokinase-type plasminogen activator and its receptor. *J Mol Biol* 363, 482-95 (2006)

27. Q. Huai, A. P. Mazar, A. Kuo, G. C. Parry, D. E. Shaw, J. Callahan, Y. Li, C. Yuan, C. Bian, L. Chen, B. Furie, B. C. Furie, D. B. Cines and M. Huang: Structure of human urokinase plasminogen activator in complex with its receptor. *Science* 311, 656-9 (2006)

28. M. Huang, A. P. Mazar, G. Parry, A. A. Higazi, A. Kuo and D. B. Cines: Crystallization of soluble urokinase receptor (suPAR) in complex with urokinase amino-terminal fragment (1-143). *Acta Crystallogr D Biol Crystallogr* 61, 697-700 (2005)

29. P. Llinas, M. H. Le Du, H. Gardsvoll, K. Dano, M. Ploug, B. Gilquin, E. A. Stura and A. Menez: Crystal

structure of the human urokinase plasminogen activator receptor bound to an antagonist peptide. *Embo J* 24, 1655-63 (2005)

30. M. A. Cahill, R. Janknecht and A. Nordheim: Signalling pathways: jack of all cascades. *Curr Biol* 6, 16-9 (1996)

31. E. Selva, D. L. Raden and R. J. Davis: Mitogen-activated protein kinase stimulation by a tyrosine kinase-negative epidermal growth factor receptor. *J Biol Chem* 268, 2250-4 (1993)
32. B. Su and M. Karin: Mitogen-activated protein kinase cascades and regulation of gene expression. *Curr Opin Immunol* 8, 402-11 (1996)

33. V. S. Sivaraman, H. Wang, G. J. Nuovo and C. C. Malbon: Hyperexpression of mitogen-activated protein kinase in human breast cancer. *J Clin Invest* 99, 1478-83 (1997)

34. S. Y. Cho and R. L. Klemke: Extracellular-regulated kinase activation and CAS/Crk coupling regulate cell migration and suppress apoptosis during invasion of the extracellular matrix. *J Cell Biol* 149, 223-36 (2000)

35. E. Lengyel, H. Wang, R. Gum, C. Simon, Y. Wang and D. Boyd: Elevated urokinase-type plasminogen activator receptor expression in a colon cancer cell line is due to a constitutively activated extracellular signal-regulated kinase-1-dependent signaling cascade. *Oncogene* 14, 2563-73 (1997)

36. M. Seddighzadeh, J. N. Zhou, U. Kronenwett, M. C. Shoshan, G. Auer, M. Sten-Linder, B. Wiman and S. Linder: ERK signalling in metastatic human MDA-MB-231 breast carcinoma cells is adapted to obtain high urokinase expression and rapid cell proliferation. *Clin Exp Metastasis* 17, 649-54 (1999)

37. C. Simon, J. Juarez, G. L. Nicolson and D. Boyd: Effect of PD 098059, a specific inhibitor of mitogen-activated protein kinase kinase, on urokinase expression and *in vitro* invasion. *Cancer Res* 56, 5369-74 (1996)

38. J. A. Aguirre-Ghiso, Y. Estrada, D. Liu and L. Ossowski: ERK (MAPK) activity as a determinant of tumor growth and dormancy; regulation by p38 (SAPK). *Cancer Res* 63, 1684-95 (2003)

39. G. Chen, M. Hitomi, J. Han and D. W. Stacey: The p38 pathway provides negative feedback for Ras proliferative signaling. *J Biol Chem* 275, 38973-80 (2000)

40. D. Liu, J. A. A. Ghiso, Y. Estrada and L. Ossowski: EGFR is a transducer of the urokinase receptor initiated signal that is required for *in vivo* growth of a human carcinoma. *Cancer Cell* 1, 445-457 (2002)

41. J. A. Aguirre-Ghiso, D. Liu, A. Mignatti, K. Kovalski and L. Ossowski: Urokinase receptor and

fibronectin regulate the ERK (MAPK) to p38 (MAPK) activity ratios that determine carcinoma cell proliferation or dormancy *in vivo*. *Mol Biol Cell* 12, 863-79 (2001)

