REVIEW / SYNTHÈSE

# Regulation of arterial remodeling and angiogenesis by urokinase-type plasminogen activator[1]

**Vsevolod A. Tkachuk, Olga S. Plekhanova, and Yelena V. Parfyonova**

**Abstract:** A wide variety of disorders are associated with an imbalance in the plasminogen activator system, including inflammatory diseases, atherosclerosis, intimal hyperplasia, the response mechanism to vascular injury, and restenosis. Urokinase-type plasminogen activator (uPA) is a multifunctional protein that in addition to its fibrinolytic and matrix degradation capabilities also affects growth factor bioavailability, cytokine modulation, receptor shedding, cell migration and proliferation, phenotypic modulation, protein expression, and cascade activation of proteases, inhibitors, receptors, and modulators. uPA is the crucial protein for neointimal growth and vascular remodeling. Moreover, it was recently shown to be implicated in the stimulation of angiogenesis, which makes it a promising multipurpose therapeutic target. This review is focused on the mechanisms by which uPA can regulate arterial remodeling, angiogenesis, and cell migration and proliferation after arterial injury and the means by which it modulates gene expression in vascular cells. The role of domain specificity of urokinase in these processes is also discussed.

*Key words:* urokinase, arterial remodeling, migration, proliferation, restenosis.

**Résumé :** Une grande variété de troubles sont associés à un déséquilibre du système d'activation du plasminogène, entre autres, les maladies inflammatoires, l'athérosclérose, l'hyperplasie intimale, le mécanisme de réponse à une lésion vasculaire et à la resténose. L'activateur du plasminogène de type urokinase (uPA) est une protéine multifonctionnelle, qui, en plus de ses capacités de dégradation matricielle et fibrinolytique, a d'autres fonctions biologiques, telles que la biodisponibilité du facteur de croissance, la modulation de la cytokine, la perte du récepteur, la stimulation de la migration et de la prolifération des cellules, la modulation du phénotype, l'expression des protéines et l'activation en cascade des protéases, des inhibiteurs, des récepteurs et des modulateurs. La protéine uPA est essentielle à la croissance de la néointima et au remodelage vasculaire. De plus, elle a été récemment impliquée dans la stimulation de l'angiogenèse, ce qui en fait une cible thérapeutique multifonctionnelle prometteuse. Cette synthèse présente les mécanismes par lesquels uPA peut réguler le remodelage artériel, l'angiogenèse, la migration et la prolifération des cellules après une lésion artérielle et moduler l'expression génique dans les cellules vasculaires, et traite du rôle de la spécificité de domaine de l'urokinase dans ces processus.

*Mots-clés :* urokinase, remodelage artériel, migration, prolifération, resténose.

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## Introduction

The plasminogen activator system is an important participant in diverse physiologic processes including blood coagulation, tissue growth and remodeling, wound healing, angiogenesis, and embryogenesis (Nicholl et al. 2006). It also plays one of the key roles in such pathologic processes as icancer, chronic inflammation, and blood vessel diseases. An imbalance in the plasminogen activator system resulting in its excessive or insufficient activation leads to serious disorders in tissue structure and function. The plasminogen activator system is composed of 2 physiologic activators, a tissue-type plasminogen activator (tPA) and a urokinase-type plasminogen activator (uPA), specific uPA cell-surface receptors, and multiple plasminogen activator inhibitors

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(PAIs). Plasminogen activators mediate the extracellular matrix (ECM) degradation, cell migration and proliferation, and activation of inflammatory mediators via chemotactic processes and integrin signaling (Bhoday et al. 2006). The dual role of the plasminogen system is presently supposed as follows: tPA is primarily involved in maintaining vessel patency and fibrin homeostasis, whilst uPA plays a pivotal role in the regulation of cell adhesion, migration, and proliferation during tissue remodeling (Carmeliet et al. 1997*a*). On the cell surface, uPA binds to the high-affinity urokinase receptor uPAR (CD87) that is located on the leading edge of the migrating cell (Blasi 1999), as well as to others binding sites (Pluskota et al. 2003; Mukhina et al. 2000). Binding of urokinase to uPAR provides a strictly localized proteolysis of ECM proteins in the direction of cell movement. The uPA–uPAR complex also activates the intracellular signaling, thus regulating cell adhesion, migration, and proliferation. Furthermore, both uPAR and the specific inhibitor PAI-1 can interact with adhesion receptors and ECM proteins, mediating the activation of intracellular signaling. Recently we obtained novel data expanding the knowledge on uPA-regulated pathways and on the unique and multifunctional nature of uPA. It was shown that uPA is able to be translocated to the nucleus, thus regulating gene expression (Stepanova et al. 2008*a*). Moreover, uPA is able to increase the expression of NAD(P)H oxidases, regulating the cellular redox state and vascular smooth muscle cell (SMC) growth as the autocrine growth factor (Menshikov et al. 2006*a*). Urokinase has generated special interest because it is a multifunctional multidomain protein that is not only a physiologic regulator of fibrinolysis, ECM remodeling, and cell migration and proliferation, but is also associated with several acute and chronic pathologic conditions. Recent attention has focused on the impact of the specific proteolytic property of uPA for tissue remodeling. Multiple in vitro and in vivo studies have elucidated the key role of uPA in inward remodeling (shrinking) of blood vessels, as well as in atherosclerosis progression, plaque instability, and restenosis (Nicholl et al. 2006; Parfyonova et al. 2004). Moreover, we reported that uPA can augment angiogenesis in ischemic skeletal and myocardial muscle, which makes it an even more promising multipurpose therapeutic target (Traktuev et al. 2007). This review discusses cellular and molecular mechanisms of the involvement of uPA in arterial remodeling, as well as its role for angiogenesis and vascular cell migration and proliferation.

## Mechanisms of uPA action

### uPA: structural and functional aspects

uPA is a serine protease that is synthesized by vascular endothelial cells and SMCs, monocytes and macrophages, fibroblasts, epithelial cells, and malignant tumors cells of different origins (Tkachuk et al. 1996; Clowes et al. 1990; Ossowski et al. 1991; Parfyonova et al. 2002). Expression of uPA is activated by various factors: inflammatory cytokines (Besser et al. 1995), growth factors (Ried et al. 1999), and tumor promoters (Stoppelli et al. 1986), whereas anti-inflammatory agents, such as glucocorticoids, inhibit the expression of uPA (Pearson et al. 1987).

The urokinase molecule consists of 3 structural domains: the N-terminal domain homologous to the epidermal growth factor-like domain (GFD), the kringle domain, and the C-terminal proteolytic domain (Fig. 1). The uPA proteolytic domain specifically cleaves plasminogen and converts it into the serine protease plasmin with wide substrate specificity. Plasmin directly degrades fibrin, leading to thrombus dissolution and activating a number of matrix metalloproteinases (MMPs). MMPs degrade ECM proteins and the components of the basal membrane, such as collagen, fibronectin, and laminin (Kuzuya and Iguchi 2003). Such proteolytic orchestra on the plasma membrane promotes targeted cell movement due to the destruction of cell-to-cell contacts and degradation of the local matrix. Moreover, the activation and (or) release of latent and matrix-bound growth factors represents the important mechanism enhancing the chemotactic and proliferative cellular responses.

uPA is secreted by cells as a single-chain polypeptide with molecular weight of 54 kDa (Stepanova et al. 2008*a*). The single-chain urokinase has no peptidase activity to synthetic substrates, but can convert plasminogen into plasmin. Plasmin, in turn, activates urokinase and converts it into the 2-chain form that reveals protease activity to both synthetic substrates and plasminogen with a rate of plasminogen cleavage 200-fold higher than that of the single-chain form (Lijnen et al. 1990). In the 2-chain urokinase, the polypeptide chains A and B (light and heavy chains, respectively) are connected by the Cys148–Cys279 disulfide bond (Fig. 1). The A chain includes the GFD and the kringle domain, whereas the proteolytic domain is part of the B chain. A 32 kDa urokinase that was isolated from adenoma cell culture (Stump et al. 1986) is the low molecular weight single-chain form of uPA containing amino acids 144–411 with the same enzymatic properties as the full-length form. The low molecular weight 2-chain urokinase is generated after the cleavage of the Lys135–Lys136 bond by plasmin in the region between the kringle and protease domains, and its proteolytic activity is comparable to the activity of the full-length 2-chain uPA (Stump et al. 1986) (Fig. 1). In addition to the proteolytic activation, plasmin can sequentially split off the N-terminal domains of urokinase resulting in the generation on the cell surface of several proteolytically active forms of 32–40 kDa that lack either the GFD or both the kringle domain and GFD (Poliakov et al. 2001) (Fig. 1). Thrombin hydrolysis provides the mechanism of proteolytic inactivation of uPA cleavage of the Arg156–Phe157 enzyme bond that does not exclude nonproteolytic functioning of such peptide forms (Ichinose et al. 1986; Braat et al. 1999).

