Epidermal growth factor-binding protein activates soluble and receptor-bound single chain urokinase-type plasminogen activator

Beni B. Wolf[a,*], Morry D. Brown[b]

[a]Department of Internal Medicine, University of California at San Diego, San Diego, CA 92103, USA
[b]Departments of Pathology and Biochemistry, University of Virginia Health Sciences Center, Charlottesville, VA 22908, USA

Received 17 September 1995

Abstract **Epidermal growth factor-binding protein (EGF-BP) is a serine proteinase that reversibly associates with epidermal growth factor (EGF). We analyzed the reaction of EGF-BP with urokinase type plasminogen activator (u-PA), a serine proteinase that promotes pericellular proteolysis and cellular migration. EGF-BP cleaved single chain u-PA (scu-PA) between Lys^{158} and Ile^{159}, converting the zymogen into enzymatically active two-chain u-PA (tcu-PA), as shown by SDS-PAGE, N-terminal sequence analysis, and enzymatic assay. The k_{cat} and K_m of the activation reaction were $(5.6 \pm 0.6) \times 10^{-2}$ s^{-1} and 2.0 ± 0.3 μM, yielding a catalytic efficiency of 2.8×10^4 $M^{-1} \cdot s^{-1}$. EGF-BP also activated scu-PA bound to receptors on U937 monocytes as demonstrated by the generation of amidase activity against a tcu-PA-specific fluorogenic substrate. By activating scu-PA, EGF-BP may initiate u-PA-dependent cell surface proteolysis and therefore enhance EGF activities that require cellular migration and/or tissue remodeling.**

Key words: Urokinase-type plasminogen activator; Urokinase-type plasminogen activator receptor; Epidermal growth factor binding protein; Epidermal growth factor

1. Introduction

Urokinase type plasminogen activator (u-PA) is the primary enzyme responsible for plasminogen activation at the cell surface (reviewed in [1]). u-PA, while bound to its cellular receptor, urokinase type plasminogen activator receptor (uPAR), activates cell-associated plasminogen, generating plasmin. Cell-associated plasmin directly degrades glycoproteins of the extracellular matrix and activates metalloproteinases that digest extracellular matrix collagen. This u-PA-dependent cell-surface proteinase cascade promotes cellular migration and extracellular matrix remodeling during several biological processes including chemotaxis, angiogenesis, neurite outgrowth, and wound repair [1].

*Corresponding author. University of California at San Diego, Medical Service 111, San Diego VAMC, 3350 La Jolla Village Dr., San Diego, CA 92161, USA. Fax: (1) (619) 552 7452.

Abbreviations: EGF, epidermal growth factor; EGF-BP, EGF-binding protein; NGF-γ, nerve growth factor-γ; scu-PA, single chain urokinase type plasminogen activator; tcu-PA, two chain urokinase type plasminogen activator; uPAR, urokinase type plasminogen activator receptor; PPACK, D-phenylalanyl-L-prolyl-arginine chloromethyl ketone; S-2444, L-pyroglutamyl-glycyl-arginine-*p*-nitroanilide hydrochloride; S-2251, *H*-D-valyl-L-leucyl-L-lysine dihydrochloride; PMA, phorbol 12-myristate 13-acetate; PNPGB, *p*-nitrophenyl *p'*-guanidinobenzoate hydrochloride; EGR-AMC, glutamyl-glycyl-arginine-7-amino-4-methyl coumarin; S.E.M., standard error of the mean.

Cells synthesize u-PA as an M_r 55,000 single chain zymogen (scu-PA) that demonstrates little or no activity against chromogenic substrates or plasminogen [2,3]. Plasmin is a potent scu-PA activator that converts the zymogen into a two-chain form (tcu-PA) that is highly active against chromogenic substrates and plasminogen [3]. Since plasmin is present in biological fluids as an inactive zymogen (plasminogen), a critical question remains regarding initiation of u-PA-dependent cell surface proteolysis. Several proteinases other than plasmin activate scu-PA, including plasma kallikrein, tumor-associated trypsin, cathepsin B, cathepsin L, nerve growth factor-γ (NGF-γ), mast cell tryptase, and human T cell-associated serine proteinase [4–10]. Although these proteinases activate scu-PA less efficiently than plasmin, they may initiate the u-PA dependent proteinase cascade which can then undergo self-amplification.