42. E. Lengyel, E. Stepp, R. Gum and D. Boyd: Involvement of a mitogen-activated protein kinase signaling pathway in the regulation of urokinase promoter activity by c-Ha-ras. *J Biol Chem* 270, 23007-12 (1995)

43. E. Lengyel, H. Wang, E. Stepp, J. Juarez, Y. Wang, W. Doe, C. M. Pfarr and D. Boyd: Requirement of an upstream AP-1 motif for the constitutive and phorbol ester-inducible expression of the urokinase-type plasminogen activator receptor gene. *J Biol Chem* 271, 23176-84 (1996)

44. N. Montuori, M. V. Carriero, S. Salzano, G. Rossi and P. Ragno: The cleavage of the urokinase receptor regulates its multiple functions. *J Biol Chem* 277, 46932-9 (2002)

45. K. Balmanno and S. J. Cook: Sustained MAP kinase activation is required for the expression of cyclin D1, p21Cip1 and a subset of AP-1 proteins in CCL39 cells. *Oncogene* 18, 3085-97 (1999)

46. L. O. Murphy, S. Smith, R. H. Chen, D. C. Fingar and J. Blenis: Molecular interpretation of ERK signal duration by immediate early gene products. *Nat Cell Biol* 4, 556-64 (2002)

47. J. D. Weber, W. Hu, S. C. Jefcoat, Jr., D. M. Raben and J. J. Baldassare: Ras-stimulated extracellular signal-related kinase 1 and RhoA activities coordinate platelet-derived growth factor-induced G1 progression through the independent regulation of cyclin D1 and p27. *J Biol Chem* 272, 32966-71 (1997)

48. M. Jo, K. S. Thomas, D. M. O'Donnell and S. L. Gonias: Epidermal growth factor receptor-dependent and -independent cell-signaling pathways originating from the urokinase receptor. *J Biol Chem* 278, 1642-6 (2003)

49. G. Melillo, T. Musso, A. Sica, L. S. Taylor, G. W. Cox and L. Varesio: A hypoxia-responsive element mediates a novel pathway of activation of the inducible nitric oxide synthase promoter. *J Exp Med* 182, 1683-93 (1995)

50. J. Pollanen, R. W. Stephens and A. Vaheri: Directed plasminogen activation at the surface of normal and malignant cells. *Adv Cancer Res* 57, 273-328 (1991)

51. M. Schmitt, N. Harbeck, C. Thomssen, O. Wilhelm, V. Magdolen, U. Reuning, K. Ulm, H. Hofler, F. Janicke and H. Graeff: Clinical impact of the plasminogen activation system in tumor invasion and metastasis: prognostic relevance and target for therapy. Thromb Haemost 78, 285-96 (1997)

52. M. Jo, K. S. Thomas, L. Wu and S. L. Gonias: Soluble urokinase-type plasminogen activator receptor inhibits cancer cell growth and invasion by direct urokinase-independent effects on cell signaling. J Biol Chem 278, 46692-8 (2003)

53. A. Estreicher, A. Wohlwend, D. Belin, W. D. Schleuning and J. D. Vassalli: Characterization of the cellular binding site for the urokinase-type plasminogen activator. J Biol Chem 264, 1180-9 (1989)

54. A. M. Weaver, I. M. Hussaini, A. Mazar, J. Henkin and S. L. Gonias: Embryonic fibroblasts that are genetically deficient in low density lipoprotein receptor-related protein demonstrate increased activity of the urokinase receptor system and accelerated migration on vitronectin. *J Biol Chem* 272, 14372-9 (1997)

55. A. Kruger, R. Soeltl, V. Lutz, O. G. Wilhelm, V. Magdolen, E. E. Rojo, P. A. Hantzopoulos, H. Graeff, B. Gansbacher and M. Schmitt: Reduction of breast carcinoma tumor growth and lung colonization by overexpression of the soluble urokinase-type plasminogen activator receptor (CD87). *Cancer Gene Ther* 7, 292-9 (2000)