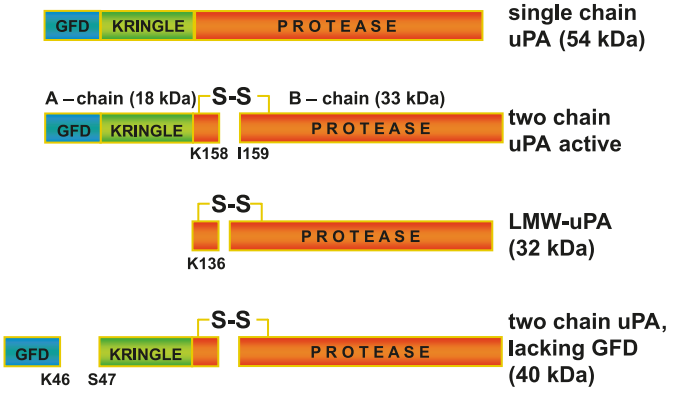
The GFD provides binding of urokinase to its receptor uPAR with high affinity ($K_d \sim 10^{-10}$–$10^{-9}$ mol/L) (Vassalli et al. 1985; Appella et al. 1987). The uPAR is concentrated in caveolae—special intrusions of the plasma membrane maintained by the membrane protein caveolin (Fig. 2). Caveolae contain glycosphingolipids, sphingomyelin, polyphosphoinositols, and cholesterol (Monier et al. 1995). The uPAR lacks a transmembrane sequence and is anchored in the plasma membrane by a glycosyl-phosphatidylinositol (GPI) moiety. Because of the GPI anchorage, the uPAR has high mobility in the plasma membrane, and its location depends on the functional state of the cell: in the resting cell,

uPAR is uniformly distributed on the surface, whereas in the migrating cell, clusters of uPARs form on the leading edge (Andreasen et al. 1997). The concentration of the proteolytic potential provides the vector movement of the cell along the chemoattractant gradient. Receptor-bound single-chain uPA is activated by plasmin more effectively than free urokinase (Cubellis et al. 1986). At the same time if urokinase is bound to uPAR, plasmin-mediated elimination of the GFD from uPA molecules occurs more slowly, as uPAR can protect bound urokinase from further degradation by plasmin. Urokinase lacking the GFD and unable to interact with uPAR undergoes rapid endocytosis and intracellular degradation (Poliakov et al. 2001). For signal transduction from uPAR, the complex formation between uPAR and transmembrane proteins such as integrins (Ossowski and Aguirre-Ghiso 2000), caveolin (Monier et al. 1995), vitronectin (Kjoller and Hall 2001; Gardsvoll and Ploug 2007), and leukocyte (L)-selectin (Sitrin et al. 2001) is necessary because the uPAR structure does not contain transmembrane or cytoplasmic domains (Fig. 2).

uPAR interaction with the ECM protein vitronectin contributes to cell adhesion (Kanse et al. 1996; Deng et al. 1996; Del Rosso et al. 2008). Urokinase stabilizes the 'vitronectin-binding' conformation of the uPAR and thus stimulates a 'vitronectin-dependent' adhesion of some cells (Kanse et al. 1996; Deng et al. 1996; Hoyer-Hansen et al. 1997). uPAR can also interact with integrins, such as the leukocyte β2 integrin Mac-1 (CD11b/CD18) (Sitrin et al. 2000) and also β1 and β3 integrins, and with the vitronectin α(v)β5 receptor (Sitrin et al. 2000; Ghosh et al. 2000) (Fig. 2). Urokinase modifies the interaction of uPAR with integrins (May et al. 1998). The interaction of urokinase with uPAR on the cell plasma membrane leads to the activation of intracellular signaling pathways that in turn result in the migration of SMCs, fibroblasts, and other cell types (Poliakov et al. 1999; Dumler et al. 1998; Nguyen et al. 1998; Busso et al. 1994; Anichini et al. 1994), as well as in cell adhesion (Waltz et al. 1993; Chang et al. 1998), proliferation, and differentiation (Rabbani et al. 1992; Furlan et al. 2007; Hildenbrand et al. 2008) (Fig. 2). uPAR is a multifunctional receptor, promoting pericellular proteolysis and matrix attachment and affecting proteinase expression during macrophage differentiation (Rao et al. 1995).

It has been shown that uPA can simultaneously bind 2 receptors on the cell surface: uPAR through the GFD and integrin Mac-1 through kringle and proteolytic domains (Pluskota et al. 2003). Moreover, the uPA kringle domain binds on the cell surface to a specific receptor that is distinct from uPAR (Mukhina et al. 2000) and contains a sequence that interacts with the inhibitor PAI-1 (Mimuro et al. 1992). The kringle domain is implicated in intracellular signaling and in the induction of cell migration and adhesion (Stepanova et al. 2008*b*). The interesting feature is the presence of an interaction between the kringle domain and the GFD. It was suggested that uPA GFD, which is not bound to uPAR, shields kringle epitopes responsible for binding with its receptor(s) on the cell surface. Exposure of kringle active sites due to the interaction of GFD with uPAR or to plasmin cleavage of GFD allows the kringle domain to bind to its receptor or several integrins and trigger migration (Stepanova et al. 2008*b*). Thus, through different domains urokinase can specifically interact with a number of target receptors and regulate cell functions.

**Fig. 1.** Proteolytic processing of urokinase leads to the generation of several uPA forms. uPA, urokinase plasminogen activator; GFD, growth factor-like domain; kringle, kringle domain of uPA; protease, proteolytic domain; LMW, low molecular weight.

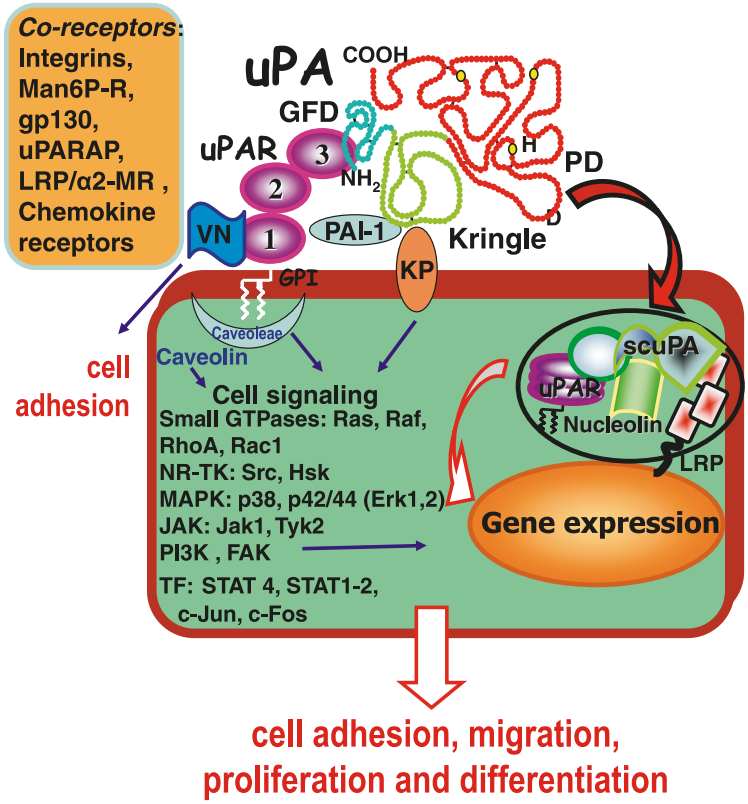



Urokinase via its A chain also binds to receptors of the low-density lipoprotein receptor (LDLR) family: to the LDLR-related protein $\alpha_2$-macroglobulin receptor (LRP/$\alpha_2$MR) (Herz et al. 1992; Conese et al. 1994, 1995) and to the very low density lipoprotein receptor (VLDLR) (Argraves et al. 1995). These receptors provide clearance of the various protease–inhibitor complexes from the cell surface by endocytosis through clathrin-coated pits. The affinity of these receptors for urokinase ($K_d$ approximately $(1–2) \times 10^{-8}$ mol/L) is lower than that of uPAR, so it has been suggested that high-affinity uPAR–uPA interaction could prevent binding of uPA to LRP/$\alpha_2$MR and subsequent intracellular degradation (Nykjaer et al. 1997). Urokinase endocytosis via LRP/$\alpha_2$MR or VLDLR can also induce intracellular signaling (Goretzki and Mueller 1998).

