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THE ROLE OF PLASMINOGEN ACTIVATOR RECEPTOR IN CANCER INVASION AND DORMANCY

[1]**LILIANA OSSOWSKI, JULIO AGUIRRE GHISO, DAVID LIU, WEN YU, AND KATHERINE KOVALSKI**

Department of Medicine, Division of Medical Oncology, Mount Sinai School of Medicine, New York NY, USA

Abstract Urokinase plasminogen activator receptor (uPAR) has been identified some 15 years ago and the anticipation was that its presence on the cell surface will provide a focus for anchoring uPA and possibly protect the enzyme from native inhibitors. The studies of the last decade have shown that uPA localized to the surface of cells by uPAR is indeed an important factor in the process of cancer cell invasion and metastasis. We developed a chick embryo model in which we showed that uPAR is crucial in invasion of stroma and in intravasation (breaching of the blood vessels walls). More recently and unexpectedly, uPAR- a protein anchored in the outer leaflet of the plasma membrane, has been shown to initiate signal transduction events and affect cell migration. We have shown that uPAR co-associates with fibronectin binding integrin, $\alpha 5\beta 1$, activates them and that this interaction leads to a greatly increased level of active ERK. When the association between uPAR and integrin or integrin and fibronectin are interrupted either by reduction of surface uPAR expression, or by other means, human carcinoma cells enter a state of protracted dormancy. We show that very high levels of active ERK are required to keep cancer cells proliferating in vivo.

Key words: urokinase plasminogen activator (uPA), uPA receptor (uPAR), cancer dormancy, invasion, intravasation, ERK, $\alpha 5b1$ integrin

Biochemical characterization of uPAR and its interaction with uPA

Urokinase-type plasminogen activator (uPA) interacts with a specific plasma membrane receptor, discovered in 1985 (Vassalli et al., 1985; Stoppelli et al., 1985). The receptor was cloned from several species including human (Roldan et al., 1990; Kristensen et al., 1991). During post-translational processing a 22 amino acid C-terminal domain is removed and a glycosyl-phospatidylinositol moiety (Ploug et al., 1991) membrane anchor is added. The mature, heavily glycosylated protein is composed of 3 homologous, independently folded extracellular domains, that bind uPA with high affinity (Kd of 0.1 to 1nM). uPA binds to domain 1 of uPAR, but interdomain interactions contribute significantly to the binding affinity (Plough et al., 1994; Behrendt et al., 1996).

uPA is secreted in the form of a pro-enzyme and is converted to an active enzyme which is composed of two-chain held by a disulfide bond. Although, under normal conditions plasmin activates pro-uPA, other means of activation must exist, as the level of active uPA is similar in wild type and plasminogen null mice (Bugge et al., 1995). Activation of pro-uPA by plasmin and activation of plasminogen by uPA proceed much faster on the surface of cells (in association with receptors) than in fluid phase (Ellis et al., 1989; Ellis et al., 1991). In addition, surface-bound plasmin generated by this interactions is believed to be at least partially shielded from inhibition by native, high molecular weight plasma inhibitors (Stephens et al., 1989).

The role of uPAR-associated uPA in invasion and metastasis

The presence of uPAR bound uPA is important for efficient generation of surface-bound plasmin which, through its direct degradation of matrix proteins as well as activation of pro-MMPs (Mazzieri et al., 1997), has been shown to be important in cancer cell invasion (Danϕ et al., 1985; Mignatti et al., 1986; Ossowski, 1988a; Cajot et al., 1989; Schlechte et al., 1989; Testa and Quigley, 1990; Ossowski et al., 1991; Mignatti and Rifkin, 1993; Blasi, 1993; Stahl and Mueller, 1994) as well as in lung colonization and spontaneous metastasis (Ossowski and Reich, 1983; Hearing et al., 1988; Axelrod et al., 1989; Crowley et al., 1993; Kobayashi et al., 1994). It appears that when the uPA concentration is limiting, the enzyme is most efficiently utilized when receptor-bound (Ossowski, 1988b; Ossowski et al., 1991; Quax et al., 1991).