56. V. Lutz, U. Reuning, A. Kruger, T. Luther, S. P. von Steinburg, H. Graeff, M. Schmitt, O. G. Wilhelm and V. Magdolen: High level synthesis of recombinant soluble urokinase receptor (CD87) by ovarian cancer cells reduces intraperitoneal tumor growth and spread in nude mice. *Biol Chem* 382, 789-98 (2001)

57. S. S. Mirshahi, K. C. Lounes, H. Lu, E. Pujade-Lauraine, Z. Mishal, J. Benard, A. Bernadou, C. Soria and J. Soria: Defective cell migration in an ovarian cancer cell line is associated with impaired urokinase-induced tyrosine phosphorylation. *FEBS Lett* 411, 322-6 (1997)

58. D. H. D. Nguyen, A. D. Catling, D. J. Webb, M. Sankovic, L. A. Walker, A. V. Somlyo, M. J. Weber and S. L. Gonias: Myosin Light Chain Kinase Functions Downstream of Ras/ERK to Promote Migration of Urokinase-type Plasminogen Activator-stimulated Cells in an Integrin-selective Manner. *J Cell Biol* 149-164 (1999).

59. D. H. D. Nguyen, I. M. Hussaini and S. L. Gonias: Binding of Urokinase-type Plasminogen Activator to Its Receptor in MCF-7 Cells Activates Extracellular Signal-regulated Kinase 1 and 2 Which Is Required for Increased Cellular Motility. *J Biol Chem* 8502-8507 (1998).

60. B. Degryse, S. Orlando, M. Resnati, S. A. Rabbani and F. Blasi: Urokinase/urokinase receptor and vitronectin/alpha (v)beta (3) integrin induce chemotaxis and cytoskeleton reorganization through different signaling pathways. *Oncogene* 20, 2032-43 (2001)

61. A. Bessard, C. Fremin, F. Ezan, A. Coutant and G. Baffet: MEK/ERK-dependent uPAR expression is required for motility via phosphorylation of P70S6K in human hepatocarcinoma cells. *J Cell Physiol* 212, 526-36 (2007)

62. S. Lee and D. M. Helfman: Cytoplasmic p21Cip1 is involved in Ras-induced inhibition of the ROCK/LIMK/cofilin pathway. *J Biol Chem* 279, 1885-91 (2004)

63. P. K. Morrow and E. S. Kim: New biological agents in the treatment of advanced non-small cell lung cancer. *Semin Respir Crit Care Med* 26, 323-32 (2005)

64. E. Vial, E. Sahai and C. J. Marshall: ERK-MAPK signaling coordinately regulates activity of Rac1 and RhoA for tumor cell motility. *Cancer Cell* 4, 67-79 (2003)

65. L. S. Gutierrez, A. Schulman, T. Brito-Robinson, F. Noria, V. A. Ploplis and F. J. Castellino: Tumor development is retarded in mice lacking the gene for urokinase-type plasminogen activator or its inhibitor, plasminogen activator inhibitor-1. *Cancer Res* 60, 5839-47 (2000)

66. Z. Ma, D. J. Webb, M. Jo and S. L. Gonias: Endogenously produced urokinase-type plasminogen activator is a major determinant of the basal level of activated ERK/MAP kinase and prevents apoptosis in MDA-MB-231 breast cancer cells. *J Cell Sci* 3387-3396 (2001).

67. L. A. Cary and J. L. Guan: Focal adhesion kinase in integrin-mediated signaling. *Front Biosci* 4, D102-13 (1999)

68. F. G. Giancotti and E. Ruoslahti: Integrin signaling. *Science* 285, 1028-32 (1999)

69. M. Yebra, L. Goretzki, M. Pfeifer and B. M. Mueller: Urokinase-Type Plasminogen Activator Binding to Its Receptor Stimulates Tumor Cell Migration by Enhancing Integrin-Mediated Signal Transduction. *Experimental Cell Research* 250, 231-240 (1999)

70. D. H. Nguyen, D. J. Webb, A. D. Catling, Q. Song, A. Dhakephalkar, M. J. Weber, K. S. Ravichandran and S. L. Gonias: Urokinase-type plasminogen activator stimulates the Ras/Extracellular signal-regulated kinase (ERK) signaling pathway and MCF-7 cell migration by a mechanism that requires focal adhesion kinase, Src, and Shc. Rapid dissociation of GRB2/Sps-Shc complex is associated with the transient phosphorylation of ERK in urokinase-treated cells. *J Biol Chem* 275, 19382-8 (2000)

71. J. A. Aguirre Ghiso, K. Kovalski and L. Ossowski: Tumor Dormancy Induced by Downregulation of Urokinase Receptor in Human Carcinoma Involves

Integrin and MAPK Signaling. *J Cell Biol* 89-104 (1999).