Cytokines and growth factors including epidermal growth factor (EGF), induce u-PA synthesis [11–14]. EGF is a small (M_r 6,000), widely distributed mitogen for cells of epithelial origin [15]. Beyond its mitogenic activity, EGF accelerates wound healing, enhances keratinocyte growth and migration, and functions as a chemoattractant for certain cell types [15–20]. In mice, EGF associates noncovalently with an Arg/Lys specific serine proteinase termed EGF-binding protein (EGF-BP) [21,22]. The multimeric complex, high molecular weight-EGF (HMW-EGF), has the stoichiometry $(EGF)_2(EGF\text{-}BP)_2$ [21,22]. The primary function of EGF-BP probably involves proteolytic processing of EGF precursors [23]; other functions of EGF-BP are less well characterized.

In the HMW-EGF complex, EGF-BP remains inactive due to association of the enzyme's active site with the C-terminal arg residue of EGF [21,22]. Dilution and changes in pH cause HMW-EGF dissociation, releasing active proteinase and growth factor in the same micro-environment [21,22]. However, it is not clear whether EGF-BP and EGF activity are coordinated in any manner. In this investigation, we show that EGF-BP activates scu-PA both in solution and at the cell surface. We hypothesize that by activating scu-PA, EGF-BP may initiate u-PA-dependent cell surface proteolysis and therefore enhance EGF-dependent activities that require cellular migration and/or tissue remodeling.

2. Materials and methods

2.1. Reagents

H-D-Valyl-L-leucyl-L-lysine-*p*-nitroanilide dihydrochloride (S-2251) and L-pyroglutamyl-glycyl-arginine-*p*-nitroanilide hydrochloride (S-2444) were from Kabi Vitrum (Stockholm, Sweden). D-Phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK) was from Chemica Alta (Edmonton, Alberta). Glutamyl-glycyl-arginine-7-amino-4-methyl coumarin (EGR-AMC) was purchased from Enzyme Systems (Livermore, CA). Phorbol 12-myristate 13-acetate (PMA) and *p*-nitrophenyl *p'*-guanidinobenzoate hydrochloride (PNPGB) were from

SSDI 0014-5793(95)01234-6

Sigma. $Na^{125}I$ was from Amersham International. Iodo-beads were from Pierce (Rockford, IL). Unless otherwise stated, reactions were conducted in buffer A (50 mM Tris-HCl, 100 mM NaCl, pH 7.4).

2.2. Proteins

EGF-BP was purified from male mouse submaxillary glands (Pel Freeze, Rogers, AZ) according to the method of Taylor et al. [21] and active site titrated with PNPGB by the method of Chase and Shaw [24]. In the presented experiments, the indicated EGF-BP concentrations are those obtained by active site titration. Dr. Jack Henkin of Abbott Laboratories provided the recombinant human scu-PA used in these studies. The scu-PA contained less than 0.5% active tcu-PA as determined by velocity of S-2444 hydrolysis. scu-PA was radiolabeled with $Na^{125}I$ and Enzymobeads according to the manufacturer's instructions. Desalting was performed on Sephadex G-25 (Pharmacia). Specific activities ranged from 1–3 μCi/μg.

2.3. Inhibition of EGF-BP by PPACK

EGF-BP (5.5–22 nM) was incubated with 1 mM S-2251 in the sample cuvette of a Hewlett Packard 8450A diode array spectrophotometer. Reactions were conducted at 37°C in buffer A. The absorbance at 406 nm was determined at 2 s intervals. After 200 s, PPACK (final concentration 60 μM) was added and absorbance monitoring continued. Absorbance measurements were transformed with the first derivative function (dA_{406}/dt) to yield substrate hydrolysis velocities that are directly proportional to the concentration of active enzyme at any given time.

2.4. Cleavage of scu-PA by EGF-BP

EGF-BP (22 nM-0.44 μM) was incubated with scu-PA (10.9 μM) in buffer A for 30 min at 37°C. Reactions were terminated with PPACK (0.4 mM final concentration) and the products subjected to SDS-PAGE (8% slabs) under reducing conditions as described previously [8].

2.5. Identification of the EGF-BP cleavage site in scu-PA

scu-PA (10.9 μM) was digested with EGF-BP (0.4 μM) in buffer A for 30 min at 37°C. The reaction was terminated with PPACK (0.4 mM final concentration) and the products analyzed by SDS-PAGE under reducing conditions [8]. Proteins were electrotransferred to Immobilon P membranes and stained with Coomassie blue (0.1% w/v). Staining revealed two new bands (33 kDa and 22 kDa). The 33 kDa band was excised and subjected to N-terminal sequence analysis (5 cycles) using an Applied Biosystems 470A gas-phase sequencer equipped with a model 120A on-line analyzer.