[1]Department of Medicine, Box 1178, 1 Levy Place, New York, NY 10029, USA
FAX: 212-996 5787 E-mail L: OSSOWSKI@SMTPLINK.MSSM.EDU

Following is a description of the experimental model we developed and used to establish the role of uPA/uPAR in different stages of the metastatic spread:

1. Tumorigenicity. Cells are inoculated into the artificial air sac on the wounded chorioallantoic membranes (CAMs) of 10 day old chick embryos and incubated for 7 days. The size of nodules produced on the CAMs is measured and recorded and the presence of tumor cells in the nodules is assessed microscopically.

2. Metastasis. Cells inoculated onto a wounded chorioallantoic membrane (CAM) of a chick embryo rapidly grow and disseminate through the vasculature into embryo organs. Lungs, the most predictable target for metastasis, are removed, minced and reinoculated on fresh CAMs for an additional week of growth. Metastatic cells present in the original lung «mince» are sufficiently enriched by the second cycle of growth and can be easily detected either microscopically or by measurement of human specific uPA levels (Ossowski and Reich, 1980; Ossowski and Reich, 1983; Ossowski 1988a,b).

3. Local invasion of connective tissue. Wounded CAMs, that were allowed to reseal in vivo, are inoculated with cells labelled with ^{125}IUdR for 24 hrs in culture. After 24 hrs of incubation, the cells which did not invade are removed by extensive wash and trypsinization and the excised CAMs, containing cells which penetrated into the tissue, and are protected from trypsin action, are counted in a gamma counter, (Ossowski, 1988b).

4. Extravasation. Extravasation is tested by injecting ^{125} IUdR - labelled cells intravenously and measuring their arrest in organs. (Ossowski, 1988a).

5. Intravasation. HEp3 tumors were dissociated with collagenase, plated at high density (3×10^6 cells per 100 mm dish) and passaged once 24 to 48 hrs prior to the experiment, detached from the culture dish with 2mM EDTA in PBS, resuspended in 50ml of PBS with Ca^{++} and Mg^{++} and inoculated (usually at 10^6 cells) onto a CAM of a 9 day old chick embryo in which an artificial air sac was created. After 50 hrs of incubation the lower half of the CAM was removed, and snap-frozen in liquid nitrogen. The genomic DNA was extracted and amplified by PCR using primers designed to recognize human alu sequences. The reaction mixture contained ^{32}P-dCTP and PCR products were separated on PAGE, the gels dried and autoradiographed. (Kim et al.,1998).

uPAR in malignant tumors

Malignant tumors over-express urokinase type plasminogen activator (uPA) and its receptor (uPAR). Depending on the type of cancer, tumor cells, stroma or infiltrating cells were shown to be the source of these proteins. Both uPA and uPAR have been shown to be predictive of early recurrence of malignancy .

Immunocytochemical and in situ hybridization analyses of human cancer tissue sections indicate that most types of malignant tumors acquire uPA either through auto- or paracrine interactions (DeBruin et al., 1987; Janicke et al., 1990; Duffy et al., 1990; Grϕndahl-Hansen et al., 1991). Similarly, the in vivo expression of uPAR has been shown in many tumors (Pyke et al., 1991; Bianchi et al., 1994; Carriero et al., 1994). These include colon and breast carcinomas (Pyke et al., 1993; Suzuki et al., 1998; Bianchi et al., 1994), several types of brain tumors (Mohanam et al., 1994; Gladson et al., 1995), melanoma and squamous cell carcinoma (De Vries et al., 1994). The prevalence of surface-bound uPA in cancer cells, and the experimental results implicating uPA in mechanisms underlying this disease suggest that surface proteolysis may represent a potentially attractive target for directed therapy.