## Urokinase and cell migration and proliferation

The polyfunctional feature of urokinase can be attributed to its multidomain structure. Hence uPA may induce the key processes of tissue remodeling and angiogenesis, such as cell migration and proliferation, through proteolysis-dependent and proteolysis-independent mechanisms. The important role of uPA and uPAR in cell migration was shown by both in vitro (Pepper et al. 1993; Estreicher et al. 1990; Mawatari et al. 1991; Odekon et al. 1992; Morimoto et al. 1993; Okada et al. 1995; Romer et al. 1991; Pepper et al. 1993) and in vivo studies (Clowes et al. 1990; Reidy et al. 1996; Carmeliet and Collen 1996). In migrating cells, the coordinated expression of uPA and uPAR exists at cell–substrate and cell–cell contact sites (Pepper et al. 1993; Estreicher et al. 1990; Mawatari et al. 1991; Odekon et al. 1992; Morimoto et al. 1993; Okada et al. 1995, 1996; Ossowski 1992), where uPA–uPAR complexes concentrate the plasmin production that provides extracellular matrix proteolysis, weakened cell–cell contact, and increased cell motility. The proteolytic mechanisms include uPA-induced plasmin generation at focal adhesion sites, which results in ECM degradation and thus facilitates the detachment of the cell's trailing edge. Plasmin inhibitors can suppress cell migration both in vitro (Okada et al. 1996; Wang et al. 1995) and in vivo (Jackson and Reidy 1992; Clowes et al.

**Fig. 2.** The interaction of urokinase with uPAR and the kringle-binding protein (KP) involved in signaling events leads to cell adhesion and migration as well as cell proliferation and differentiation. PD, proteolytic domain of uPA; GPI, glycosyl-phosphatidylinositol moiety; VN, vitronectin; PAI-1, plasminogen activator inhibitor; scuPA, single-chain uPA; LDLR, low-density lipoprotein receptor; LRP/$\alpha_2$MR, LDLR-related protein $\alpha_2$-macroglobulin receptor; MAPK, mitogen-activated protein kinases; JAK, tyrosine protein kinases; STAT, DNA-binding signal transducers and activators of transcription; TF, transcription factors; PI3K, phosphoinositide 3-kinase; FAK, focal adhesion kinase; NR-TK, nonreceptor tyrosine kinases; Man6P-R, mannose 6-phosphate receptor; gp130, interleukin-6 signal transducer; uPARAP, urokinase receptor-associated protein.

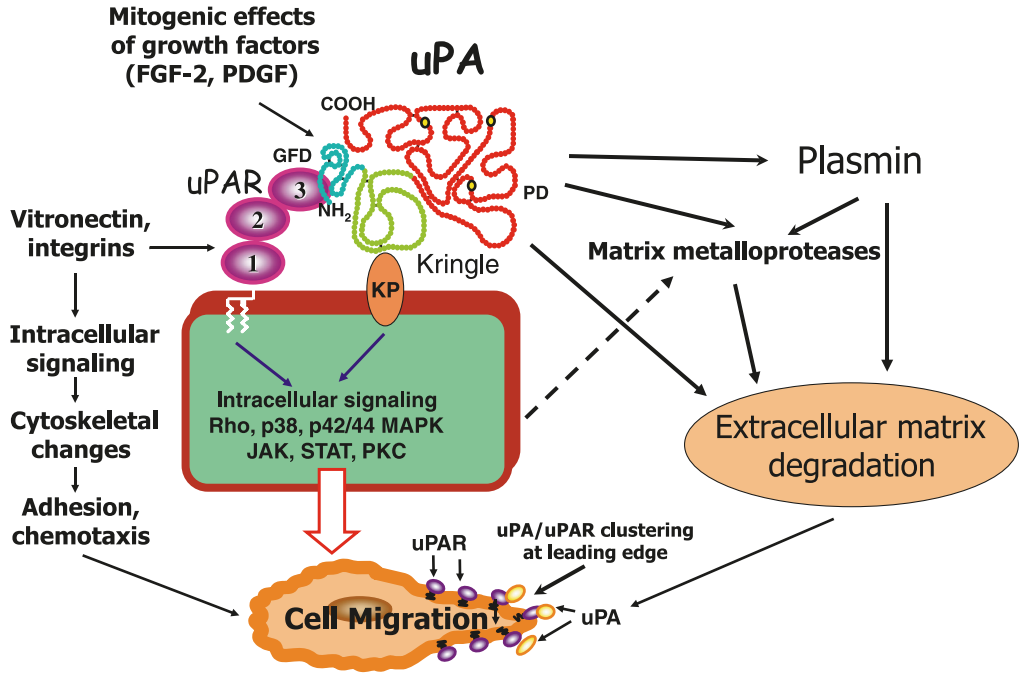



1992), suggesting an important role of plasmin-induced proteolysis in this process. Urokinase proteolytically modifies the ECM environment and affects matrix proteins that are the ligands of the integrin receptors associated with the intracellular signaling systems, thus regulating cytoskeleton rearrangements, adhesive contacts, and chemotaxis (Dumler et al. 1999*a*; Andreasen et al. 1997).

At the same time, urokinase forms that can bind with high affinity to uPAR but are proteolytically inactive or lacking the proteolytic domain can also stimulate migration of certain cells in vitro (Okada et al. 1996; Lu et al. 1996; Stepanova et al. 1997). The ability of uPA to induce chemotaxis in SMCs was attributed to the binding of the uPA–kringle domain to the surface of cells and the association of uPA with uPAR (Mukhina et al. 2000). Urokinase via its GFD appears necessary for fibroblast growth factor 2 (FGF-2) and platelet-derived growth factor (PDGF) to exert their chemotactic and mitogenic effects on vascular SMCs that might be important for arterial remodeling (Herbert et al. 1997; Stepanova et al. 1997; Ding et al. 2001). uPA stimulates cell migration via nonproteolytic mechanisms by enhancing adhesion at the leading edge of the cell, stimulating binding of uPAR to vitronectin, modulating uPAR–integrin interaction, and initiating signal transduction cascades that result in cytoskeleton reorganization and in the cell 'dragging up' to the leading edge. The pro-migrational effect can also be caused by uPA and uPAR endocytosis and by ensuring the uPAR recovery on the leading edge that accelerates a new cycle of adhesion and of cytoskeleton reorganization that are required for cell movement along the substrate. The relative significance of the proteolytic and nonproteolytic mechanisms of the uPA effect on cell migration depends on the expression by migrating cells of uPA, uPAR, integrins, and the endocytosis receptors, and on the ECM composition and plasminogen concentration. Both mechanisms could be operating simultaneously in the discrete migrating cell (Fig. 3). On the basis of studies with transgenic mice (Carmeliet et al. 1997*a*, 1998) and our in vivo studies with the use of recombinant uPA forms and uPA neutralizing antibodies (Plekhanova et al. 2000, 2001), we may suggest that proteolysis-dependent rather than uPAR-dependent mechanisms are likely more crucial for uPA-induced vascular cell migration during arterial remodeling in vivo.

Urokinase stimulates proliferation of various cell types (Fig. 4). uPA can affect mitogenesis via the direct or plasmin-mediated proteolytic activation of growth factors, such as FGF-2, latent transforming growth factor β (TGFβ), hepatocyte growth factor (HGF), and vascular endothelial growth

**Fig. 3.** uPA affects vascular cell migration by both proteolysis-dependent and proteolysis-independent modes. uPAR, urokinase receptor (CD87); Rho, small GTP-binding protein; p38, p42/44 MAPK, mitogen-activated protein kinases; PKC, protein kinase C; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor.