2.6. Amidase activities

Hydrolysis of S-2244 (0.125–3 mM) by EGF-BP (28 nM) was monitored at 406 nm (4 s intervals) for 500 s. Reactions were conducted at 37°C in buffer A. An extinction coefficient of 10^4 $M^{-1} \cdot cm^{-1}$ was used to determine the concentration of *p*-nitroaniline generated from S-2444 hydrolysis.

2.7. Activation of scu-PA in solution

scu-PA activation by EGF-BP was demonstrated with a coupled enzyme assay that detects the generation of tcu-PA amidase activity [8]. Briefly, scu-PA (0.3–3 μM) and S-2444 (1 mM, in buffer A) were equilibrated at 37°C in the spectrophotometer sample cuvette. Reactions were initiated by the addition of 28 nM EGF-BP. Absorbance readings at 406 nm were taken at 4 s intervals for 200–500 s. Absorbance measurements were transformed with the first derivative function (dA_{406}/dt) to yield S-2444 hydrolysis velocities that are directly proportional to the concentration of active tcu-PA at any given time. Rates of scu-PA activation were determined from the slopes in graphs of tcu-PA concentration against time [8].

Kinetic parameters (k_{cat}, K_m) for the activation reaction were determined from double-reciprocal plots of velocity and concentration. Since EGF-BP demonstrates a low level of amidase activity against S-2444 (see section 3), S-2444 acts as a weak competitive inhibitor of EGF-BP in the coupled enzyme assay described above. Therefore, we corrected the apparent K_m value for scu-PA activation by EGF-BP with the following expression:

$$K_M = \frac{K_{Mapp}}{\left(1 + \frac{[I]}{K_I}\right)}$$

[I] is the concentration of S-2444. $K_I = K_m$ for the hydrolysis of S-2444 by EGF-BP.

2.8. Cell culture

U937 monocyte-like cells [25] were obtained from the American Type Culture Collection and cultured as described previously [8]. Cells were treated with 150 nM PMA for 72 h before use. This treatment caused greater than 90% of the cells to become adherent. PMA also increases the number of u-PA receptors on the surfaces of U937 cells [26].

2.9. Cleavage and activation of cell-associated scu-PA by EGF-BP

Cleavage of cell associated scu-PA by EGF-BP was analyzed after binding [^{125}I]scu-PA to U937 monocytic cells [8]. Briefly, PMA-stimulated U937 cells were treated with binding buffer (Earles Balanced Salt Solution containing 2 mg/ml BSA and 20 mM HEPES pH 7.4) containing 10 mM ε-aminocaproic acid and 1000 units/ml aprotinin to dissociate any endogenously bound plasmin(ogen). The cells were subsequently treated with 50 mM glycine, 100 mM NaCl, pH 3.0 for 15 min at 22°C to remove endogenously bound scu-PA. Cells were washed three times with binding buffer and then equilibrated to 37°C. [^{125}I]scu-PA (5 nM) was incubated with the cells for 20 min at 37°C. After washing, cells were treated with EGF-BP (2–200 nM) for 15 min at 37°C. Reactions were terminated with PPACK. Cells were then washed twice with binding buffer, lysed, and subjected to SDS-PAGE (reducing conditions) and autoradiography. Cleavage of scu-PA by EGF-BP was quantitated by slicing the gels into 3 mm sections and counting the slices in a gamma counter [8].

To detect activation of cell-associated scu-PA by EGF-BP, we used the fluorogenic assay described by Wolf et al. [8]. PMA-stimulated U937 cells were treated to remove endogenously bound plasmin(ogen) and u-PA. scu-PA (5 nM) was then incubated with the cells for 20 min at 37°C. After washing to remove unbound scu-PA, 25 nM EGF-BP was added for 15 min at 37°C. The cells were washed again and cell-associated active tcu-PA was detected by the rate of hydrolysis of EGR-AMC (0.5 mM in buffer A) using a CytoFluor Fluorescence Measurement System. Excitation was at 380 nm and emission was at