Approaches to therapy

The accumulated evidence from experimental models, and the tight correlation that exists between the level of uPA/uPAR and the aggressiveness of cancer show that the uPA/uPAR complex plays an important role in cancer invasion. Most experimental approaches taken to date are either aimed at interfering with the uPA association with uPAR or at directly reducing the levels of uPAR. The latter may be a more effective approach as uPAR itself may have pro-malignant influence. We first tested whether anti-human uPA antibodies that inhibited the catalytic activity of uPA will have an effect on malignancy. This treatment blocked metastasis in a chick embryo model (Ossowski and Reich, 1983). Prostate cancer metastasis (PC3) in nude mice was shown to be inhibited by overexpression in these cells of a non-cleavable form of uPA (Crowley et al., 1993). To block uPA binding to its receptor a random peptide bacteriophage display library (Goodson et al., 1994) was examined and one peptide with an IC_{50} of 10 nM was identified, but peptide with higher affinity would probably be needed for therapeutic applications. Several additional approaches have also been tried, including the use of amino terminal fragment of uPA, or DFP-inactivated uPA to displace active uPA from its receptor. Most have been found effective in experimental models.

To directly down-regulate uPAR expression antisense oligonucleotides, degradation of the mRNA by a specific ribozyme, or proteolytic cleavage of the receptor uPA-binding domain have been tried. «Switching off» the uPAR gene expression by an anti-messenger oligo-nucleotide have been shown to reduce invasion through Matrigel in human fibroblasts transformed with SV-40 virus (Quattrone et al., 1994). A successful delivery of anti-human uPAR-RNA ribozyme to cultured human

osteosarcoma cells has been described (Kariko et al., 1994) but no follow-up to this approach has been published.

Our own choice of intervention was to stably disable the uPAR gene expression by transfecting a highly malignant human epidermoid carcinoma cells, HEp3, (Toolan, 1954), with a construct producing uPAR antimessenger RNA. We prepared a construct which contained a 300 bp PCR-amplified 5' fragment of uPAR-cDNA, which included the ATG codon, in antisense orientation. Several clones with reduced uPAR level were tested and found not to differ in their rate of growth in culture, and the amount and type of protease content; all clones produced high levels (~ 0.5 Ploug Units/10^6 cells) of uPA and 2 gelatinases, the 72 and 92 kDa. Therefore, except for blocking of uPAR, the transfection or the clonal selection did not alter other specific phenotypic properties usually associated with malignancy. The reduction in pro-uPA binding paralleled reduced levels of uPAR-protein and uPAR mRNA (Yu et al., 1997).

We found that the diminished expression of surface-uPAR leads to a severe reduction in invasiveness of tumor cells.

uPAR in signal transduction

In addition to its matrix-degrading function, the binding of uPA or pro-uPA to its receptor has been shown to stimulate chemotaxis and/or chemokinesis in a variety of cell types such as neutrophils, tumor cells and endothelium (Gudewicz and Gilboa, 1987; Fibbi et al., 1988; Odekon et al, 1992; Del Rosso et al., 1993; Busso et al., 1994; Stahl and Mueller, 1994, Resnati et al., 1996). Not unlike the case for other GPI-linked proteins, which are known to transduce signals through as yet unidentified pathways, the possibility of several signal transduction pathways through this receptor has also been considered. These involve both tyrosine phosphorylation, PKC, de novo DAG generation etc. (Del Rosso et al., 1993; Dumler et al., 1993; Anchini et al., 1994; Dumler et al., 1994, 1998; Busso et al., 1994; Koshelnick et al., 1996*).*

uPAR in tumor dormancy

Clinical experience in cancer patients indicates that some primary cancers and most metastatic lesions un-