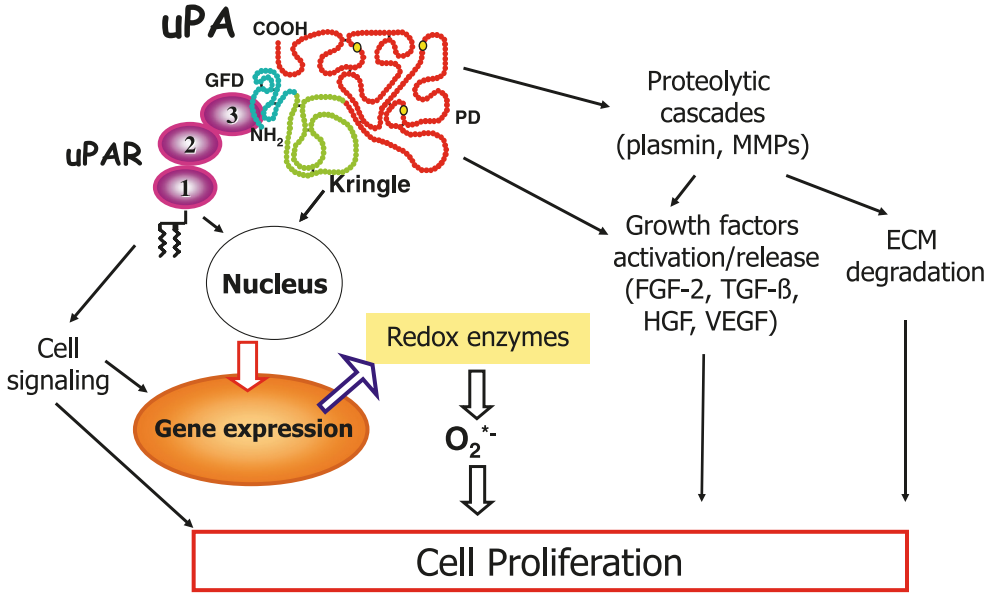



factor (VEGF) (Tkachuk et al. 1996; Agrotis and Bobik 1996; Plouet et al. 1997; Saksela and Rifkin 1990). In vascular SMCs, uPA proteolytic activity and uPA-dependent plasmin generation were required for stimulation of DNA synthesis (Stepanova et al. 1999). To induce proliferation of human fibroblasts, human kidney cells, and mesothelial cells, the interaction of proteolytically active uPA with uPAR is necessary, but in such cases the effect of uPA is not mediated by plasmin generation (Anichini et al. 1994; De Petro et al. 1994; Shetty et al. 1995). uPA stimulates proliferation of prostate cancer cells (PC-3 cell line), of human glomerular epithelial cells as well as of osteoblasts via uPAR occupancy, while uPA catalytic activity is not required (He et al. 1991; Rabbani et al. 1990), which confirms the existence of nonproteolytic mechanisms of uPA-induced proliferation. In some cases, the mitogenic effect of urokinase was observed upon the fucosylation of its GFD at Thr18 (Rabbani et al. 1992). To maintain some tumor cell proliferation in vivo, uPAR is required to form a complex with fibronectin and the integrin receptor of fibronectin (Ossowski et al. 1999). It was demonstrated that uPA stimulates the proliferation of fibrosarcoma HT-1080 cells by interaction with a signal complex of nucleolin and casein kinase 2 and by subsequent activation of casein kinase 2 (Dumler et al. 1999*b*). Recently we found the novel mechanism by which uPA may stimulate mitogenesis: we showed that the enhanced generation of reactive oxygen species (ROS) is essential for vascular SMC growth mediated by both exogenous and endogenous uPA, proving uPA as an autocrine growth factor (Fig. 4) (Menshikov et al. 2006*a*).

## Urokinase signaling

Urokinase activates a number of signaling pathways. The signaling effects of urokinase are suggested to be mediated by uPAR, LRP/$\alpha_2$MR, or other membrane uPA-binding proteins (Binder et al. 2007). It was shown that urokinase-induced cell migration is associated with the activation of Src and Janus kinases (Dumler et al. 1998, 1999*a*; Resnati et al. 1996). It has also been demonstrated that uPAR can be coprecipitated with the following tyrosine protein kinases: Hck, Fyn, Lyn, Frg, Jak1, and Tyk2 (Dumler et al. 1998; Resnati et al. 1996; Bohuslav et al. 1995; Konakova et al. 1998). uPAR-dependent chemotaxis could be observed on the wild-type cells, but not on the cells with the *Src* gene knockout (Fazioli et al. 1997). It has also been shown that the heterotrimeric GTP-binding proteins (G proteins) mediate the urokinase-induced cell chemotaxis (Resnati et al. 1996). uPA–uPAR binding results in the activation of Hsk kinase, focal adhesion kinase (FAK), paxillin, the mitogen-activated protein (MAP) kinases p38 and p42/44, protein kinase Cε (PKCε), and serine phosphorylation of cytokeratins 8 and 18, and it promotes the phosphorylation of p130CAS protein and of DNA-binding activators of transcription STAT-1 and STAT-2 proteins (Dumler et al. 1999*a*; Resnati et al. 1996; Nguyen et al. 1998; Tang et al. 1998; Busso et al. 1994; Dumler et al. 1993). Such intracellular signal transduction pathways could theoretically mediate the expression of MMPs in the vessel wall induced by urokinase, which might be important for vascular remodeling. In human vascular SMCs, the PDGF receptor may also be involved in urokinase-induced signaling, thereby participating in regulation of SMC migration (Kiyan et al. 2005). The binding of urokinase and of some other ligands to LRP/$\alpha_2$MR results in the activation of protein kinase A with the involvement of $G_s$ protein (Goretzki and Mueller 1998). LDLR family members are involved in the activation of MAP kinases and in the regulation of cell adhesion (Gotthardt et al. 2000). uPA–uPAR binding acti-

**Fig. 4.** The putative mechanisms of uPA-stimulated cell proliferation. MMPs, matrix metalloproteinases; TGF, transforming growth factor; HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factor; ECM, extracellular matrix.




vates the intracellular signaling and cell migration. For uPA's kringle-induced migration, a specific activation of the p38 MAP kinase cascade through the activation of small GTP-binding protein Rho is required. The inhibition of this signaling pathway blocked both the uPA and uPA's kringle-stimulated cell migration (Goncharova et al. 2002). In endothelial cells, uPA activates MAP kinase signaling via uPAR occupancy, as well as via PKC activation due to uPA proteolytic activity, leading to both stimulation of endothelial cell migration and angiogenesis (Tang et al. 1998). Functional analysis has shown that Mac-1 (complement receptor 3 or CD11b/CD18) can also mediate the signaling effects of the uPAR (Todd and Petty 1997). On several cell types, uPAR appears to be stably associated with β1 and β3 integrins (Xue et al. 1997; May et al. 1998; Ghosh et al. 2000; Wei et al. 1999) that interact with the cellular cytoskeleton (Andreasen et al. 1997). uPA-induced monocytic cell migration was shown to require the interaction of uPAR with integrins (Sitrin et al. 2000; Wang et al. 1995; Gyetko et al. 2000). uPAR is also a high-affinity receptor for the ECM protein vitronectin, and uPA binding to uPAR strongly promotes vitronectin–uPAR binding (Hoyer-Hansen et al. 1997; Kanse et al. 1996; Wei et al. 1994). uPAR-bound vitronectin binds integrins α(v)β3 and α(v)β5 and provides adhesion of cells on vitronectin. In vascular SMCs, vitronectin is selectively phosphorylated by ectoprotein kinase casein kinase 2 (CK2) in a uPA/uPAR-dependent manner (Dumler et al. 1999*b*). The phosphorylated vitronectin is a better ligand for integrins and uPAR than is the unphosphorylated form. The uPA-dependent cell adhesion is a function of selective vitronectin phosphorylation by the ectokinase CK2, whose activity appears to be regulated by uPA. uPA can also induce cell proliferation through the activation of the complex that includes uPAR, CK2, and a shuttle protein nucleolin (Dumler et al. 1999*b*). Nucleolin regulates recombinant DNA (rDNA) transcription, DNA replication, cell growth, and angiogenesis (Stepanova et al. 2008*a*). Recently we revealed the novel pathway by which uPA is rapidly translocated to the nucleus in a nucleolin-dependent manner, where it might participate in regulating gene expression (Stepanova et al. 2008*b*). The nuclear transport was mediated by the kringle domain of uPA, and neither uPAR nor LRP/$\alpha_2$MR were required. Our preliminary data raise the possibility that uPA in association with nucleolin and (or) transcription factors may possess transcriptional activities. Moreover, in vascular SMCs, uPA increases ROS production (Menshikov et al. 2006*b*). Using small interfering RNA (siRNA), we proved that the NAD(P)H oxidases Nox1 and Nox4 are the primary source of ROS generation stimulated by uPA in vascular SMCs, providing a novel signaling mechanism involved in regulation of cell proliferation and vascular remodeling.