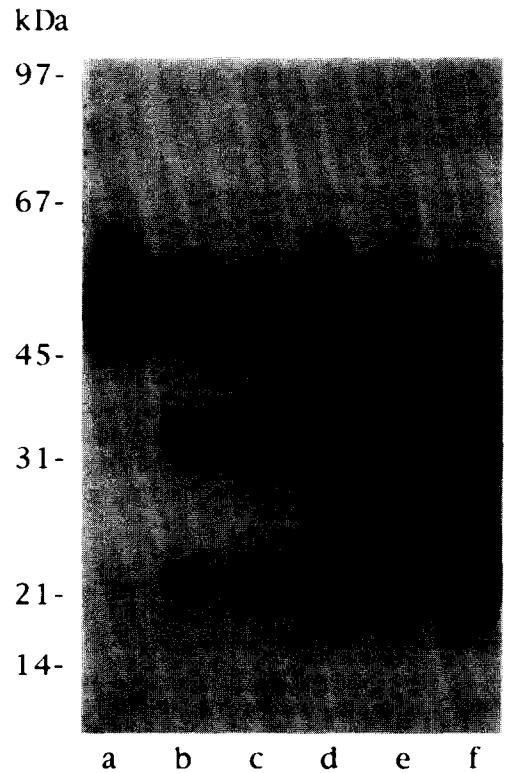

Fig. 1. Cleavage of scu-PA by EGF-BP in solution. scu-PA (10.9 μM) was reacted with EGF-BP for 0.5 h at 37°C. The products were analyzed by SDS-PAGE on 8% slabs under reducing conditions. The concentrations of EGF-BP were 0 nM (lane a), 22 nM (lane b), 44 nM (lane c), 0.11 μM (lane d), 0.22 μM (lane e), and 0.44 μM (lane f).

480 nm (slit widths of 5 nm). Fluorescence was monitored at 1 min intervals for 12 min at 22°C. Background fluorescence (determined in the absence of scu-PA) was subtracted.

3. Results and discussion

3.1. Inhibition of EGF-BP by PPACK

To determine if PPACK inhibited EGF-BP, we monitored S-2251 hydrolysis by EGF-BP in the absence and presence of the inhibitor. EGF-BP (5.5–22 nM) cleaved S-2251 at 37°C with substrate hydrolysis rates of 166 to 467 $nM \cdot s^{-1}$. Addition of 60 μM PPACK completely inhibited EGF-BP amidase activity within the time required for mixing (2–3 s) in 3 separate experiments. We subsequently employed PPACK to rapidly inhibit EGF-BP in the experiments that follow.

3.2. Cleavage of scu-PA by EGF-BP

EGF-BP cleaved scu-PA into a two-chain form as demonstrated by SDS-PAGE under reducing conditions (Fig. 1). The molecular masses of the two new u-PA chains were 33 kDa and 22 kDa. 22 nM EGF-BP cleaved significant amounts of scu-PA within 30 minutes. Almost complete scu-PA digestion was achieved with 0.44 μM EGF-BP. There was no evidence for secondary EGF-BP cleavage sites or further digestion of the 33-and 22-kDa products, even at the higher EGF-BP concentrations. Prior incubation of EGF-BP with PPACK completely prevented scu-PA degradation (not shown).

3.3. Cleavage of scu-PA by EGF-BP occurs between Lys^{158} and Ile^{159}

To identify the EGF-BP cleavage site(s), we subjected the 33 kDa digestion product to N-terminal sequence analysis. After five cycles, the only N-terminal sequence obtained was Ile-Ile-Gly-Gly-Glu. The initial yield was 21 pmol and the repetitive yield was 90%. No secondary cleavage sites were detected. By comparison with the known sequence of scu-PA [27,28], this new N-terminal sequence resulted from cleavage of Lys^{158}–Ile^{159}, which is the same peptide bond cleaved when plasmin activates scu-PA [2].