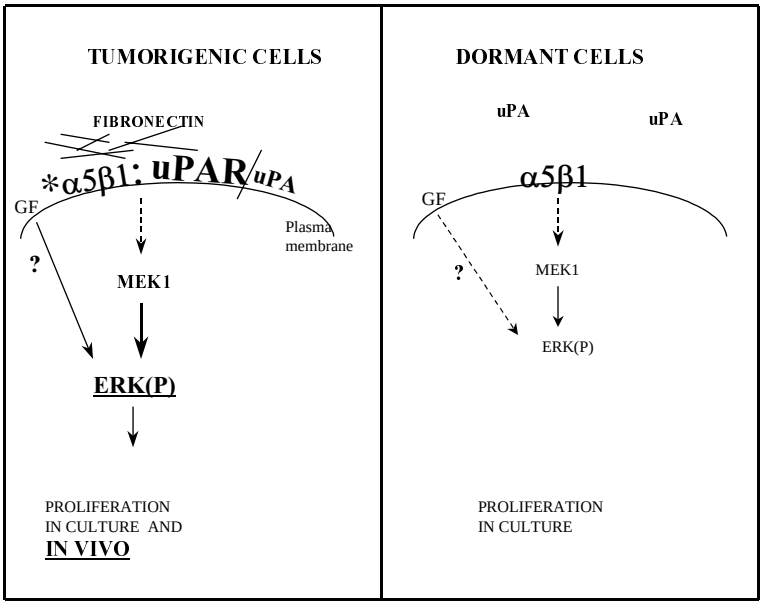

Figure 1. Schemes explaining our current understanding of the role of uPAR down-modulation in induction of dormancy. *Left panel:* When high uPAR and high uPA activity is present, the chance of physical interaction with α5β1 is high. This interaction "activates" the integrin (indicated by a star) and when cells are exposed to fibronectin a signal is initiated which leads to MEK1 and ERK1/2 activation. Only activation above a certain threshold allows cancer cells to divide in vivo. *Right panel.* In cells with low or no uPAR, the chance of uPAR/α5β1 interaction is drastically reduced. The signal through MEK1 to ERK is weak, only sufficient to allow cell proliferation in culture. It is likely that additional signals ei. growth factor (GF) induced, converge on the ERK pathway.

dergo a period of dormancy prior to entering a stage of progressive growth. Although this may be the most crucial step in cancer progression, the mechanisms underlying the conversion from a dormant to an actively growing state have not been elucidated. A prevalent hypothesis envisions that in order to grow, cancer cells must acquire the ability to induce angiogenesis (Folkman, 1995) and that the pro-angiogenic phenotype must be dominant. Another model of tumor dormancy involves experimental B-cell lymphoma in which antibodies directed to surface immunoglobulins act as agonists of signal transduction pathways leading to a block in cancer cell growth (Marches *et al.*, 1998).