## Urokinase and vessel wall remodeling

### Vessel wall remodeling and restenosis after arterial injury

After percutaneous transluminal coronary angioplasty (PTCA), one of the major techniques of revascularization, an acute recoil response can be responsible for about 30% immediate loss of the vessel lumen at the end of the procedure. Restenosis (defined as >50% diameter stenosis on follow-up angiography) is the late (within 6–9 months) loss of the lumen of the artery due to vessel shrinkage and an intense proliferative and migrative response to the local injury (Chandrasekar and Tanguay 2000; Kantor et al. 1999; Schwartz and Holmes 2001). About 30% to 60% of patients suffer recurrent ischemia within 6 months of angioplasty because of renarrowing of treated arteries (Rajagopal and Rockson 2003; Bhoday et al. 2006). Restenosis occurs after angioplasty as a result of the complex healing response of the vessel wall to injury and demonstrates a cascade of cellular and molecular events and a release of a number of vas-

oactive, thrombogenic, and mitogenic factors (Schwartz and Henry 2002). Current evidence suggests that restenosis is a maladaptive response of the coronary artery to trauma induced during angioplasty consisting of thrombosis, inflammation, cellular migration and proliferation, and ECM production that together contribute to postprocedural lumen loss (Fig. 5) (Schwartz and Henry 2002; Nikol et al. 1996). Lumen loss after balloon angioplasty can be separated into 3 distinct stages: early loss associated with elastic recoil, late loss due to negative remodeling, and neointimal hyperplasia (Rajagopal and Rockson 2003). At the site of vessel injury the power release of mitogens and pro-migratory proteins from activated platelets and SMCs promotes cell proliferation and migration (Pakala et al. 1997; Miano et al. 1993). Neointimal hyperplasia is largely a result of migration of activated medial SMCs to the site of injury and ECM synthesis by these cells (Weintraub 2007). Intimal hyperplasia is associated with a transition of the vascular SMCs from a contractile to a synthetic phenotype of actively proliferating cells (Ross 1993; Yamamoto et al. 1993). This phenotypic transformation is associated with an increased production of ECM components such as sulfated proteoglycans and collagen, which possess a regulatory effect on cell proliferation and differentiation (Nagler et al. 1997; Sakata et al. 1990; MacLeod et al. 1994). Some authors also suggested that bone marrow-derived progenitor cells contribute to the neointimal hyperplasia after injury (Tanaka et al. 2003, 2008; Zernecke et al. 2005; Soda et al. 2007).

Negative arterial remodeling is a major determinant of late luminal narrowing after nonstent interventions (Mintz et al. 1996*a*; Lansky et al. 1998; Kimura et al. 1997). In animal models of arterial injury, compensatory arterial enlargement is an important determinant of the lumen area after balloon angioplasty (Kakuta et al. 1994). Furthermore, a growing body of evidence indicates that vessel constriction may be largely responsible for restenosis (Lafont et al. 1995; Post et al. 1994; Mintz et al. 1996*b*). Post and colleagues, using 3 different animal models (normal rabbits and normal and atherosclerotic Yucatan pigs) and 2 different types of injury (standard balloon and thermal angioplasty), found that remodeling, and not neointimal hyperplasia, is the most important determinant of restenosis. These experimental findings are consistent with clinical observations with serial intracoronary ultrasonographic evaluations (Mintz et al. 1996*a*, 1996*b*). Histomorphometric studies confirm that constrictive remodeling determines the long-term outcome of PTCA (Sangiorgi et al. 1999).

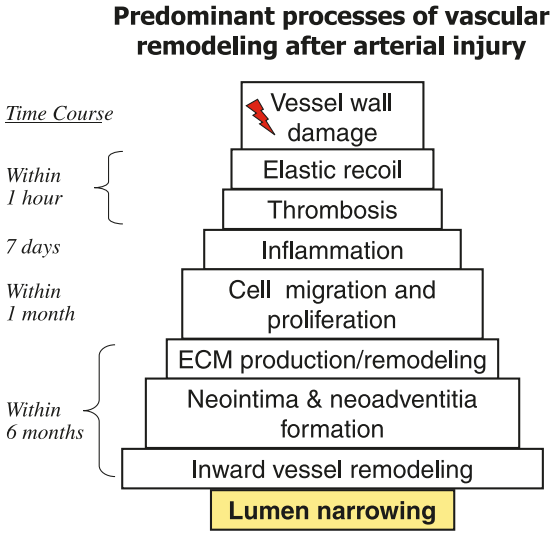
However, the mechanisms providing the post-PTCA constrictive remodeling are not fully understood. In a previous necropsy study, 2 subgroups of histologic findings (presence or absence of intimal proliferation) were documented among the restenotic coronary arteries (Waller et al. 1991). The authors suggested that restenotic lesion morphology is reflective of differing mechanisms of restenosis (neointimal increase and vessel recoil). Also, this restenotic lesion-specific difference may in part explain the discrepancy in the reported mechanisms of restenosis after interventions (Lansky et al. 1998; Di Mario et al. 1995; Okura et al. 2002). Lafont et al. (1995) evaluated the contribution of different layers of the vessel wall to restenosis after balloon injury in rabbit femoral arteries. They found a significant relationship between the adventitial area (normalized to intimal area) and chronic constriction. The response to stretch-induced adventitial injury (Scott et al. 1996; Shi et al. 1996*b*) results in adventitial fibrosis and contraction, with circumferential arterial constriction at the injury site (Mintz et al. 1996*a*; Shi et al. 1996*b*). Shi et al. (1996*a*) demonstrated that deep arterial injury in pig coronary arteries caused stretching of the adventitia, resulting in increased adventitial thickness, myofibroblast proliferation, and extracellular collagen formation. This adventitial fibrotic response after balloon injury may play a role in restenosis by either circumferential compression of the vessel (Mintz et al. 1996*b*) or by preventing external elastic laminae (EEL) enlargement. In heavily atherosclerotic human coronary arteries, a univariate relationship between adventitial thickness and histologic PTCA outcome was found (Sangiorgi et al. 1999).

A number of molecular systems have been implicated in neointimal development and arterial remodeling after vessel wall injury, including growth factors VEGF (Carmeliet and Collen 1997), TGF (Agrotis et al. 2005), PDGF (Mallawaarachchi et al. 2006; Raines 2004), tumor necrosis factor alpha (TNF-α) (Murayama et al. 2008), the angiotensin system (Heeneman et al. 2007), chemokines and the corresponding receptors (Schober and Zernecke 2007), the MMPs system, the coagulation system, and the plasminogen system (Bobik and Tkachuk 2003).

**Urokinase and arterial remodeling**

Plasminogen activators tPA and uPA can both activate plasminogen to plasmin, but their impact in vascular remodeling is different. After balloon catheter injury to a vessel, tPA and uPA expression by the medial SMCs is rapidly increased (Clowes et al. 1990). Correlations have been reported between the early expression of uPA and SMC proliferation and tPA and SMC migration in balloon catheter-injured arteries (Clowes et al. 1990). We showed that the increases in uPA expression became apparent as early as 6 h after injury and persist for at least 4 days in the media and developing neointima (Plekhanova et al. 2001), that is, throughout the time that the SMCs proliferate rapidly in the media and migrate to form the neointima (Clowes et al. 1990). We also found that in flow-induced models of vascular remodeling, the content of uPA correlated with neointimal growth, whereas tPA content correlated with outward arterial remodeling (Korshunov et al. 2004). Other studies in transgenic mice (Carmeliet et al. 1997*b*) and primate arterial tissue (Kenagy et al. 1996) suggest that uPA is the key participant in neointimal development. Studies in knockout mice showed that lack of uPA or uPA and tPA leads to impaired neointimal healing. These mice therefore had a significantly decreased intima/media ratio compared with that of wild-type mice or mice deficient only in tPA. They also had decreased luminal stenosis and fewer intimal cells compared with wild-type mice or mice deficient only in tPA (Carmeliet et al. 1997*b*). On the other hand, other studies showed that uPA-deficient mice had increased luminal stenosis, although these mice also had fewer intimal cells than did wild-type or tPA-deficient mice (Schafer et al. 2002). The increased luminal stenosis was due to a large amorphous, acellular, and fibrin-rich thrombus (Schafer et al. 2002). In uPA-deficient mice, neointimal SMCs had not migrated to the centre of

**Fig. 5.** Progression of vascular remodeling after arterial injury.




the lesion. The role of uPA in breaking down the ECM appears to be important in enabling vascular SMC migration (Carmeliet et al. 1997*c*) and hence neointimal formation. Expression of uPAR enables uPA binding, providing a mechanism by which uPA can be localized for proteolysis, for example along the leading edge of migrating cells. Moreover, uPAR-deficient mice showed no difference in their response to vascular injury compared with wild-type mice (Carmeliet et al. 1998). uPAR deficiency does not seem to affect the response to vascular injury, indicating the predominant role of uPAR-independent uPA-induced signal transduction pathways.

