Factor Xa has been reported to elicit smooth muscle cell proliferation via autocrine release of platelet-derived growth factor. However, this study has shown that factor Xa-induced mitogenesis of rat aortic smooth muscle cell is independent of platelet-derived growth factor. We also could not observe any platelet-derived growth factor isoforms in the cultured medium of factor Xa-stimulated cells. Our finding that the cultured medium of factor Xa-stimulated cells strongly induces rat aortic smooth muscle cell mitogenesis in the absence of factor Xa activity led us to explore the existence of a novel autocrine pathway. The autocrine growth factor was purified from the cultured medium and was identified to be epiregulin. Recombinant epiregulin was also able to induce the mitogenesis. The secretion of epiregulin from factor Xa-stimulated rat aortic smooth muscle cell required mRNA expression and protein synthesis of the growth factor. The mitogenic effect of factor Xa on rat aortic smooth muscle cell was significantly reduced by anti-epiregulin antibody or by antisense oligodeoxynucleotide to epiregulin. Several lines of experimental evidence clearly indicate that the autocrine production of epiregulin, an epidermal growth factor-related ligand, is induced in the factor Xa-stimulated mitogenic process of rat aortic smooth muscle cell.

Vascular smooth muscle cell (SMC) migration and proliferation are necessary events that contribute to the formation of neointimal hyperplasia in atherosclerosis, restenosis, and venous bypass graft disease (1, 2). SMC mitogenesis is triggered by a large number of growth factors, cytokines, and extracellular matrix proteins that participate in the pathogenic process (3). The induction of various mitogenic factor expressions in mechanically injured arteries (4–7) suggests that hemostatic and thrombotic events occurring in the early phase of vascular response to injury might be involved. After arterial injury, tissue factor is expressed in SMCs (8) and forms a high affinity complex with factor VII/VIIa, thereby initiating an extrinsic blood coagulation pathway leading to the formation of factor Xa and thrombin (9). In addition to its procoagulant effects, thrombin itself can act as a mitogen for fibroblasts (10), lymphocytes (11), mesenchymal cells (12) and SMCs (13–15). Thrombin also regulates cellular responses during inflammation (16) and inhibits the migration and invasion of breast cancer cells (17). After stimulation by thrombin, SMCs produce and secrete autocrine growth factors, including basic fibroblast growth factor (18), PDGF (19), heparin-binding epidermal growth factor (EGRF) (20), and transforming growth factor-β (21). Factor Xa acts as the major mediator of thrombus formation at the sites of vascular injury by activating prothrombin to thrombin and as a potent mitogen for endothelial cells (22), fibroblasts (23), and aortic SMCs (24–26). Factor Xa also induces the production of cytokines and adhesion molecules such as interleukin-6, interleukin-8, monocyte chemotactic protein-1, E-selectin, intercellular adhesion molecule 1, and vascular cell adhesion molecule-1 in human umbilical vein endothelial cells, leading to pro-inflammatory responses (27, 28). Although some aspects of the effect of factor Xa on aortic SMCs through PDGF have been investigated (25, 26), the PDGF isomorph that mediates factor Xa-induced SMC mitogenesis has not been identified yet. Moreover, no information is available about any particular autocrine production that is associated with factor Xa-induced SMC mitogenesis. In this work we have demonstrated that factor Xa-induced mitogenesis of rat aortic smooth muscle cells (RASMCs) is independent of PDGF. More importantly, we have identified epiregulin as the major element responsible for factor Xa-induced RASMC mitogenesis. It was also revealed that autocrine production of epiregulin is essential in the mitogenesis. Epiregulin is the newest member of the family of EGF-like ligands, and it was first identified from the cultured medium of mouse fibroblast-derived tumor cell line NIH3T3/clone T7 (29). It was subsequently shown that epiregulin acts as a mitogen for various cell types, including fibroblasts, aortic SMCs, hepatocytes (29), keratinocytes (30), and pancreatic cancer cells (31). Epiregulin was also purified from the cultured medium of angiotensin II-stimulated RASMCs and was identified to be a major mitogenic constituent (32).