We discovered that uPAR down-regulation renders a human epidermoid carcinoma, HEp3, dormant (Kook, *et al.*, 1994; Yu *et al.*, 1997). These cancer cells, expressing less than 50% of the normal level of uPAR, grew indistinguishably from parental cells in culture but, when inoculated onto the chorioallantoic membrane (CAM) of chick embryos and maintained by serial *in vivo* passages, entered a period of protracted (~4 months) dormancy. We determined that dormancy was the result of a reduced proliferation rate rather than an increased apoptotic rate (Yu *et al.,* 1997). These and other published results allow us to propose that, in addition to the well established role of uPA/uPAR as an activator of proteolytic cascade, this complex also participates in signal transduction. In this capacity it affects cell motility, (Nguyen et al., 1998) and, as we recently showed, (Aguirre Ghiso et al., 1999) it affects tumor dormancy. We found that a reduction in surface uPAR expression in HEp3 carcinoma cells, that induces the state of protracted dormancy (Yu *et al.,* 1997), causes G_0/G_1 arrest of the cell cycle *in vivo.* The uPAR-rich, tumorigenic cells had a much higher basal level of active ERK1/2 that could be further activated by incubating the cells with single chain uPA (scuPA). The response to scuPA of cells with reduced uPAR (dormant) was weak and substantially delayed. Several reports have shown that binding of uPA to uPAR in tumor or endothelial cells activates the mitogen activated protein kinase (MAPK), ERK1 and 2 (Konakova *et al.,* 1998; Nguyen *et al.,* 1998; Tang *et al.,* 1998). However, these reports neither addressed how uPAR activates signaling nor considered its *in vivo* consequence. In trying to identify the possible signal responsible for ERK activation we found that in cells expressing the full complement of uPAR, $\alpha5\beta1$ integrins were in an active conformation and that these cells adhered efficiently to fibronectin (FN) inducing a robust and persistent activation of ERK, and a growth stimulation not present in uPAR-deficient cells. We hypothesized that interactions between uPA/uPAR and $\alpha5\beta1$ integrin mediate the FN-dependent ERK-activation, and that a threshold level of uPA/uPAR/$\alpha5\beta1$ complex had to be surpassed for FN-dependent ERK-activation to stimulate *in vivo* growth. In support of this hypothesis we found that anti-$\beta1$ antibodies co-precipitated uPAR, that soluble uPAR induced ERK activation in uPAR-deficient cells, and that anti-uPAR antibodies and a peptide that hinders uPAR/$\beta1$ interactions, diminished FN-induced ERK activation in uPAR-rich cells. Moreover, we found that in uPAR-rich malignant cells high ERK activation persisted *in vivo*, and that blocking the signal to ERK by anti-uPAR or anti-$\beta1$ antibodies, transiently inhibited tumor growth. We conclude that by rendering $\alpha5\beta1$ integrins inactive, uPAR downregulation reduces FN-dependent ERK activation, forcing cancer cells into dormancy.

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References

Aguirre Ghiso J., Kovalski K, Ossowski L. (1999) Tumor dormancy induced by down regulation of urokinase receptor in human carcinoma involves integrin and MAPK signaling. The journal of Cell Biology 147: 89-103.

Anchini E, Fibbi G, Pucci M, Caldini R, Chevanne M, Del Rosso M. (1994) Production of second messengers following chemotactic and mitogenic urokinase-receptor interaction in human fibroblasts transfected with human urokinase receptor. Exp. Cell Res. 213: 438-48.

Axelrod JH, Reich R, Miskin R (1989) Expression of human recombinant plasminogen activators enhances invasion and experimental metastasis of H-ras-transformed NIH 3T3 cells. Mol. Cell. Biol 9: 2133-41.

Bianchi E, Cohen RL, Thor AT, Todd RF 3rd, Mizukami IF, Lawrence DA, Ljung BM, Shuman MA, Smith HS (1994) The urokinase receptor is expressed in invasive breast cancer but not in normal breast tissue Cancer Res. 54: 861-6.

Bugge T.H., Flick M.J., Daugherty C.C., and Degen J.A. (1995) Plasminogen deficiency causes severe thrombosis but is compatible with development and reproduction. Genes and Dev. 9: 794-807.

Blasi F. (1993) Urokinase and urokinase receptor: a paracrine/autocrine system regulating cell migration and invasiveness. Bioessays. 1993 15): 105-11. Review.

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

Cajot J-F., Schleuning W-D., Medcalf R.L., Bamat J., Testuz J., Libermann . and Sordat B. (1989) Mouse L-cells expressing human pro-urokinase type plasminogen activator: effect on extracellular matrix degradation and invasion. J. Cell Biol. 109: 915-25.

Carriero MV, Franco P, Del-Vecchio S, Massa O, Botti G, D'Aiuto G, Stoppelli MP, Salvatore M (1994) Tissue distribution of soluble and receptor bound urokinase in human breast cancer using a panel of monoclonal antibodies Cancer Res. 54: 5445-54.