## uPA properties and arterial remodeling

Our previous studies showed that uPA is an important factor regulating the healing responses of balloon catheter-injured arteries (Plekhanova et al. 2000). Although cell culture studies indicate that uPA stimulates SMC DNA biosynthesis and migration by mechanisms apparently not dependent on proteolysis or uPAR occupancy (Stepanova et al. 1997, 1999; Poliakov et al. 1999; Tang et al. 1998; Kanse et al. 1997), our in vivo observations of the effects of uPA neutralizing antibodies in the injured arteries suggest that uPA's proteolytic property also contributes to neointimal formation by increasing SMC proliferation and migration (Plekhanova et al. 2000). By examining the effects of recombinant forms of native (i.e., wild type) and mutated uPA forms (proteolytically inactive uPA and uPA with modified growth factor-like domain) on SMC proliferation and the size of the developing neointima, we found that the predominant structural region of uPA responsible for regulating SMC proliferation, neointimal formation, and probably SMC migration is the proteolytic domain (Plekhanova et al. 2001). In balloon catheter-injured arteries, the proteolytic property of uPA, independent of the interaction with uPAR, is the prime mechanism by which uPA enhances neointimal growth. Whilst uPAR can be involved in SMC migration, in its absence other non-uPAR-dependent mechanisms appear to take over (Yu et al. 1997). Moreover, the ability to stimulate neointimal formation and inward arterial remodeling is a specific property for uPA that cannot be mimicked by tPA. By examining the effects of recombinant uPA and tPA on vessel structure, we found the contrasting effects of plasminogen activators on neointimal formation and arterial narrowing (Parfyonova et al. 2004). Recombinant tPA attenuated early neointimal formation and neointimal SMC accumulation, contrasting markedly with the effects of proteolytically active uPA. tPA also significantly increased the area encompassed by the EEL, whereas uPA decreased it. These results reflect differential early vessel remodeling stimulated by plasminogen activators: tPA inducing outward remodeling and uPA inducing inward remodeling, as defined by Mulvany et al. (1996). At later stages (28 days after injury), the effect of tPA on neointimal formation was similar to that observed early after injury: recombinant tPA attenuated intima–media thickness, although its effects on lumen size and the area encompassed by the EEL were not evident. In contrast, inward arterial remodeling and stimulation of neointimal growth induced by uPA at early stages remained significant at later time points, indicating that different mechanisms provide plasminogen activator effects on arterial remodeling.

## uPA and adventitial response to injury

It is possible that uPA might augment neointimal growth and inward arterial remodeling, affecting adventitial fibroblasts. In post-angioplasty restenosis, adventitial cells are involved in both the process of inward arterial remodeling and neointimal formation after vessel injury (Scott et al. 1996; Shi et al. 1996*b*; De Leon et al. 2001). Gabbiani and co-workers (Gabbiani 2003; Gabbiani et al. 1971) have established that wound healing is associated with the transition from fibroblasts to myofibroblasts, which then proliferate, migrate, and synthesize ECM components such as collagen type I and III. An important role of uPA for myofibroblast function in tissue repair was suggested by findings of enhanced expression of uPA during wound healing inflicted on cultured human fibroblasts (Hatane et al. 1998). Other in vitro studies have demonstrated the elevated uPA production by myofibroblasts, which are capable of extensive migration (Sieuwerts et al. 1999; Romer et al. 1991). In transplanted hearts, high uPA expression by adventitial myofibroblasts during arterial remodeling of coronary arteries was shown by Garvin and co-workers (1997). We demonstrated that uPA could augment myofibroblast accumulation and adventitial growth in injured rat carotid artery adventitia in vivo by mechanisms dependent on its proteolytic properties (Plekhanova et al. 2006*a*). Recently, we revealed the mechanism of fibroblast-to-myofibroblast transformation induced by uPA (Stepanova et al. 2008*a*). We found that nucleolin-dependent nuclear translocation of uPA is required for myofibroblastic modulation induced by proteolytically active (but not proteolytically inactive) uPA, indicating the importance of both nuclear translocation as well as proteolysis.

## uPA and vascular inflammatory response to injury

Vascular inflammation induced by proinflammatory cytokines is suggested to be one of the key mechanisms in the development of neointimal hyperplasia, restenosis, and atherosclerosis (Okamoto et al. 2001; Shah 2003). An important

role for leukocyte accumulation is postulated in neointimal formation and restenosis (Shah 2003). In particular, leukocytes contribute to the generation of ROS and augment oxidative stress and restenosis (Cipollone et al. 2001). Moreover, macrophages express multiple proteases, including MMPs and urokinase that degrade the ECM and secrete numerous other effectors, including ROS, TNF-α, and interleukin-1 (IL-1) (Boyle 2005). Delayed monocyte recruitment into experimental venous thrombus was associated with absence of uPA (Singh et al. 2003). Antibodies to uPAR or selective blockade of uPAR expression by antisense oligonucleotides completely abrogates monocyte chemotaxis (Okada et al. 1996; Gyetko et al. 1995). Moreover, uPAR is known to take part in the release of both inflammatory cytokines TNF-α, IL-1β, and IL-6 and chemokines IL-8 and monocyte chemoattractant protein (MCP)-1 from human blood mononuclear cells through p38, c-Jun N-terminal kinases (JNKs), and NF-κB activation (Khan et al. 2006; Del Rosso et al. 2008). In our studies in the rat model of balloon catheter injury of the carotid artery, we found that uPA but not tPA augmented inflammatory cell accumulation in the arterial adventitia (Parfyonova et al. 2004). In injured adventitia, uPA treatment contributed to ED1-positive cell accumulation, whereas uPA neutralizing antibody induced the opposite effect (Plekhanova et al. 2006*b*). These data are consistent with findings in uPA knockout mice that uPA mediated infiltration of leukocytes during arterial remodeling and neointimal formation after injury (Carmeliet et al. 1997*d*) and during arteriogenesis (Deindl et al. 2003). Adventitial inflammatory response and subsequent constrictive fibrosis have been proposed to be the major causes of constrictive inward remodeling after balloon injury (Mori et al. 2002; Wilcox and Scott 1996). It was shown that inhibition of the inflammatory response in the adventitia limited the development of arterial constrictive remodeling by reducing adventitial fibrosis (Mori et al. 2002). The inflammatory reaction was also suggested to underlie the pathogenesis of hypertension and the associated lesion formation (Suematsu et al. 2002).

Recently we found that uPA is able to stimulate the expression of inflammatory mediators including IL-1β-converting enzyme, TNF-α, and TNF-α-converting enzyme (TACE) (Plekhanova et al. 2008). Since IL-1β and TNF-α are central mediators in the cytokine network, they may act as the potent activators of cardiovascular cells involved in pathogenesis of cardiovascular diseases, including atherosclerosis and restenosis (Bhoday et al. 2006). The selective upregulation of TACE in uPA-treated vessels compared with vehicle-treated arteries deserves special attention because TNF-α is one of the most potent proinflammatory cytokines secreted by monocytes and macrophages. TNF-α has pleiotropic functions in cardiovascular diseases (Levine et al. 1990). The periprocedural release of plaque-derived TNF-α was supposed to represent the amount and activity of the atherosclerotic process and to be a predictor for restenosis (Bose et al. 2007). Moreover, a number of studies demonstrated the role of TNF-α signaling for proliferative arterial diseases and restenosis (Li et al. 2007; Kubica et al. 2005). The upregulation of TNF-α represents a potent mechanism for leukocyte recruitment to the injured region (Seki et al. 2005). Moreover TNF-α stimulates SMC migration, one of the key processes of vascular stenotic lesion formation (Ono et al. 2004). It was also shown that TNF-α may stimulate proliferation of vascular SMCs (Selzman et al. 1998) and enhance their proliferation induced by monocytic cells (Voisard et al. 2001), which is important for neointimal growth and vessel remodeling. Finally, TNF-α was shown to be a key transcriptional inducer of several MMPs (Overall and Lopez-Otin 2002; Siwik and Colucci 2004), active participants in the arterial response to intravascular injury.