72. J. A. Aguirre Ghiso: Inhibition of FAK signaling activated by urokinase receptor induces dormancy in human carcinoma cells *in vivo*. *Oncogene* 21, 2513-24 (2002)

73. K. K. Wary, F. Mainiero, S. J. Isakoff, E. E. Marcantonio and F. G. Giancotti: The adaptor protein Shc couples a class of integrins to the control of cell cycle progression. *Cell* 87, 733-43 (1996)

74. Y. Wei, M. Lukashev, D. I. Simon, S. C. Bodary, S. Rosenberg, M. V. Doyle and H. A. Chapman: Regulation of integrin function by the urokinase receptor. *Science* 273, 1551-5 (1996)

75. Y. Wei, X. Yang, Q. Liu, J. A. Wilkins and H. A. Chapman: A role for caveolin and the urokinase receptor in integrin-mediated adhesion and signaling. *J Cell Biol* 144, 1285-94 (1999)

76. S. Shaya, A. L. Kindzelskii, J. Minor, E. C. Moore, R. F. Todd, 3rd and H. R. Petty: Aberrant integrin (CR4; alpha (x)beta2; CD11c/CD18) oscillations on neutrophils in a mild form of pyoderma gangrenosum. *J Invest Dermatol* 111, 154-8 (1998)

77. R. G. Sitrin, R. F. Todd, 3rd, E. Albrecht and M. R. Gyetko: The urokinase receptor (CD87) facilitates CD11b/CD18-mediated adhesion of human monocytes. *J Clin Invest* 97, 1942-51 (1996)

78. A. Stahl and B. M. Mueller: The urokinase-type plasminogen activator receptor, a GPI-linked protein, is localized in caveolae. *J Cell Biol* 129, 335-44 (1995)

79. Y. Wei, D. A. Waltz, N. Rao, R. J. Drummond, S. Rosenberg and H. A. Chapman: Identification of the urokinase receptor as an adhesion receptor for vitronectin. *J Biol Chem* 269, 32380-8 (1994)

80. Y. Koshelnick, M. Ehart, P. Hufnagl, P. C. Heinrich and B. R. Binder: Urokinase Receptor Is Associated with the Components of the JAK1/STAT1 Signaling Pathway and Leads to Activation of This Pathway upon Receptor Clustering in the Human Kidney Epithelial Tumor Cell Line TCL-598. *J Biol Chem* 28563-28567 (1997).

81. F. Fazioli, M. Resnati, N. Sidenius, Y. Higashimoto, E. Appella and F. Blasi: A urokinase-sensitive region of the human urokinase receptor is responsible for its chemotactic activity. *Embo J* 16, 7279-86 (1997)

82. I. Dumler, A. Kopmann, K. Wagner, O. A. Mayboroda, U. Jerke, R. Dietz, H. Haller and D. C. Gulba: Urokinase Induces Activation and Formation of Stat4 and Stat1-Stat2 Complexes in Human Vascular Smooth Muscle Cells. *J Biol Chem* 24059-24065 (1999).

83. I. Dumler, A. Weis, O. A. Mayboroda, C. Maasch, U. Jerke, H. Haller and D. C. Gulba: The Jak/Stat pathway and urokinase receptor signaling in human aortic vascular smooth muscle cells. *J Biol Chem* 273, 315-21 (1998)

84. S. Shetty, G. N. Rao, D. B. Cines and K. Bdeir: Urokinase induces activation of STAT3 in lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol* L772-780 (2006).

