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Regulatory Effects of Urokinase on Mesenchymal Stromal Cell Migration, Proliferation, and Matrix Metalloproteinase Secretion

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We studied the effect of urokinase, its recombinant forms, and domain fragments on migration and proliferation of adipose tissue mesenchymal stromal cells (MSCs) and MMP secretion by these cells. Urokinase, but not its recombinant forms, slightly induced directed migration of MSCs. Spontaneous migration of MSCs increased under the action of urokinase or its isolated kringle domain. Migration induced by platelet-derived growth factor was inhibited by proteolytically inactive form of urokinase, the kringle domain, and blocking antibody to urokinase receptor. Urokinase, its proteolytically inactive form, and kringle domain produced no effect on MSC proliferation. In contrast to platelet-derived growth factor, all urokinase forms induced secretion of MMP-9 by MSCs.

Key Words: *urokinase; matrix metalloproteinases; cell migration; cell proliferation; adipose-derived mesenchymal stromal cells*

Multipotent mesenchymal stromal cells (MSCs) are present in the vascular compartment of virtually all tissues and play the key role in the regenerative processes via secretion of a broad spectrum of bioactive factors, differentiation into mesodermal cells, modulation of immune reactions, migration into the focus of injury, and remodel the extracellular matrix by secreting proteases [10]. These properties are extremely important for transplantation of MSCs in regenerative medicine.

In MSC physiology, the key role is played by growth factors, *e.g.* platelet-derived growth factor (PDGF) that stimulates their proliferative activity and chemotaxis mediated by interaction with integrins (in particular, with integrin β1) [11].

En essential role in providing directed cell migration is played by urokinase-type plasminogen activator (uPA or urokinase) and its receptor uPAR attached to the cell membrane with glycosylphosphatidylinositol anchor and thereby determines location of uPA on the leading edge of migrating cell where it disintegrates the extracellular matrix [9]. uPA is expressed and secreted as a single-chain molecule consisting of N-terminal domain structurally homologous to epidermal growth factor (EGF) responsible for interaction with uPAR, kringle domain (KD), and C-terminal proteolytic domain. The interaction between uPA and uPAR is needed for cell migration, extracellular matrix remodeling, and cell invasion [2]. An important condition for directed cell migration is uPAR interaction with integrins, which promotes cytoskeleton rearrangement [3].

In many respects, the cell invasion potential is determined by expression and secretion of MMPs that along with their basic function (remodeling of extracellular matrix) are implicated in proteolytic processing and activation of a number of bioactive molecules such as growth factors, chemokines, cytokines,

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membrane receptors, and apoptosis regulators [12]. In cooperation with plasmin, uPA can activate MMPs [7] and trigger expression of MMP-9 in monocytes [8], which can be an additional pathway employed by uPA to regulate the cell activity. It is noteworthy that uPA exerts the proliferative effect on vascular smooth muscle cells [4].

Despite the presence of uPARs on the surface of MSCs, the effect of uPA on functional activity of MSCs is little known, so our aim was to study the effects of uPA, its recombinant forms, domains, and fragments on migration and proliferation of MSCs and on secretion of MMPs, gelatinase-A (MMP-2) and gelatinase-B (MMP-9).

MATERIALS AND METHODS

Full-length wild-type uPA (uPAwt), proteolytically inactive uPA with His204Gln mutation (uPA-H/Q), uPA lacking the growth factor-like domain (ΔGFD), KD (ΔKD), and aminoterminal fragment (ATF) of uPA consisting of EFD and KD were expressed in *E. coli* and purified as described previously [1].

MSCs were isolated from the adipose tissue; migration assay was performed in a 24-well transwell system with porous membrane inserts (8 μ pore size) (BD) as described previously [14]. The seeding density was 3.5×10^4 cells per well. The following agents were added to the lower chambers as the chemoattractants: medium for migration (control), fetal calf serum (FCS; positive control), uPA or its forms (100 nM), or PDGF-BB (10 ng/ml). Migration was assayed for 20 h in a CO_2 incubator at 37°C.

The effects of uPA and its recombinant forms on directed migration of adipose tissue-derived MSCs towards PDGF or migration medium were assessed by adding them at concentration of 10 nM into the upper chambers of the 24-well system inserts (in this case migration assay was performed over 4 h). Then, the non-migrated cells were scraped off; the membranes were stained with a Diff-Quick Kit (Dade Behring) according to the product guide. The cells were photo-