Crowley CW, Cohen R, Lucas BK, Liu G, Shuman MA, Levinson

AD (1993) Prevention of metastasis by inhibition of the urokinase receptor. PNAS 90: 5021-5.

Cubellis MV, Andreasen P, Ragno P, Mayer M, Dano K, Blasi F (1989) Accessibility of receptor-bound urokinase to type-1 plasminogen activator inhibitor. Proc.Natl.Acad.Sci USA 86: 4828-34.

Danϕ K, Andreasen PA, Grondahl-Hansen J, Kristensen P, Nielsen L, Skriver L Plasminogen activators, tissue degradation and cancer. (1985) Adv. Cancer Res. 44: 139-266

DeBruin PA, Griffioen G, Verspaget HV, Verheijen JH (1987) Plasminogen activators and tumor development in the human colon. Activity levels in normal mucosa, adenomateous tissue, polyps and adenocarcinomas. Cancer Res. 47: 4654-7.

Del Rosso M, Anichini E, Pedersen N, Blasi F, Fibbi G, Pucci M, Ruggiero M (1993) Urokinase-urokinase receptor interaction: non-mitogenic signal transduction in human epidermal cells. Biochem. Biophys. Res. Commun. 190: 347-52.

de Vries TJ, Quax PH, Denijn M, Verrijp KN, Verheijen JH, Verspaget HW, Weidle UH, Ruiter DJ, van Muijen,GN (1994) Plasminogen activators, their inhibitors, and urokinase receptor emerge inlate stages of melanocytic tumor progression. Am J Pathol 144: 70-81.

Duffy MJ, Reilly D, O'Sullivan C, O'Higgins N, Fennely JJ (1990) Urokinase plasminogen activator and prognosis in breast cancer. Lancet 13: 108-11.

Dumler I, Petri T, Schleuning W-D (1993) Interaction of urokinase-type plasminogen activator (uPA) with its cellular receptor (uPAR) induces phosphorylation on tyrosine of a 38 kDa protein. FEBS Lett. 322: 37-40 .

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

Ellis V., Scully M.F. and Kakar V.V. (1989) Plasminogen activation initiated by single-chain urokinase type plasminogen activator. Potentiation by U937 monocytes. J. Biol. Chem. 264: 2184-8.

Ellis V., Behrendt N. and Danϕ K. (1991) Plasminogen activation by receptor-bound urokinase. A kinetic study with both cell-associated and isolated receptor. J.Biol. Chem. 266: 12752-8.

Fibbi G, Ziche M, Morbidelli, L., Magnelli, L., Del Rosso, M. (1988) Interaction of urokinase with specific receptors stimulates mobilization of bovine adrenal capillary endothelial cells. Exp. Cell Res. 179: 385-95.

Folkman J. (1995) Angiogenesis in cancer, vascular, rheumatoid and other diseases. Nat Med., 1: 27-31.

Gladson C.L., Pijuan-ThompsonV., Olman M.A., Gillespie G.Y. and Yacoud I.Z. (1995) Up-regulation of urokinase and urokinase receptor genes in malignant astrocytoma. Am J Pathol. 146: 1150-60.

Grϕndahl-Hansen J, Ralfkiaer E, Kirkeby L, Kristensen P, Lund LR, Dano K (1991) Localization of urokinase-type plasminogen activator in stromal cells in adenocarcinomas of the colon in humans Am. J. Pathol. 138: 111-7.

Goodson RJ, Doyle MV, Kaufman SE, Rosenberg S (1994) High affinity urokinase receptor antagonists identified with bacteriophage peptide display. PNAS 91: 7129-33.

Gudewicz PW, Gilboa N (1987) Human urokinase-type plasminogen activator stimulates chemotaxis of human neutrophils. Biochem. Biophys. Res. Commun. 147: 1176-81.

Hearing VJ, Law LW, Corti A, Appella E, Blasi F (1988) Modulation of metastatic potential by cell surface urokinase of murine melanoma cells. Cancer Res. 48: 1270-8.