85. M. Jo, K. S. Thomas, N. Marozkina, T. J. Amin, C. M. Silva, S. J. Parsons and S. L. Gonias: Dynamic Assembly of the Urokinase-type Plasminogen Activator Signaling Receptor Complex Determines the Mitogenic Activity of Urokinase-type Plasminogen Activator. *J Biol Chem* 17449-17457 (2005).

86. I. Dumler, T. Petri and W. D. Schleuning: Induction of c-fos gene expression by urokinase-type plasminogen activator in human ovarian cancer cells. *FEBS Lett* 343, 103-6 (1994)

87. S. A. Rabbani, J. Gladu, A. P. Mazar, J. Henkin and D. Goltzman: Induction in human osteoblastic cells (SaOS2) of the early response genes fos, jun, and myc by the amino terminal fragment (ATF) of urokinase. *J Cell Physiol* 172, 137-45 (1997)

88. J. Bohuslav, V. Horejsi, C. Hansmann, J. Stockl, U. H. Weidle, O. Majdic, I. Bartke, W. Knapp and H. Stockinger: Urokinase plasminogen activator receptor, beta 2-integrins, and Src-kinases within a single receptor complex of human monocytes. *J Exp Med* 181, 1381-90 (1995)

89. M. Resnati, M. Guttinger, S. Valcamonica, N. Sidenius, F. Blasi and F. Fazioli: Proteolytic cleavage of the urokinase receptor substitutes for the agonist-induced chemotactic effect. *Embo J* 15, 1572-82 (1996)

90. T. David-Pfeuty and Y. Nouvian-Dooghe: Immunolocalization of the cellular src protein in interphase and mitotic NIH c-src overexpresser cells. *J Cell Biol* 111, 3097-116 (1990)

91. T. Erpel and S. A. Courtneidge: Src family protein tyrosine kinases and cellular signal transduction pathways. *Curr Opin Cell Biol* 7, 176-82 (1995)

92. K. B. Kaplan, K. B. Bibbins, J. R. Swedlow, M. Arnaud, D. O. Morgan and H. E. Varmus: Association of the amino-terminal half of c-Src with focal adhesions alters their properties and is regulated by phosphorylation of tyrosine 527. *Embo J* 13, 4745-56 (1994)

93. K. B. Kaplan, J. R. Swedlow, D. O. Morgan and H. E. Varmus: c-Src enhances the spreading of src-/- fibroblasts on fibronectin by a kinase-independent mechanism. *Genes Dev* 9, 1505-17 (1995)

94. K. B. Kaplan, J. R. Swedlow, H. E. Varmus and D. O. Morgan: Association of p60c-src with endosomal membranes in mammalian fibroblasts. *J Cell Biol* 118, 321-33 (1992)

95. B. Degryse, M. Resnati, S. A. Rabbani, A. Villa, F. Fazioli and F. Blasi: Src-dependence and pertussis-toxin sensitivity of urokinase receptor-dependent chemotaxis and cytoskeleton reorganization in rat smooth muscle cells. *Blood* 94, 649-62 (1999)

96. H. Tang, D. M. Kerins, Q. Hao, T. Inagami and D. E. Vaughan: The urokinase-type plasminogen activator receptor mediates tyrosine phosphorylation of focal adhesion proteins and activation of mitogen-activated protein kinase in cultured endothelial cells. *J Biol Chem* 273, 18268-72 (1998)

97. M. D. Schaller, C. A. Otey, J. D. Hildebrand and J. T. Parsons: Focal adhesion kinase and paxillin bind to peptides mimicking beta integrin cytoplasmic domains. *J Cell Biol* 130, 1181-7 (1995)

98. D. D. Schlaepfer, M. A. Broome and T. Hunter: Fibronectin-stimulated signaling from a focal adhesion kinase-c-Src complex: involvement of the Grb2, p130cas, and Nck adaptor proteins. *Mol Cell Biol* 17, 1702-13 (1997)
99. D. D. Schlaepfer, S. K. Hanks, T. Hunter and P. van der Geer: Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. *Nature* 372, 786-91 (1994)