**uPA-associated cascades and arterial remodeling**

Proteolytic cascades initiated via uPA in injured arteries have the potential to stimulate SMC proliferation and migration by a number of highly coordinated mechanisms (Tkachuk et al. 1996; Agrotis and Bobik 1996). Urokinase itself proteolytically activates proforms of HGF, macrophage stimulating protein (MSP), and an isoform of VEGF189 (Naldini et al. 1992; Plouet et al. 1997). uPA-generated plasmin activates latent TGFβ and other VEGF isoforms (Godar et al. 1999) and promotes the release of basic FGF (bFGF) from ECM (Saksela and Rifkin 1990). These growth factors bind to their receptors on the cell surface and activate intracellular signaling pathways that have the potential to further augment cell proliferation and migration (Lindner and Reidy 1993). TGFβ1 was shown to augment neointimal formation after balloon catheter injury to carotid arteries (Smith et al. 1999). Moreover, uPA due to its own proteolytic activity (independently of plasmin generation) can cleave certain matrix proteins (particularly fibronectin) and activate MMPs, thus affecting ECM remodeling and cell migration (Carmeliet et al. 1997*c*). Because of its wide substrate specificity, once plasmin is activated by uPA, it may digest some matrix proteins or convert proforms of MMPs to active MMPs, which, in turn, further degrade the components of the ECM proteins, including collagens (Keski-Oja et al. 1992). MMPs are important for SMC migration into the intima and for their replication (Kuzuya and Iguchi 2003; Jenkins et al. 1998; Cho and Reidy 2002). MMP-9-deficient mice have decreased neointimal lesion formation, as well as SMC migration and proliferation after arterial injury (Bhoday et al. 2006; Galis et al. 2002). Moreover, MMP-2 and MMP-9 can modulate interleukin activity, processing IL-1β precursor to its active form and being agonists of the inflammatory response (Schonbeck et al. 1998). After experimental balloon angioplasty, perivascular uPA increased the content and activity of MMPs (MMP-2 and MMP-9) in the arterial wall in vivo, whereas tPA did not induce such changes (Plekhanova et al. 2006*a*). We also showed that urokinase upregulates MMP-9 expression in monocytes via MMP-9 gene transcription and protein biosynthesis (Menshikov et al. 2002), thus providing the mechanism by which uPA could be responsible for continual MMP-9 upregulation in vivo.

Of great interest is the potential ability of uPA to regulate oxidative stress after arterial injury, which may represent one of the important mechanisms of arterial remodeling induced by uPA (Plekhanova et al. 2008). Oxidative stress is a signaling mechanism of tissue repair involving mainly cell proliferation and early apoptosis (Pollman et al. 1999; Azevedo et al. 2000). Moreover, oxidative stress induced by

injury contributes to unfavorable vessel remodeling and plays an important role in a number of cardiovascular pathologies (Berk 1999, 2007; Alexander 1995). Previous studies showed an increased superoxide production after balloon injury and demonstrated the beneficial effects of antioxidant administration on vascular remodeling (Nunes et al. 1997). ROS are the important signaling molecules regulating vascular SMC proliferation and vascular tone (Berk 2001; Ellis and Triggle 2003). It was reported that uPA contributes to superoxide production (Cao et al. 1995) and release (Sitrin et al. 2000) by neutrophils. uPA increases ROS production in SMCs by stimulating both the expression and activity of NAD(P)H oxidases (Nox1 and Nox4, equally) (Menshikov et al. 2006*b*). Moreover, uPA stimulates SMC proliferation in an ROS-dependent manner. An autocrine role for uPA in ROS production may be an important novel mechanism regulating the redox state and remodeling of the vessel wall. An in vivo study showed changes in oxidation-related gene expression after recombinant uPA treatment compared with that of control arteries, suggesting that uPA has the potential ability to regulate oxidative stress in the injured artery (Plekhanova et al. 2008). The cross-interaction between inflammation-related and oxidation-related gene patterns may occur in the injured artery; for example, TNF-α is able to cause oxidative stress in a variety of cell types (Nakano et al. 2006) and to increase mitochondrial ROS production (Nishikawa and Araki 2007). Novel findings suggest that ROS and TACE/TNF-α signaling pathways in the injured artery could act synergistically, affecting neointimal formation and vascular inflammation in an additive manner (Tanaka et al. 2008). In a recent study, a model was proposed for the interaction between ROS signaling and pathways activated by upregulation of the TACE/TNF-α system, leading to vascular remodeling (Ungvari et al. 2006). As uPA influences both ROS production and TACE/TNF-α expression, we may hypothesize that uPA could affect this cross-interaction.

## Urokinase and coronary artery disease

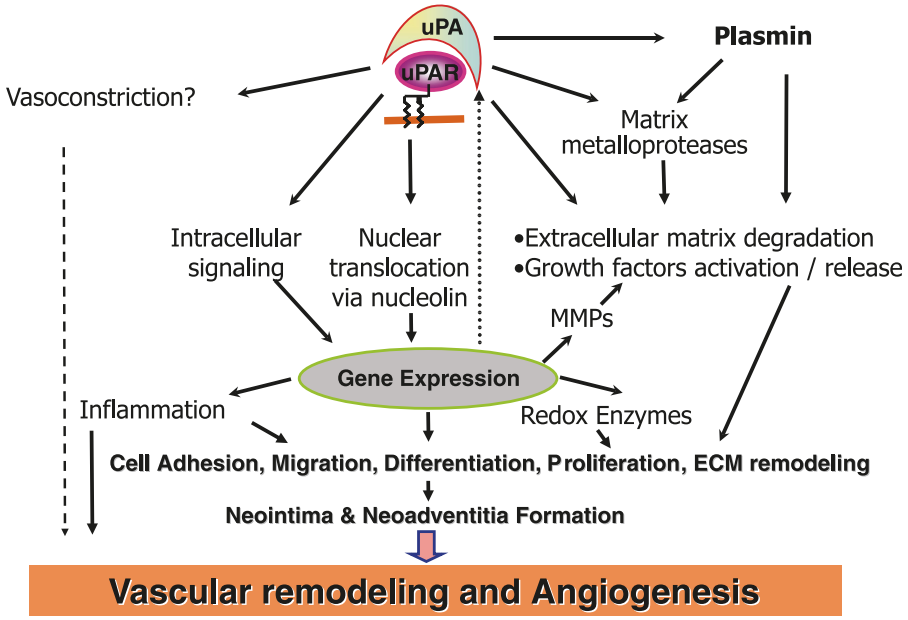
Elevated urokinase level is the predictor for angiographic coronary restenosis (Strauss et al. 1999). We found that plasma uPA antigen and activity levels are significantly higher in patients with stable angina than in healthy volunteers (Krasnikova et al. 1999). uPA expression and activity are increased in SMCs and macrophages of atherosclerotic and restenotic lesions of human arteries (Lupu et al. 1995; Kienast et al. 1998; Padro et al. 1995; Schneiderman et al. 1995; Raghunath et al. 1995). The increased levels of uPA are found in the neointima and in more mature plaques, and in macrophage-rich areas close to the necrotic core where both macrophages and foam cells highly express uPA (Lupu et al. 1995; Kienast et al. 1998). In vivo studies demonstrated that overexpression of uPA in macrophages accelerates atherosclerotic progression and early death in transgenic mice (Cozen et al. 2004).

Different roles of uPA and tPA in restenosis development and atherosclerosis progression are suggested. tPA has been implicated in maintaining vessel patency, perhaps owing to its specificity for fibrin, whilst uPA is a key regulator of pericellular proteolysis-related events and cell migration, adhesion, and proliferation—the integral parts of vessel wall repair (Fay et al. 2007). The impaired fibrinolysis appears to be an important contributor to atherosclerosis progression because of thrombus formation on the damaged vessel wall section that may accelerate atherosclerotic processes in nontarget vessels and contribute to restenosis development (Raghunath et al. 1995). In atherosclerotic lesions, the increased tPA expression was observed in macrophages and SMCs,and its intensity correlated with the severity of the lesion, reflecting the protective mechanism for maintaining vessel patency. Changes in tPA plasma levels and activity are not associated with restenosis (Christ et al. 1999; Brack et al. 1994). Moreover, in a rabbit model of hypercholesterolemia, the infusion of tPA after arterial injury suppressed SMC proliferation and attenuated intimal hyperplasia (Kanamasa et al. 2001).