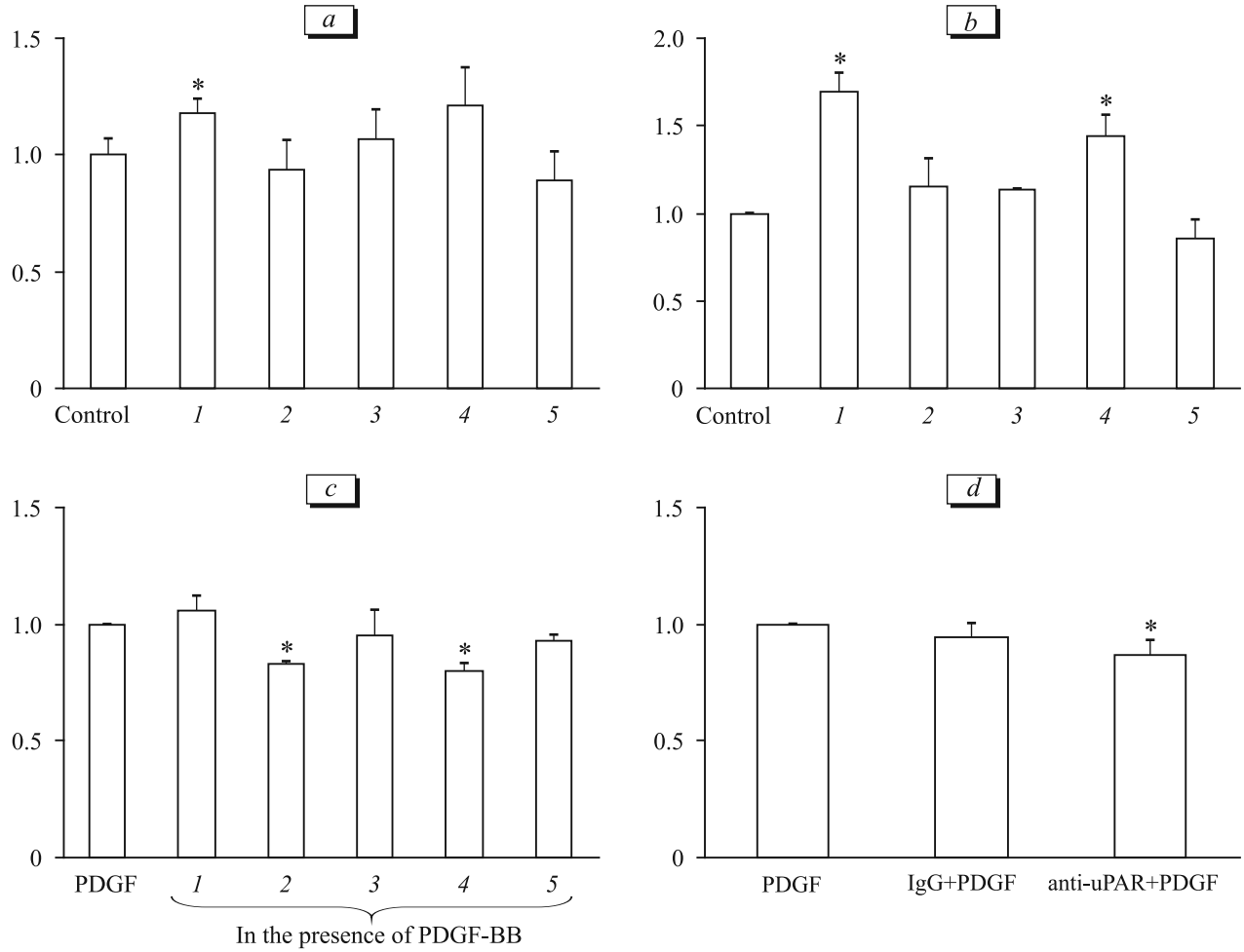

Fig. 1. Effect of uPA and its recombinant forms on MSC migration. *a*) induction of chemotaxis; *b*) spontaneous migration; *c*) PDGF-dependent chemotaxis; *d*) effects of IgG and anti-uPAR antibodies on PDGF-induced migration. *1*) uPAwt; *2*) uPA-H/Q; *3*) ΔKD; *4*) KD; *5*) ATF. Ordinate, migration index. **p*<0.05 in comparison with migration in control (*a*, *b*) or migration in the presence of PDGF-BB alone (*c*, *d*).

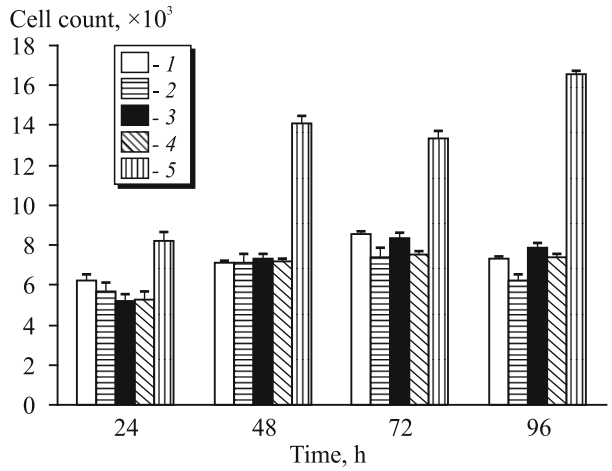

Fig. 2. Effect of uPA and its domains on MSC proliferation. *1*) 1% FCS; *2*) 1% FCS+uPA (100 nM); *3*) 1% FCS+ATF (100 nM); *4*) 1% FCS+KD (100 nM); *5*) 10% FCS.

graphed and counted in at least 6 randomly selected field of view. Migration index was determined as number of cells that had migrated to the chemoattractants normalized to the number of cells migrated toward the assay medium.