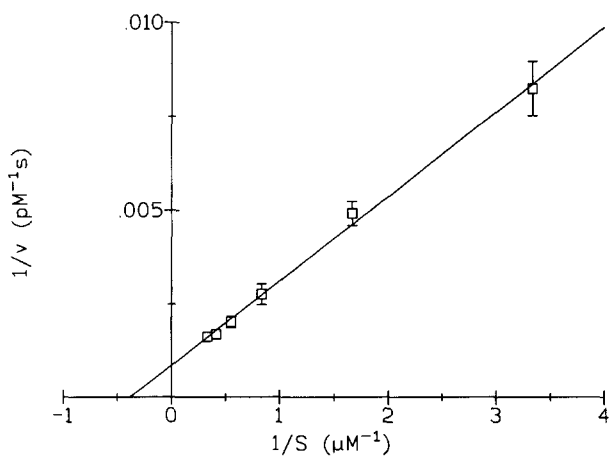

Fig. 2. Activation of scu-PA by EGF-BP. scu-PA was activated at 37°C in 50 mM Tris-HCl, 100 mM NaCl, pH 7.4 containing 1 mM S-2444. The concentration of active EGF-BP was 28 nM. Each value represents the avg ± S.E.M. (n = 6).

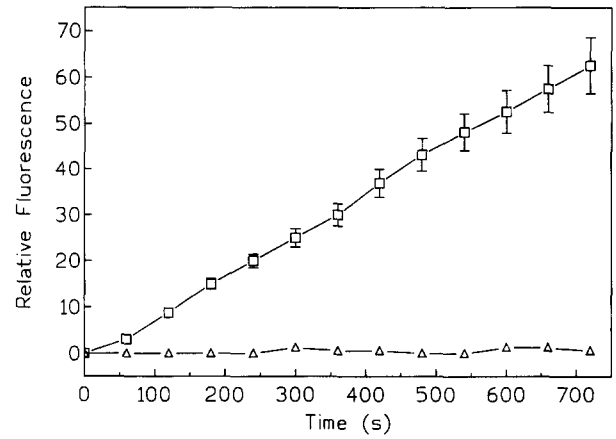

Fig. 3. Activation of receptor-bound scu-PA by EGF-BP. scu-PA was bound to PMA-stimulated U937 cells as described in section 2. Cells that were treated with 20 nM EGF-BP for 15 min at 37°C (□) or untreated cells (△) were washed two times with binding buffer. Subsequently, 0.5 mM EGR-AMC was added to each well. Fluorescence was monitored at 480 nm at 1 min intervals for 12 min at 22°C. Each value represents the avg ± S.E.M. (n = 3).

3.4. S-2444 Hydrolysis by EGF-BP

EGF-BP demonstrated a low level of amidase activity against S-2444 in control experiments. Double reciprocal plots of S-2444 hydrolysis velocities versus S-2444 concentration were linear, indicating that EGF-BP cleaved S-2444 according to simple Michaelis–Menten kinetics (not shown). The k_{cat} and K_m for this reaction were 5.0 ± 0.1 s^{-1} and 0.84 ± 0.08 mM, respectively, yielding a catalytic efficiency (k_{cat}/K_m) of 5.8×10^3 $M^{-1} \cdot s^{-1}$. tcu-PA hydrolyzes S-2444 with a catalytic efficiency over 150-fold greater than with EGF-BP under identical conditions [8]. We accounted for the low level of EGF-BP activity against S-2444 in the activation studies discussed below.

3.5. Activation of scu-PA by EGF-BP

Cleavage of scu-PA by EGF-BP resulted in u-PA activation as demonstrated by the generation of amidase activity. The linear Lineweaver-Burke plot shown in Fig. 2 indicates that EGF-BP activates scu-PA according to simple Michaelis–Menten kinetics in the substrate concentration range studied. The k_{cat} and K_m for the activation reaction were $(5.6 \pm 0.6) \times 10^{-2}$ s^{-1} and 2.0 ± 0.3 μM, yielding a catalytic efficiency (k_{cat}/K_m) of 2.8×10^4 $M^{-1} \cdot s^{-1}$ (Table 1). Table 1 also shows the kinetic parameters for the activation of scu-PA by NGF-γ and plasmin under identical conditions [8]. Plasmin activated scu-PA with a catalytic efficiency 20-fold greater than that demonstrated

Table 1
Kinetic parameters (avg ± S.E.M., n = 6) for the activation of scu-PA by EGF-BP, NGF-γ, and plasmin[a]

Proteinase	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1} \cdot s^{-1}$)
EGF-BP	2.0 ± 0.3	$(5.6 \pm 0.6) \times 10^{-2}$	2.8×10^4
NGF-γ[b]	2.3 ± 0.4	$(4.1 \pm 0.6) \times 10^{-2}$	1.3×10^4
Plasmin[b]	4.6 ± 1.4	2.6 ± 0.8	6.2×10^5

[a] Substrate cleavage was at 37°C in 50 mM Tris-HCl, 100 mM NaCl, pH 7.4 in the presence of 1 mM S-2444.
[b] These values are taken from [8].

