BIOPHYSICS AND BIOCHEMISTRY

The Role of Urokinase in Cell Migration Induced by Growth Factors

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 3, pp. 285-287, March, 1999
Original article submitted April 27, 1998

The role of urokinase (serine protease converting plasminogen into plasmin) and its receptor in cell migration was studied on cultured smooth muscle cells from rat aorta. Platelet-derived growth factor, fibroblast growth factor-2, and urokinase activated migration of smooth muscle cells. Antibodies against urokinase inhibited cell migration induced by both urokinase and other growth factors. It is assumed that urokinase is involved in vascular remodeling induced by growth factors.

Key Words: *urokinase; growth factors; smooth muscle cells*

A number of growth factors secreted by blood cells and cells of the vascular wall induce migration of vascular smooth muscle cells (SMC) [4,7,14]. The chemoattractants for SMC are platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), fibroblast growth factor-2 (FGF-2), and fibronectin (protein component of intercellular matrix). Cell motility in tissues is controlled by not only growth factors and matrix proteins, but also local secretion of proteases such as plasmin produced under the effect of urokinase- and tissue-types plasminogen activators, and plasmin-activated metalloproteinases [2,13,14]. Synthesis of urokinase-type plasminogen activator (urokinase) and its receptor in SMC and endothelial cells is enhanced in the presence of PDGF and FGF-2 [6, 10,12]. These growth factors also induce secretion of plasminogen activator inhibitor by vascular cells thus participating in the regulation of urokinase activity [9].

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MATERIALS AND METHODS

Rat aorta SMC were cultured in Petri plastic dishes in an incubator at 37°C and 5% CO_2, in DMEM medium containing 4 mM glutamine, 20 mM HEPES (pH 7.3), 100 U/ml penicillin, 100 U/ml streptomycin, and 10% calf embryonic serum. The immunohistochemical characterization of isolated cells, which were positive to SMC-specific α-actin and negative to factor VIII, was performed in the primary culture. The cells were passivated by treating the monolayer with 0.25% trypsin and 0.02% EDTA. The cells of passages 18-21 were used in the experiments.

Migration of SMC was assessed using a Boyden microchamber as described elsewhere [1]. To evaluate the effect of anti-urokinase antibodies and urokinase receptor antagonist on cell migration induced by urokinase and growth factors, the cell suspension was preincubated with polyclonal antibodies against urokinase (120 μg/ml) or with modified recombinant urokinase (10 nM) for 1 h at 37°C in a CO_2-incubator. The migrated cells were stained by Diff Quick (Baxter) dye and the intensity of migration was estimated [1].

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Recombinant urokinase was iodated with Na-^{125}I and iodogen. Binding of ^{125}I-urokinase with SMC surface was performed as described elsewhere [1].

RESULTS

The PDGF-induced cell migration depends on FGF-2, because antibodies against FGF-2 block the effect of PDGF [3]. Migration of endothelial cells induced by FGF-2 is urokinase-dependent, because it is suppressed in the presence of urokinase antibodies [8]. The mechanism of urokinase participation in cell migration induced by growth factors is not clear. In our experiments SMC migration was increased 4-fold by PDGF (PDGF-BB isoform was used) and 2.5-fold by FGF-2 (Fig. 1). The maximum enhancement of SMC migration under the effect of urokinase was 3-fold (Fig. 1). The data on urokinase-induced cell migration closely agree with its binding characteristics at SMC surface. This binding was saturable and reversible, it was characterized by high affinity (K_d=7.2×10^{-9} mM) and a comparatively great number of binding sites (3×10^5).

Polyclonal antibodies against urokinase (120 μg/ml) completely inhibited SMC migration induced by FGF-2. In contrast, under these conditions cell migration induced by PDGF-BB was inhibited by only 50% (Fig. 2).

Previously we showed that recombinant urokinase with modified receptor-binding domain acted as a urokinase receptor antagonist [1], because it competed for the binding with native ligand and suppressed cell migration induced by native urokinase. In this work we show that this form of recombinant urokinase inhibits SMC migration induced by not only urokinase, but also PDGF-BB (Fig. 2).

Our findings indicate that SMC migration induced by FGF-2 and PDGF-BB depends on endogenous urokinase and its interaction with specific receptor. Growth factors are known to increase the number of urokinase receptors on SMC and endothelial cells [6,10] and stimulate the synthesis and secretion of urokinase by endothelial cells [5]. In addition, urokinase and its specific receptor are the key elements in the activation of vascular SMC migration induced by growth factors.

We are grateful to R. Sh. Bibilashvili and P. J. Little for discussion of these data. This work is a part of Russian-Australian Government Program on Medicine and Health Protection and is supported by the Russian Foundation for Basic Research (grant No. 96-04-50714).

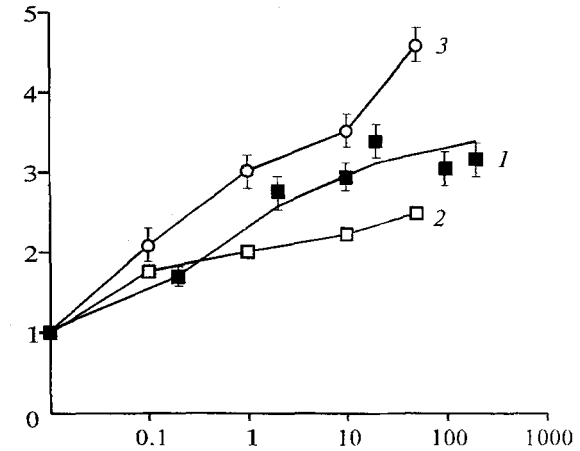

Fig. 1. Effect of urokinase (*1*), fibroblast growth factor-2 (*2*), and (*3*) platelet-derived growth factor (PDGF-BB) on smooth muscle cell migration. Stimulation intensity is calculated as the ratio of cell migration in the presence of stimulators to the control value obtained in the absence of the chemoattractant. Abscissa: concentrations of urokinase (nM) and growth factors (ng/ml); ordinate: activation of migration (rel. units).

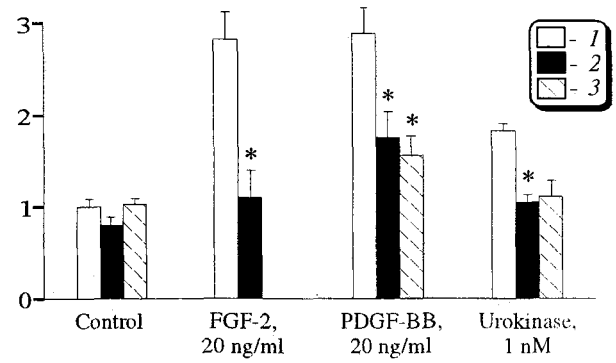

Fig. 2. Effect of antibodies against urokinase and its modified form on smooth muscle cell migration induced by fibroblast growth factor-2 (FGF-2), platelet-derived growth factor (PDGF-BB), and urokinase. Ordinate: activation of migration (rel. units); *1*) without antibodies; *2*) in the presence of antibodies against urokinase; *3)* in the presence of modified urokinase.

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