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Invitrogen, and [3H]thymidine was from PerkinElmer Life Sciences. Bovine factor Xa, bovine thrombin, and pGEX-2T vector were purchased from Novagen, Inc., and Pefabloc® TH (Pefa-3204) was from Pentapharm Ltd. (Switzerland). Recombinant tick anticoagulant protein (rTap) was kindly provided by Dr. Y. Jang (Cardiovascular Research Institute, Yonsei University College of Medicine, Seoul, Korea). NuPAGE™ 4–12% Bis-Tris gel was purchased from Invitrogen, and DC protein assay reagent from Bio-Rad. An antibody to phospho-p44/42 mitogen-activated protein kinase (ERK-1/2) and an antibody that recognizes both phosphorylated and nonphosphorylated forms of p44/42 mitogen-activated protein kinase were obtained from Cell Signaling Technology. ECL reagent, benzamidine-Sepharose, and heparin-Sepharose were purchased from Amersham Biosciences. Anti-PDGF-A (E-10) and -B (H-55) antibodies came from Santa Cruz Biotechnology Inc.
Tyrophostin AG 1296 was purchased from Sigma-Aldrich, whereas neutralizing anti-PDGFB-AB antibodies PDGF-AB and PDGFBB-antibody were purchased from Upstate Biotechnology. Quantikine human PDGF-AB and human PDGF-BB immunoassay kits and anti-mouse epiregulin antibody came from R&D Systems. Ultraspec-II RNA system was purchased from Biotex Laboratories, Inc., whereas avian myeloblastosis virus reverse transcription and pGEM-T Easy vector were obtained from Promega. PCR primers and phosphorothioate oligonucleotides (ODNs) were synthesized from Genotec, Inc. (Korea). All other reagents were of the highest commercial purity.

Cell Culture—RASMCs were isolated by the method of the method of Chamley-Campbell et al. (33). The thoracic aortas from six- to eight-week-old Sprague-Dawley rats were removed and transferred onto ice in serum-free Dulbecco’s modified Eagle’s medium (DMEM) containing 1% penicillin/streptomycin. The aorta was minced and then suspended in DMEM containing 0.2% FBS for 48 h. After stimulation with agonists or antibodies before stimulation. After an additional 24-h incubation, the cells were then pulsed with 2 Ci of [3H]thymidine for 16 h at 37 °C

Reverse Transcription-PCR Analysis—The expression level of epiregulin mRNA was analyzed using the reverse transcription-PCR method. For the RNA preparation, RASMCs were incubated for 48 h in DMEM containing 0.2% FBS and then treated with factor Xa for the times indicated. Total RNA was prepared by Ultraspec-II RNA system, and single-stranded cDNA was then synthesized from the isolated total RNA using avian myeloblastosis virus reverse transcriptase. A 20-μl reverse transcription reaction mixture containing 1 μg of total RNA, 1× reverse transcription buffer (10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 0.1% benzamidine-Sepharose column (1.5 × 4 cm) equilibrated with buffer A containing 0.15 M NaCl. The adsorbed proteins were eluted with a linear gradient of 0.15 to 2 M NaCl in buffer A. Active fractions were pooled, concentrated, and separated in a Superose 12 HR 10/30 gel filtration column (Amersham Biosciences) equilibrated with buffer A. Protein concentrations were determined using Quantikine™ 4–12% Bis-Tris gel for each lane. The gel was stained with Coomassie blue. The highly purified protein was used for N-terminal amino acid sequencing after alkylation with iodoacetamide.

Expression Construct for Rat Epiregulin—The rat epiregulin cDNA was modified by PCR for GEX-2T vector. The N-terminal primer (5'-GGATCCGTGTTGATTACAAAGTGTAGCTCTG-3') and a BamHI site. The C-terminal primer (5'-GAATTCTCCAGAAGAAGGTGTTCACTCGAGACGAC-3') contains a stop codon and an EcoRI restriction site. The thermal cycling reaction was performed with 30 cycles of steps (denaturation at 94 °C for 30 s, annealing at 61 °C for 30 s, and extension at 72 °C for 1 min). The amplified PCR product was subcloned into pGEM-T Easy Vector, and its nucleotide sequence was analyzed using the ALF automatic sequencing system (Amersham Biosciences).