100. D. D. Schlaepfer and T. Hunter: Evidence for *in vivo* phosphorylation of the Grb2 SH2-domain binding site on focal adhesion kinase by Src-family protein-tyrosine kinases. *Mol Cell Biol* 16, 5623-33 (1996)

101. D. D. Schlaepfer and T. Hunter: Focal adhesion kinase overexpression enhances ras-dependent integrin signaling to ERK2/mitogen-activated protein kinase through interactions with and activation of c-Src. *J Biol Chem* 272, 13189-95 (1997)

102. E. Monaghan, V. Gueorguiev, C. Wilkins-Port and P. J. McKeown-Longo: The receptor for urokinase-type plasminogen activator regulates fibronectin matrix assembly in human skin fibroblasts. *J Biol Chem* 279, 1400-7 (2004)

103. E. Monaghan-Benson and P. J. McKeown-Longo: Urokinase-type Plasminogen Activator Receptor Regulates a Novel Pathway of Fibronectin Matrix Assembly Requiring Src-dependent Transactivation of Epidermal Growth Factor Receptor. *J Biol Chem* 9450-9459 (2006).

104. M. Ploug, E. Ronne, N. Behrendt, A. L. Jensen, F. Blasi and K. Dano: Cellular receptor for urokinase plasminogen activator. Carboxyl-terminal processing and membrane anchoring by glycosyl-phosphatidylinositol. *J Biol Chem* 266, 1926-33 (1991)

105. D. Cao, I. F. Mizukami, B. A. Garni-Wagner, A. L. Kindzelskii, R. F. Todd, 3rd, L. A. Boxer and H. R. Petty: Human urokinase-type plasminogen activator primes neutrophils for superoxide anion release. Possible roles of complement receptor type 3 and calcium. *J Immunol* 154, 1817-29 (1995)

106. R. G. Sitrin, P. M. Pan, H. A. Harper, R. A. Blackwood and R. F. Todd, III: Urokinase Receptor (CD87) Aggregation Triggers Phosphoinositide Hydrolysis and Intracellular Calcium Mobilization in Mononuclear Phagocytes. *J Biol Chem* 6193-6200 (1999).

107. S. P. Christow, R. Bychkov, C. Schroeder, R. Dietz, H. Haller, I. Dumler and D. C. Gulba: Urokinase activates calcium-dependent potassium channels in U937 cells via calcium release from intracellular stores. *The FEBS J* 264-272 (1999).

108. Y. Le, W. Gong, H. L. Tiffany, A. Tumanov, S. Nedospasov, W. Shen, N. M. Dunlop, J. L. Gao, P. M. Murphy, J. J. Oppenheim and J. M. Wang: Amyloid (beta)42 activates a G-protein-coupled chemoattractant receptor, FPR-like-1. *J Neurosci* 21, RC123 (2001)

109. G. Deng, S. A. Curriden, S. Wang, S. Rosenberg and D. J. Loskutoff: Is plasminogen activator inhibitor-1 the molecular switch that governs urokinase receptor-mediated cell adhesion and release? *J Cell Biol* 134, 1563-71 (1996)

110. S. M. Kanse, C. Kost, O. G. Wilhelm, P. A. Andreasen and K. T. Preissner: The urokinase receptor is a major vitronectin-binding protein on endothelial cells. *Exp Cell Res* 224, 344-53 (1996)

111. K. T. Preissner, S. M. Kanse and A. E. May: Urokinase receptor: a molecular organizer in cellular communication. *Curr Opin Cell Biol* 12, 621-8 (2000)

112. N. Sidenius and F. Blasi: Domain 1 of the urokinase receptor (uPAR) is required for uPAR-mediated cell binding to vitronectin. *FEBS Lett* 470, 40-6 (2000)

113. L. Kjoller and A. Hall: Rac mediates cytoskeletal rearrangements and increased cell motility induced by urokinase-type plasminogen activator receptor binding to vitronectin. *J Cell Biol* 152, 1145-57 (2001)

114. N. Sidenius, C. F. Sier and F. Blasi: Shedding and cleavage of the urokinase receptor (uPAR): identification and characterisation of uPAR fragments *in vitro* and *in vivo*. *FEBS Lett*, 475, 52-6 (2000)