Janicke F, Schmidt M, Hollrieder A, Babic R, Ulm K, Gossner W, Graeff H (1990) Urokinase-type plasminogen activator (uPA) is a predictor of early relapse in breast cancer. Fibrinolysis 4: 69-78.

Kariko K, Megyeri K, Xiao Q, Barnathan ES (1994) Lipofectin aided cell delivery of ribozyme targeted to human urokinase receptor mRNA. FEBS-Lett. 352: 41-4.

Kim J, Yu W., Kovalski K., and Ossowski L. (1998) Requirement for specific proteases in cancer cell intravasation as revealed by a novel semiquantitative PCR-based assay. Cell. 1998 94: 353-62.

Konakova M., Hucho F. and Schleuning W.D. (1998) Downstream targets of urokinase- type plasminogen-activator-mediated signal transduction. Eur. J. Biochem. 253: 421-9.

Kook Y.H., Adamski J., Zelent A. and Ossowski L. (1994) The effect of antisense inhibition of urokinase receptor in human squamous cell carcinoma on malignancy. EMBO J. 13: 3983-91.

Koshelnick Y, Ehart M, Hufnagl P, Heinrich PC, Binder BR. (1997). Urokinase receptor is associated with the components of the JAK1/STAT1signaling pathway and leads to activation of this pathway upon receptor clustering in the human kidney epithelial tumor cell line TCL-598. J Biol Chem 1997 272: 28563-7.

Kristensen P., Eriksen J., Blasi F. and Dano K. (1991) Two alternatively spliced mouse urokinase receptor mRNAs with different histological localization in the gastrointestinal tract. J. Cell Biol. 115: 1763-71.

Marches R., Scheuermann R.H. and Uhr J.W. (1998) Cancer dormancy: role of cyclin-dependent kinase inhibitors in induction of cell cycle arrest mediated via membrane IgM. Cancer Res. 58: 691-7.

Mazzieri R., Masiero L., Zanetta L., Monea S., Onisto M., Garbisa S., and Mignatti P. (1997) Control of type IV collagenase activity by components of the urokinase-plasmin system: a regulatory mechanism with cell bound reactants. EMBO J., 16: 2319-32.

Mignatti P, Robbins E, Rifkin DB (1986) Tumor invasion through the human amniotic membrane: Requirement for proteinase cascade Cell 47: 487-98.

Mignatti P, Rifkin DB (1993) Biology and biochemistry of proteinases in tumor invasion. Physiol. Reviews. 73: 161-95.

Mohanam S, Sawaya RE, Yamamoto M, Bruner JM, Nicholson GL, Rao JS (1994) Proteolysis and invasiveness of brain tumors: role of urokinase-type plasminogen activator receptor. Neurooncol 22: 153-60.

Nguyen D.H., Hussaini I.M. and Gonias S.L. (1998) 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. 273: 8502-7.

Odekon LE, Sato Y, Rifkin DB (1992) Urokinase-type plasminogen activator mediates basic fibroblast growth factor-induced bovine endothelial cell migration independent of its proteolytic activity. J. Cell. Physiol. 150: 258-63.

Ossowski L, Reich E (1980) Experimental model for quantitative study of metastasis Cancer Res. 40: 2300-9 .

Ossowski L, Reich E (1983) Antibodies to plasminogen activator inhibit human tumor metastasis. Cell 35: 611-9.

Ossowski L (1988a) Plasminogen activator dependent pathways in the dissemination of human tumor cells in the chick embryo. Cell 52: 321-8.

Ossowski L (1988b) In vivo invasion of modified membrane by tumor cells: the role of cell surface-bound urokinase. J. Cell Biol. 107: 2437-45.

Ossowski L, Clunie G, Masucci T, Blasi F (1991) In vivo paracrine interaction between urokinase and its receptor: effect on tumor cell invasion. J. Cell Biol. 115: 1107-12.