Expression and Purification of Recombinant Rat Epiregulin—The expression vector was transformed into Escherichia coli strain BL21. The transformants were grown in LB medium containing ampicillin to an absorbance of 0.5 at A600um, induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside, and incubated for an additional 4 h at 37 °C. The expressed inclusion bodies of glutathione S-transferase to the mature form of rat epiregulin.

Antisense Oligodeoxynucleotide to Epiregulin—Sense (S)- 5' to 3') and antisense (AS)- (3' to 5') ODNs with phosphorothioate linkages were designed to be complementary to the translation initiation sites of rat epiregulin. For rat epiregulin, the AS-ODN sequence was 5'-AAGCTTCATCCTTCCTC-3', and S-ODN was 5'-GAGAAGGATGCTCTTC-3'. The specificity of the AS-ODN for rat epiregulin was tested by Western blot analysis.

Statistical Analysis—Data represented the mean ± S.E. of n experiments. Statistical analysis was performed using an unpaired t test. A p value less than 0.05 was considered to be statistically significant.
RESULTS

Mitogenesis of Factor Xa-stimulated RASMCs—Factor Xa-induced mitogenesis of RASMCs was determined by measuring $[^{3}H]$thymidine incorporation and the cell number in RASMCs stimulated by the protease. As expected, factor Xa markedly increased $[^{3}H]$thymidine incorporation into the cell and increased the cell number compared with unstimulated RASMCs. A similar mitogenic effect was also observed with thrombin-stimulated RASMCs (Fig. 1). To determine whether the mitogenic effect of factor Xa on RASMCs is dependent on its proteolytic activity, a competitive factor Xa inhibitor, rTAP, was employed. As shown in Fig. 1, rTAP significantly reduced the mitogenesis of RASMCs that was induced by factor Xa, indicating that the induction of RASMC mitogenesis is closely associated with the proteolytic enzyme activity of factor Xa. rTAP on its own did not influence the $[^{3}H]$thymidine incorporation and proliferation of unstimulated cells (data not shown). To rule out the possibility that the mitogenic function of factor Xa may be caused by the local production of thrombin, the factor Xa-induced mitogenesis was examined in the presence of Pefabloc®TH, a specific thrombin inhibitor. Experimental results clearly indicate that Pefabloc®TH did not affect factor Xa-induced mitogenesis, whereas the thrombin-stimulated mitogenic effect was markedly suppressed by the inhibitor (Fig. 1). Pefabloc®TH did not influence the $[^{3}H]$thymidine incorporation and proliferation of unstimulated RASMCs (data not shown).

PDGF Independence of Factor Xa-induced $[^{3}H]$Thymidine Incorporation and ERK-1/2 Activation—To determine whether the factor Xa-induced mitogenesis is associated with the secretion of PDGF, RASMCs were stimulated with factor Xa in the presence of anti-PDGF-AB polyclonal antibody, which neutralizes all three isoforms of PDGF, i.e. PDGF-AA, -AB, and -BB. As demonstrated in Fig. 2, the antibody failed to affect the increase in $[^{3}H]$thymidine incorporation and ERK-1/2 phosphorylation in factor Xa-stimulated cells. To further investigate the requirement of autocrine PDGF for this particular mitogenic pathway, RASMCs were stimulated by factor Xa in the presence of Tyrophostin AG 1296, a selective tyrosine kinase inhibitor of PDGF receptors (34). Experimental results indicate that neither the factor Xa-induced $[^{3}H]$thymidine incorporation nor ERK-1/2 activation was influenced by the reagent (Fig. 2). In control experiments, it was demonstrated that the neutralizing antibody or tyrophostin AG 1296 completely suppressed the mitogenic effects induced by PDGF-BB (Fig. 2). The influence of the antibody or the inhibitor itself in the unstimulated cells was negligible on such mitogenic effects (data not shown).