115. D. A. Waltz and H. A. Chapman: Reversible cellular adhesion to vitronectin linked to urokinase receptor occupancy. *J Biol Chem* 269, 14746-50 (1994)

116. N. Sidenius, A. Andolfo, R. Fesce and F. Blasi: Urokinase regulates vitronectin binding by controlling urokinase receptor oligomerization. *J Biol Chem* 277, 27982-90 (2002)

117. C. D. Madsen, G. M. Ferraris, A. Andolfo, O. Cunningham and N. Sidenius: uPAR-induced cell adhesion and migration: vitronectin provides the key. *J Cell Biol* 177, 927-39 (2007)

118. C. A. Hebert and J. B. Baker: Linkage of extracellular plasminogen activator to the fibroblast cytoskeleton: colocalization of cell surface urokinase with vinculin. *J Cell Biol* 106, 1241-7 (1988)

119. H. T. Myohanen, R. W. Stephens, K. Hedman, H. Tapiovaara, E. Ronne, G. Hoyer-Hansen, K. Dano and A. Vaheri: Distribution and lateral mobility of the urokinase-receptor complex at the cell surface. *J Histochem Cytochem* 41, 1291-301 (1993)

120. J. Pollanen, K. Hedman, L. S. Nielsen, K. Dano and A. Vaheri: Ultrastructural localization of plasma membrane-associated urokinase-type plasminogen activator at focal contacts. *J Cell Biol* 106, 87-95 (1988)

121. S. A. Wilcox, T. Reho, P. J. Higgins, E. Tominna-Sebald and P. J. McKeown-Longo: Localization of urokinase to focal adhesions by human fibrosarcoma cells synthesizing recombinant vitronectin. *Biochem Cell Biol* 74, 899-910 (1996)

122. W. Xue, I. Mizukami, R. F. Todd, 3rd and H. R. Petty: Urokinase-type plasminogen activator receptors associate with beta1 and beta3 integrins of fibrosarcoma cells: dependence on extracellular matrix components. *Cancer Res* 57, 1682-9 (1997)

123. L. Kjoller: The urokinase plasminogen activator receptor in the regulation of the actin cytoskeleton and cell motility. *Biol Chem* 383, 5-19 (2002)

124. I. Kiian, N. Tkachuk, H. Haller and I. Dumler: Urokinase-induced migration of human vascular smooth muscle cells requires coupling of the small GTPases RhoA and Rac1 to the Tyk2/PI3-K signalling pathway. *Thromb Haemost* 89, 904-14 (2003)

125. N. Wang, E. Planus, M. Pouchelet, J. J. Fredberg and G. Barlovatz-Meimon: Urokinase receptor mediates mechanical force transfer across the cell surface. *Am J Physiol* 268, C1062-6 (1995)

126. N. Busso, S. K. Masur, D. Lazega, S. Waxman and L. Ossowski: Induction of cell migration by pro-urokinase binding to its receptor: possible mechanism for signal transduction in human epithelial cells. *J Cell Biol* 126, 259-70 (1994)

127. N. Chandrasekar, S. Mohanam, M. Gujrati, W. C. Olivero, D. H. Dinh and J. S. Rao: Downregulation of uPA inhibits migration and PI3k/Akt signaling in glioblastoma cells. *Oncogene* 22, 392-400 (2003)

128. J. Sturge, J. Hamelin and G. E. Jones: N-WASP activation by a {beta}1-integrin-dependent mechanism supports PI3K-independent chemotaxis stimulated by urokinase-type plasminogen activator. *J Cell Sci* 699-711 (2002).