Table 2
Cleavage of cell-associated scu-PA by EGF-BP (avg ± S.E.M., $n = 3$)[a]

	EGF-BP		
	2.0 nM	20 nM	200 nM
% scu-PA cleaved	2.6 ± 0.1	6.5 ± 0.9	16.7 ± 0.5

[a] [^{125}I]scu-PA was bound to PMA-stimulated U937 cells. The cells were then washed and treated with the indicated concentrations of EGF-BP for 15 min at 37°C. Reactions were terminated with PPACK. Cells were washed again and then lysed with sample buffer. Cleavage of scu-PA was assessed by SDS-PAGE and autoradiography. The % scu-PA was determined by slicing the gels and counting the sections in a gamma counter.

with EGF-BP. Therefore, EGF-BP is probably not a significant scu-PA activator once substantial levels of plasmin are generated. By contrast, EGF-BP demonstrated a 2-fold greater catalytic efficiency than the related proteinase NGF-γ (Table 1) and over a 10-fold greater catalytic efficiency than mast cell tryptase (2.4×10^3 $M^{-1} \cdot s^{-1}$, [9]). Comparison of EGF-BP with other scu-PA activating enzymes such as plasma kallikrein, tumor-associated trypsin, and cathepsins B and L, is difficult since formal kinetic parameters (k_{cat}, K_m) have not been determined for these enzymes [4–7]. Since all these proteinases activate scu-PA less efficiently than plasmin, they most likely function in the initiation of the u-PA-dependent cascade and not in the amplification phase.

3.6. Cleavage and activation of cell-associated scu-PA by EGF-BP

Since activation of receptor-bound scu-PA is essential for pericellular proteolysis, we examined the possibility that EGF-BP might also activate cell-associated scu-PA. EGF-BP cleaved cell-associated scu-PA, generating products that were identical to those formed when the scu-PA was free in solution. The extent of scu-PA cleavage was EGF-BP concentration-dependent (Table 2). We obtained similar results with undifferentiated U937 cells in suspension (not shown).

EGF-BP activated cell-associated scu-PA as shown by the generation of amidase activity against the tcu-PA specific fluorogenic substrate EGR-AMC (Fig. 3). After incubation with EGF-BP, significant substrate hydrolysis was observed within 5 min at 22°C. In the absence of EGF-BP, u-PA activity was equal to background levels. Treatment of EGF-BP with PPACK prevented scu-PA activation at the cell surface (not shown). These data show that EGF-BP activates scu-PA both in solution and at the cell surface. Besides EGF-BP, only plasmin, cathepsin B, and NGF-γ are known to activate cell-associated scu-PA [2,6,8].

Growth factors, proteinases, and proteinase inhibitors act in a concerted fashion to mediate the extensive cellular proliferation, cellular migration, and tissue remodeling necessary for wound healing. EGF plays a pivotal role in this process by promoting keratinocyte proliferation, migration, and u-PA synthesis [14,18,19]. Keratinocyte uPAR selectively localizes the increased levels of u-PA secreted during wound healing to the leading edge of migrating keratinocytes [29–33]. We hypothesize that by activating keratinocyte scu-PA, EGF-BP may initiate u-PA-dependent cell surface proteolysis and thus potentiate EGF activity during wound healing by promoting cellular migration and tissue remodeling.

Acknowledgements: This work was supported by Grant HL-45786 from the National Institutes of Health. M.D.B. was supported in part by the Medical Scientist Training Program (GM 07267). We thank Dr. Jack Henkin of Abbott Laboratories for providing the recombinant scu-PA used in these studies and Dr. Steven L. Gonias for helpful comments concerning this work.