Analysis of Autocrine PDGF in the Cultured Medium—To assess whether factor Xa stimulates the secretion of PDGF from RASMCs, Western blot analysis was carried out. For this investigation, the cultured supernatant from untreated or factor Xa-treated RASMCs was concentrated and analyzed by Western blot using anti-PDGF-A or -B antibody. The obtained results indicate that the immunoreactive band recognized by the PDGF-A or -B antibody was not detected in the supernatants (data not shown). To further confirm the presence of PDGF-AB and -BB in the cultured medium, the concentrations of these growth factors were measured using Quantikine® immunoassay kits. The stimulation of RASMCs with factor Xa for up to 48 h did not result in any detectable secretion of PDGF-AB and -BB in the cultured medium (data not shown). The detection limits of all enzyme-linked immunosorbent assay systems used in the experiments were below 15 pg/ml.

Autocrine-dependent DNA Synthesis and Cell Proliferation—To determine whether the factor Xa-induced mitogenesis is autocrine-dependent, the cultured supernatant was harvested from the factor Xa-stimulated RASMCs at various time intervals up to 6 h and then added to the serum-starved RASMCs after rTAP treatment to prevent the direct effect of factor Xa. The cells were then assayed for $[^{3}H]$thymidine incorporation and cell proliferation. Experimental results demonstrate that the factor Xa-treated conditioned medium was successful in significantly increasing DNA synthesis and cell number in RASMCs (Fig. 3), whereas the medium of unstimulated cells failed to exert such mitogenic effects (data not shown). Anti-PDGF-AB antibody or tyrophostin AG 1296 did not affect the $[^{3}H]$thymidine incorporation induced by the cultured medium containing rTAP, indicating that there is no requirement for autocrine PDGF in the particular mitogenesis induced by factor Xa (Fig. 3A). The mitogenic activity appeared 1 h after factor Xa stimulation and reached its plateau in 4 h (Fig. 3).

Purification and Identification of the Autocrine Factor—Autocrine factor, which is responsible for the factor Xa-stimulated mitogenesis of RASMCs, was purified to homogeneity from two liters of the conditioned medium in four steps. After dialfiltration against buffer A, the cultured medium was fractionated by affinity chromatography on a column of benzamidine-Sepha-
Mitogenic activity was recovered in the washing step of the column with buffer A, whereas factor Xa was retained in the column. When the pooled active fractions were applied to a heparin-Sepharose column, the mitogenic activity was recovered by washing the column with buffer A containing 0.15 M NaCl. The active fractions were then concentrated and fractionated in a gel filtration column (Fig. 4A). Finally, the mitogenic activity was further purified to homogeneity in a C8 reversed phase HPLC column (Fig. 4B). The protein eluted in a gradient of 28 ~ 29% acetonitrile migrated as a single band with an apparent molecular mass of 5 kDa under the reducing condition of SDS-PAGE (Fig. 4C). When the N-terminal amino acid sequence of the purified 5-kDa protein was determined through Edman degradation, it was revealed to be VLITKC-SSDM, which is identical to that of epiregulin.

Expression of Epiregulin mRNA in Factor Xa-stimulated RASMCs—Reverse transcription-PCR was used to analyze the mRNA level of epiregulin in factor Xa-stimulated RASMCs.
PCR primers were designed on the basis of the complete sequence of the rat epiregulin gene. After stimulation of the quiescent RASMCs with factor Xa, it was interesting to observe a rapid and transient induction of epiregulin mRNA. Expression of the mRNA reached its maximal level at 1 h and returned to the basal level in 6 h (Fig. 5).