129. S. Godar, V. Horejsi, U. H. Weidle, B. R. Binder, C. Hansmann and H. Stockinger: M6P/IGFII-receptor complexes urokinase receptor and plasminogen for activation of transforming growth factor-beta1. *Eur J Immunol* 29, 1004-13 (1999)

130. A. Nykjaer, E. I. Christensen, H. Vorum, H. Hager, C. M. Petersen, H. Roigaard, H. Y. Min, F. Vilhardt, L. B. Moller, S. Kornfeld and J. Gliemann: Mannose 6-phosphate/insulin-like growth factor-II receptor targets the urokinase receptor to lysosomes via a novel binding interaction. *J Cell Biol* 141, 815-28 (1998)

131. C. R. Dass, A. P. Nadesapillai, D. Robin, M. L. Howard, J. L. Fisher, H. Zhou and P. F. Choong: Downregulation of uPAR confirms link in growth and metastasis of osteosarcoma. *Clin Exp Metastasis* 22, 643-52 (2005)

132. S. S. Lakka, C. S. Gondi, D. H. Dinh, W. C. Olivero, M. Gujrati, V. H. Rao, C. Sioka and J. S. Rao: Specific interference of urokinase-type plasminogen activator receptor and matrix metalloproteinase-9 gene expression induced by double-stranded RNA results in decreased invasion, tumor growth, and angiogenesis in gliomas. *J Biol Chem* 280, 21882-92 (2005)

133. S. S. Lakka, R. Rajagopal, M. K. Rajan, P. M. Mohan, Y. Adachi, D. H. Dinh, W. C. Olivero, M. Gujrati, F. Ali-Osman, J. A. Roth, W. K. Yung, A. P. Kyritsis and J. S. Rao: Adenovirus-mediated antisense urokinase-type plasminogen activator receptor gene transfer reduces tumor cell invasion and metastasis in non-small cell lung cancer cell lines. *Clin Cancer Res* 7, 1087-93 (2001)

134. F. Margheri, S. D'Alessio, S. Serrati, M. Pucci, F. Annunziato, L. Cosmi, F. Liotta, R. Angeli, A. Angelucci, G. L. Gravina, N. Rucci, M. Bologna, A. Teti, B. Monia, G. Fibbi and M. Del Rosso: Effects of blocking urokinase receptor signaling by antisense oligonucleotides in a mouse model of experimental prostate cancer bone metastases. *Gene Ther* 12, 702-14 (2005)

135. P. M. Mohan, S. K. Chintala, S. Mohanam, C. L. Gladson, E. S. Kim, Z. L. Gokaslan, S. S. Lakka, J. A. Roth, B. Fang, R. Sawaya, A. P. Kyritsis and J. S. Rao: Adenovirus-mediated delivery of antisense gene to urokinase-type plasminogen activator receptor suppresses glioma invasion and tumor growth. *Cancer Res* 59, 3369-73 (1999)

136. S. Nozaki, Y. Endo, H. Nakahara, K. Yoshizawa, Y. Hashiba, S. Kawashiri, A. Tanaka, K. Nakagawa, Y. Matsuoka, M. Kogo and E. Yamamoto: Inhibition of invasion and metastasis in oral cancer by targeting urokinase-type plasminogen activator receptor. *Oral Oncol* 41, 971-7 (2005)

137. Y. Wang, X. Liang, S. Wu, G. A. Murrell and W. F. Doe: Inhibition of colon cancer metastasis by a 3'-end antisense urokinase receptor mRNA in a nude mouse model. *Int J Cancer* 92, 257-62 (2001)

Abbreviations: GPI: glycosylphosphatidylinositol; EGFR: epidermal growth factor receptor; FPRL-1: formyl peptide receptor like 1; MEFs: mouse embryonic fibroblasts; MEK: map-erk kinase; STAT: signal transducer and activators of transcription; PTKs: protein tyrosine kinases; FRET: fluorescence resonance energy transfer; PI3-K: phosphoinositide-3 kinase; AKT: protein kinase B; ODN: oligodeoxynucleotide

Key Words: uPA, uPAR, Signal Transduction, Tumor Invasion, Cell Signalling, Cell Adhesion, Cell Migration, EGFR, integrins, MAPkinases, Review

Send correspondence to: Silvia D'Alessio, Department of Molecular Biology and Functional Genomics, DIBIT, Università Vita-Salute San Raffaele, Via Olgettina 58, 20132 Milan, Italy, Tel: 39 02 2643 4758, Fax: 39 02 2643 4844, E-mail: dalessio.silvia@hsr.it

http://www.bioscience.org/current/vol14.htm