Ploug M., Ronne E., Behrendt N., Jensen A.L., Blasi F. and Danϕ, K. (1991) Cellular receptor for urokinase plasminogen activator. Carboxyl-terminal processign nad membrane anchoring by glycosyl-phosphtidyl inositol. J.Biol. Chem. 266: 12524-34.

Ploug M., Ellis V., and Danϕ K. (1994) Ligand interaction between urokinase-type plasminogen activator and its receptor probed with 8-anilino-1-naphtalenesulfonate. Evidence for a hydrophobic binding site exposed only on the intact receptor. FEBS Lett.349: 163-8.

Pyke C, Graem N, Ralfkiaer E, Ronne E, Hoyer-Hansen G, Brunner N, Dano K (1993) Receptor for urokinase is present in tumor-associated macrophages in ductal breast carcinoma Cancer Res. 53: 1911-1915

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

Quattrone A, Fibbi G, Anichini E, Pucci M, Zamperini A, Capaccioli S, Del Rosso M (1995) Reversion of the invasive phenotype of transformed human fibroblasts by anti-messenger oligonucleotide inhibition of urokinase receptor gene expression. Cancer Res. 55: 90-5.

Quax PHA, Pedersen N, Masucci MT, Weeningverhoeff EJD, Dano K, Verheijnen JH, Blasi F (1991) Complementation between urokinase-producing and receptor-producing cells in extracellular matrix degradation. Cell Regul. 2: 793-803.

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

Roldan AL, Cubellis MV, Masucci MT, Behrendt N, Lund LR, Danϕ K, Appella E, Blasi F (1990) Cloning and expression of the receptor for human urokinase plasminogen activator, a central molecule in cell surface plasmin-dependent proteolysis. EMBO J. 9: 467-74.

Schlechte W, Murano G, Boyd D (1989) Examination of the role of urokinase receptors in human colon cancer mediated laminin degradation. Cancer Res. 49: 6064-9.

Stahl A, Mueller BM (1994) Binding of urokinase to its receptor promotes migration and invasion of human melanoma cells in vitro Cancer Res. 54: 3066-71.

Stephens, R.W., Pollanen,J., Tapiovara, H., et al., (1989) Activationof pro-urokinase and plasminogen on human sarcoma cells: a proteolytic system with with surface-bound reactants. J.Cell Biol. 1108: 1987-95.

Stoppelli MP, Corti A, Sofientini A, Cassani G, Blasi F, Assoian RK (1985) Differentiation-enhanced binding of the amino terminal fragment of human urokinase plasminogen activator to a specific receptor on U937 monocytes. Proc. Natl. Acad. Sci USA 82: 4939-43.

Suzuki S., Hayashi Y., Wang T., Morita Y., Kawasaki K., Ohta K., Aoyama N., Kim S.R., Itoh H., Kuroda Y. and Doe W.F (1998) Urokinase type plasminogen activator receptor expression in colorectal carcinoma neoplasms. Gut 43: 798-805.

Tang H., Kerins D.M., Hao Q., Inagami T. and Vaughan D.E. (1998) 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.

Testa JE, Quigley JP (1990) The role of plasminogen activator in aggressive tumor behavior. Cancer Metastasis Rev. 9: 353-67.

Toolan HW (1954) Transplantable human neoplasms maintained in cortisone-treated laboratory animals: HS#1, H.Ep.#1, H.Ep.#2, H.Ep.#3 and H.Emb.#1. Cancer Res. 14: 660-6.

Vassalli J-D, Baccino D, Belin (1985) A cellular binding site for the Mr 55,000 form of the human plasminogen activator, urokinase. J. Cell Biol. 100: 86-92.

Yu W, Kim J, Ossowski L (1997) Reduction in surface urokinase receptor forces malignant cells into a protracted state of dormancy. J Cell Biol 137: 767-77.