## Urokinase and angiogenesis

Tissue neovascularization includes vasculogenesis (vessel formation by differentiation of cell precursors, angioblasts, and stem cells into endothelial cells), angiogenesis (the sprouting of new capillaries from the preexisting vasculature), and arteriogenesis (the remodeling of capillaries or of preexisting collateral vessels into muscle arteries) (Parfyonova et al. 2002). To initiate angiogenesis, vessel destabilization is necessary, and this includes weakening of the intercellular contacts between endothelial cells, destruction of the basement membrane, and local proteolysis of matrix proteins to allow the endothelial cells to migrate and produce new capillaries (van Weel et al. 2008; Lamalice et al. 2007). The involvement of uPA and uPAR in angiogenesis is well established (Parfyonova et al. 2002). Endothelial cells overexpressing uPA possess higher invasiveness than do intact cells in vitro (Gualandris et al. 1997). uPAR expression is associated with the mostly invasive edges of the tumor and might be a marker of invasive disease, tumor angiogenesis, and metastasis (Nakata et al. 1998; Duffy et al. 1999). uPA stimulates endothelial cell tube formation on fibrin matrix, whereas the antibody blocking urokinase binding to uPAR and to soluble uPAR inhibits the effects of uPA (Lansink et al. 1998; Koolwijk et al. 1996). In a hindlimb ischemia model, uPA-deficient mice demonstrated impaired endogenous ability to restore blood flow compared with wild-type mice (Deindl et al. 2003). The infiltration of tissue by monocytes and macrophages secreting proangiogenic factors is known to stimulate angiogenesis (Davis et al. 2003; Arras et al. 1998). Circulating monocytes are suggested to play a substantial role in collateral vessel development (Heil et al. 2002). Urokinase and its receptor stimulate macrophage accumulation and participate in the inflammatory response in tissue during both arterial remodeling and arteriogenesis (Carmeliet et al. 1997*d*; Deindl et al. 2003). Extracellular proteolysis due to uPA-dependent generation of plasmin on the cell surface is necessary for angiogenesis. uPA and plasmin initiate the strictly directed degradation of the basement membrane proteins, such as fibronectin and laminin. Urokinase and uPA-generated plasmin can activate and (or) release the latent MMPs and elastase, as well as growth factors involved in angiogenesis, especially VEGF, bFGF, HGF, TGFβ, and PDGF, further contributing to the endothelial cell migration, invasion, and proliferation (Saksela and

**Fig. 6.** The putative mechanisms of the involvement of urokinase in regulation of arterial remodeling and angiogenesis.




Rifkin 1990; Tkachuk et al. 1996; Carmeliet et al. 1997*a*; Plouet et al. 1997; Mazar et al. 1999; van Hinsbergh et al. 2006). Urokinase can mediate the angiogenic effect of some growth factors not only by localization of cell surface proteolysis but also by nonproteolytic mechanisms. The antibody blocking uPA–uPAR binding suppressed bFGF- and VEGF-induced tube formation in fibrin matrix. In uPA-deficient mice, angiogenesis in infarcted hearts was significantly impaired and failed to be stimulated by VEGF, suggesting a critical dependence of VEGF angiogenic activity on uPA (Heymans et al. 1999). This effect may be dependent on activation of VEGF isoforms by uPA (Naldini et al. 1992; Plouet et al. 1997) and by uPA-generated plasmin activity (Godar et al. 1999). Moreover, VEGF induces uPA in vascular endothelial cells (Mandriota et al. 1995; Ferrara 2004). We may suppose that the activation of VEGF by uPA is essential for the realization of VEGF angiogenic properties in vivo.

HGF-stimulated retinal angiogenesis was mediated by increased urokinase activity (Colombo et al. 2007). HGF increased the migratory and invasive capacity of retinal endothelial cells, which could be inhibited by the disruption of urokinase–uPAR interactions. The proteolytic cleavage of urokinase between Lys135–Lys136 by plasmin or by uPA itself, or the cleavage of the Glu143–Leu144 bond by MMPs, results in generation of the 'connecting peptide', inhibiting the interaction of uPA with uPAR and thus suppressing endothelial cell migration stimulated by bFGF and angiogenesis in vivo (Ugwu et al. 1998). This connecting peptide also inhibits tumor cell invasion and attenuates the growth and metastasis of tumors (Guo et al. 2000). uPA may also influence angiogenesis by the activation of intracellular signaling pathways in endothelial cells. uPA via uPAR occupancy activates MAP kinase signaling and the phosphorylation of focal adhesion proteins, whereas uPA proteolytic activity via matrix degradation and the disruption of integrin ligation activates PKC (Tang et al. 1998).

Recently we demonstrated that plasmid-based uPA overexpression stimulated vessel growth and tissue perfusion, limiting myocardial damage and subsequent remodeling after infarction (Traktuev et al. 2007). Moreover, in hearts treated with uPA plasmid, highly significant increases in the density of both capillaries and SMC-containing arterioles were observed. uPA did not induce hypervascularization of uninjured nonischemic regions nor did it induce any tissue edema, indicating safety of such proangiogenic therapy. Our findings suggest the effectiveness of this novel strategy of using uPA for stimulation of vessel growth in both ischemic heart and skeletal muscle, exerting its effects both directly and indirectly through the activation of growth factors, cytokines, and proteases.

## Perspectives

A number of studies suggest that urokinase provides strict regulation of ECM degradation, cell adhesion, migration, proliferation, vascular remodeling, and angiogenesis. It accomplishes this as a multifunctional multidomain protein through specific proteolytic activity, plasmin generation, and receptor-binding interactions between its specific structural domains and matrix proteins, integrins, endocytosis receptors, and activation of intracellular signal pathways. The long-term effects of uPA in vivo might be related to its action on protein expression and its nuclear translocation and possible interaction with transcription factors. Moreover, uPA induces the local proteolytic cascade that is also of great importance for both vascular remodeling and angiogenesis. We may summarize the putative mechanisms of the involvement of urokinase in regulation of angiogenesis and arterial remodeling into the scheme shown in Fig. 6. However, additional studies are required to address several questions in this field. For instance, better understanding of the cross-interactions between ROS, TACE/TNF-α, and uPA

signaling pathways involved in vascular disease will be helpful in establishing strategies to deal with pathologies. The question of whether uPA affects the activity of transcription factors deserves further study. Clarification of these issues will bring us to a possible solution to the urokinase enigma.

Urokinase constitutes one of the critical participants in the response mechanism to cardiovascular injury, which makes it an interesting and promising therapeutic target in vascular diseases. Arterial response to injury is characterized by cell proliferation, migration, and intimal hyperplasia and plays a key role in arterial remodeling during vascular disorders such as atherosclerosis and restenosis. We may suppose that blocking uPA signaling pathways or neutralizing its proteolytic activity could attenuate neointimal development and cell migration. In addition, the therapeutic potential of siRNA targeted to the uPA promoter represents a potentially powerful new approach not only to cancer therapy (Pulukuri and Rao 2007) but also to prevention of restenosis and unfavourable arterial remodeling. The inhibition of vessel wall thickening and cell proliferation may be achieved by influencing intracellular uPA targets, for example, by the local knockdown of NAD(P)H oxidases Nox1 and Nox4. At the same time, uPA provides enhanced blood flow and tissue protection in ischemic tissue because of its ability to induce both angiogenesis and arteriogenesis as effectively as VEGF, but without the side effect of local edema. uPA gene therapy or local enhancement of uPA production might be a promising tool for local stimulation of angiogenesis and arteriogenesis and the treatment of tissue ischemia.

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## Abbreviation list

| | |
|---|---|
| $\alpha_2$MR | $\alpha_2$-macroglobulin receptor |
| bFGF | Basic fibroblast growth factor |
| ECM | Extracellular matrix |
| GFD | Growth factor-like domain, N-terminal domain of urokinase similar to the epidermal growth factor |
| HGF | Hepatocyte growth factor |
| LDLR | Low-density lipoprotein receptor |
| LRP | LDLR-related protein |
| MAP | Mitogen-activated protein |
| MMPs | Matrix metalloproteinases |
| PAI | Plasminogen activator inhibitor |
| PDGF | Platelet-derived growth factor |
| PTCA | Percutaneous transluminal coronary angioplasty |
| ROS | Reactive oxygen species |
| SMC | Smooth muscle cell |
| TACE | TNF-α-converting enzyme |
| TGFβ | Transforming growth factor β |
| TNF-α | Tumor necrosis factor alpha |
| tPA | Tissue-type plasminogen activator |
| uPA | Urokinase-type plasminogen activator |
| uPAR | uPA high-affinity receptor (CD87) |
| VEGF | Vascular endothelial growth factor |
| VLDLR | Very low density lipoprotein receptor |