References

[1] Vassalli, J.D., Sappino, A.P. and Belin, D. (1992) J. Clin. Invest. 88, 1067–1072.
[2] de Munk, GAW. and Rijken, D.C. (1990) Fibrinolysis 4, 1–9.
[3] Gurewich, V. (1988) Semin. Throm. Hemostas. 14, 110–115.
[4] Ichinose, A., Fujikawa, K. and Suyama, T. (1986) J. Biol. Chem. 261, 3486–3489.
[5] Koivunen, E., Huhtala, L. and Stenman, U.-H. (1989) J. Biol. Chem. 264, 14095–14099.
[6] Kobayashi, H., Schmitt, M., Goretzki, L., Chucholowski, N., Calvete, J., Kramer, M., Gunzler, W.A., Janicke, F. and Graeff, H. (1991) J. Biol. Chem. 266, 5147–5152.
[7] Goretzki, L., Schmitt, M., Mann, K., Calvete, J., Chucholowski, N., Kramer, M., Gunzler, W.A., Janicke, F. and Graeff, H. (1992) FEBS Lett. 297, 112–118.
[8] Wolf, B.B., Vasudevan, J., Henkin, J. and Gonias, S.L. (1993) J. Biol. Chem. 268, 16327–16331.
[9] Stack, M.S. and Johnson, D. (1994) J. Biol. Chem. 269, 9416–9419.
[10] Brunner, G., Simon, M.M. and Kramer, M.D. (1990) FEBS Lett. 260, 141–144.
[11] Boyd, D. and Brattain, M. (1989) Cancer Res. 49, 1948–1953.
[12] Niedbala, M.J. and Sartorelli, A.C. (1989) Cancer Res. 49, 3302–3309.
[13] Kessler, T.L. and Markus, G. (1991) Semin. Throm. Hemostas. 17, 217–224.
[14] Jensen, P.J. and Rodeck, U. (1993) J. Cell. Physiol. 155, 333–339.
[15] Carpenter, G. and Cohen, S. (1990) J. Biol. Chem. 265, 7709–7712.
[16] Buckley, A., Davidson, J.M., Kamerath, C.D., Wolt, T.B. and Woodward, S.C. (1985) Proc. Natl. Acad. Sci. USA 82, 7340–7344.
[17] Brown, G.L., Curtsinger, L., Brightwell, J.R., Ackerman, D.M., George-Nascimento, C., Valenzuela, P. and Schultz, G.S. (1986) J. Exp. Med. 163, 1319–1324.
[18] Barrandon, Y. and Green, H. (1987) Cell 50, 1131–1137.
[19] Ando, Y. and Jensen, P.J. (1993) J. Invest. Dermatol. 100, 633–639.
[20] Blay, J. and Brown, K.D. (1985) J. Cell. Physiol. 124, 107–112.
[21] Taylor, J.M., Cohen, S. and Mitchell, W.M. (1970) Proc. Natl. Acad. Sci. USA 67, 164–171.
[22] Server, A.C., Sutter, A. and Shooter, E.M. (1976) J. Biol. Chem. 251, 1188–1196.
[23] Frey, P., Forand, R., Maciag, T. and Shooter, E.M. (1979) Proc. Natl. Acad. Sci. USA 76, 6294–6298.
[24] Chase, T. and Shaw, E. (1967) Biochem. Biophys. Res. Commun. 29, 508–514.
[25] Sundstrom, C. and Nilsson, K. (1976) Int. J. Cancer 17, 565–577.
[26] Picone, R., Kajtaniak, E.L., Nielsen, L.S., Behrendt, N., Mastronicola, M.R., Cubellis, M.V., Stoppelli, M.P., Pedersen, S., Dano, K. and Blasi, F. (1989) J. Cell. Biol. 108, 693–702.
[27] Gunzler, W.A., Steffens, G.J., Otting, F., Kim, S.M.A., Frankus, E. and Flohe, L. (1982) Hoppe-Seyler's Z. Physiol. Chem. 363, 1155–1181.
[28] Steffens, G.J., Gunzler, W.A., Otting, F., Frankus, E., and Flohe, L. (1982) Hoppe-Seyler's Z. Physiol. Chem. 363, 1043–1058.
[29] Grondahl-Hansen, J., Lund, L.R., Ralfkiaer, E., Ottevanger, V. and Dano, K. (1988) J. Invest. Dermatol. 90, 790–795.
[30] Jensen, P.J., John, M. and Baird, J. (1990) Exp. Cell Res. 187, 162–169.
[31] Romer, J., Lund, L.R., Eriksen, J., Ralfkiaer, E., Zehe, R., Gelehrter, T.D., Dano, K. and Kristensen, P. (1991) J. Invest. Dermatol. 97, 803–811.
[32] Morioka, S., Lazarus, G.S., Baird, J.L. and Jensen, P.J. (1987) J. Invest. Dermatol. 88, 418–423.
[33] McNeil, H. and Jensen, P.J. (1990) Cell Reg. 1, 843–852.