Proliferative activity of MSCs was evaluated by MTT assay. Secretion and activity of MMPs in MSCs were assessed with zymography [8] in the cell media collected after a 24-h incubation of MSCs in a CO_2 incubator in the presence of PGDF and the recombinant forms of uPA.

The results are summarized as $m \pm SEM$ (n=3-5 independent measurements). Significance was assessed with unpaired Student's t test at $p<0.05$.

RESULTS

Among all examined uPA recombinant forms, only uPAwt significantly (though slightly) enhanced migration of MSCs (Fig. 1, *a*). This fact showed that all three major constituents of uPA are needed to trigger cell migration.

Both uPAwt and isolated KD enhanced spontaneous migration of MSCs (Fig. 1, *b*). At the same time, assessment of the effects of uPA and its recombinant forms on PDGF-induced migration of MSCs revealed *1*) a slight and insignificant potentiating action of uPAwt and *2*) significant inhibition of migration in the presence of uPA-H/Q and KD (Fig. 1, *c*). uPA lacking KD (ΔKD) or ATF alone produced no effect on PDGF-induced migration of MSCs (Fig. 1, *c*). We also found a slight but significant inhibition of PDGF-induced migration of MSCs by anti-uPAR antibodies (Fig. 1, *d*).

The effect of PDGF on the cells is mediated via activation of its receptor, which exists in several molecular forms [11]. PDGF-dependent stimulation of the cells leads to redistribution of β1 and β3 integrins needed for triggering cell migration [5]. The available data showed that the effect of PDGF on cell migration is mediated via uPAR activation and its interaction with β1 integrin [6]. In its turn, uPA also stimulates the formation of uPAR complex with integrin α3β1 on fibronectin and collagen I [13].

Moderation of PDGF-dependent migration of MSCs under the effect of catalytically inactive uPA or its KD can be explained by the fact that these uPA-derived forms compete with endogenous uPA for binding to uPAR on the cell surface and thus prevent association with PDGF receptor and possibly with the integrins, which can interact with the receptors for growth factor and uPA.

In addition to positive regulation of migration activity of MSCs, the growth factors such as PDGF, EGF, bFGF, *etc*. stimulate their proliferation [10]. Actually, proliferation of MSCs was significantly elevated by the growth factors of FCS (Fig. 2). At the same time, uPA, ATF, and KD produced no significant effect on cell proliferation during 24-96 h cell culturing.

One of the features characterizing the cell motility is their ability to secrete MMPs. Actually, irrespective of the presence of PDGF or uPA, the adipose tissue-derive MSCs secreted MMP-2 (gelatinase-A, 72 kDa,

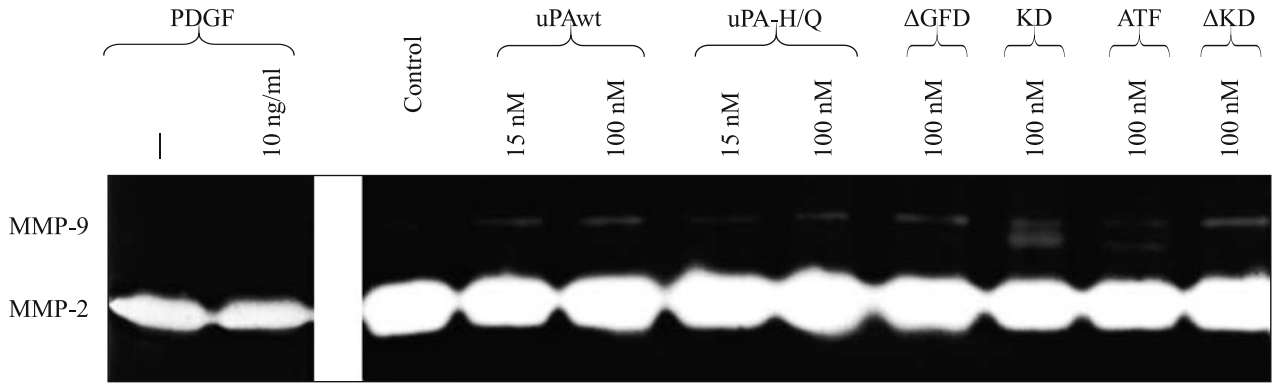

Fig. 3. Stimulatory effects of uPA and its recombinant forms on secretion of MMPs by adipose-derived MSCs.

Fig. 3). Moreover, uPA, its recombinant forms, ATF, and KD induced secretion of MMP-9 (gelatinase-B, 92 kDa).

In addition to up-regulation of MMP-9 secretion, uPA fragments containing KD induced proteolytic activation of MMP-9 manifested in the appearance of low-molecular-weight form of this enzyme with a molecular weight of 82 kDa. At concentration sufficient to stimulate cell migration, PDGF did not induce secretion of MMP-9 (Fig. 3). Neither uPA, nor PDGF affected MMP-2 secretion.

The present findings attest to implication of uPA system in the control of the regenerative processes with involvement of MSCs; they characterize it as a possible target in novel approaches to cell therapy.

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