Cloning, Expression, and Purification of Recombinant Rat Epiregulin—The rat epiregulin cDNA was cloned and expressed in E. coli as a form of inclusion body. The expressed fusion protein containing glutathione S-transferase was refolded, adsorbed to the glutathione-Sepharose column, and then enzymatically cleaved at a specific site to remove the fusion partner. Recombinant epiregulin was further purified to homogeneity using C8 reversed phase HPLC column. The finally purified recombinant epiregulin migrated as a single band, which corresponded to native epiregulin, on SDS-PAGE (Fig. 4C). The molecular identity of the recombinant epiregulin with the native protein was confirmed by mass spectrometric analysis and by N-terminal amino acid sequencing. Then we examined the recombinant epiregulin for its functional ability to stimulate RASMC mitogenesis under serum-free conditions. As expected, the recombinant epiregulin was able to successfully stimulate the RASMC mitogenesis in a dose-dependent manner (Fig. 6).
FIG. 6. Recombinant epiregulin-stimulated mitogenesis of RASMCs. Quiescent RASMCs were stimulated with various concentrations of recombinant epiregulin. The mitogenesis was estimated by measuring \(^{3}H\)thymidine incorporation into DNA (panel A) and by measuring the number of cells (panel B) as described under “Experimental Procedures.” Data are mean ± S.E. of \(n = 5\) experiments. *, \(p < 0.05\) versus unstimulated control.
Epiregulin Dependence of Factor Xa-induced RASMC Mitogenesis—To investigate the role of epiregulin in factor Xa-stimulated mitogenesis, we examined the effect of anti-epiregulin antibody on factor Xa-induced \[^{3}H\]thymidine incorporation into RASMCs. Serum-starved RASMCs were stimulated by factor Xa in the presence of anti-epiregulin antibody or normal goat IgG. The anti-epiregulin antibody significantly reduced \[^{3}H\]thymidine incorporation into the cell, whereas the normal goat IgG did not affect the mitogenic effect induced by factor Xa (Fig. 7). In control experiments, the anti-epiregulin antibody completely inhibited the mitogenic effect of the recombinant epiregulin. The anti-epiregulin antibody alone had little effect on \[^{3}H\]thymidine incorporation into the unstimulated cells (data not shown). To examine the involvement of other growth factors in factor Xa-induced mitogenesis, serum-starved RASMCs were stimulated with factor Xa for 6 h, and then the cultured medium was analyzed for RASMC mitogenesis after the addition of rTAP and anti-epiregulin antibody to prevent the direct roles of factor Xa and epiregulin, which were present in the cultured medium. The conditioned medium that was recovered after the culture exhibited little effect on RASMC mitogenesis, indicating that epiregulin is the major autocrine factor in factor Xa-stimulated RASMCs (data not shown).

To further clarify the involvement of epiregulin in factor Xa-induced RASMC mitogenesis, we employed an AS-ODN to block the protein expression of epiregulin. When RASMCs were treated with epiregulin AS-ODN to suppress the expression of its encoding protein, a complete inhibition of epiregulin production was observed by immunoblotting the cultured medium with anti-epiregulin antibody (Fig. 8A). The AS-ODN also significantly reduced \[^{3}H\]thymidine incorporation into the factor Xa-stimulated RASMCs (Fig. 8B). On the contrary, the S-ODN was not effective in reducing the mitogenic function of factor Xa. The AS-ODN itself was nearly ineffective in unstimulated RASMC mitogenesis in serum-free media.

**DISCUSSION**

This work shows that factor Xa triggers the mitogenesis of RASMCs via autocrine release of epiregulin. It has been well established that factor Xa promotes the mitogenesis of aortic SMCs (24–26) and saphenous vein SMCs (35). Likewise, the present study demonstrated that factor Xa markedly stimulates both DNA synthesis and the proliferation of RASMCs compared with unstimulated cells. Factor Xa-induced mitogenesis of RASMCs was greatly suppressed by its specific protease inhibitor but not by the thrombin inhibitor, suggesting that thrombin is not associated with the mitogenic pathway. Because factor Xa has been claimed to elicit SMC proliferation via autocrine stimulation involving a yet unidentified isoform of PDGF (25), it was investigated to identify the PDGF isoform that mediates factor Xa-induced SMC mitogenesis. All three isoforms of PDGF (AA, AB, BB) are able to stimulate SMC proliferation with individually differing potencies. However, each of the PDGF isoforms exhibits characteristic cellular effects on vascular SMCs (36). PDGF-BB and -AB are distinguished from PDGF-AA in stimulating the chemotaxis of SMCs. The individual isoform can also activate ERK-1/2 with an intensity that corresponds to its proliferative function. Unlike PDGF-AA, PDGF-BB or -AB is able to phosphorylate tyrosine residues of tensin and p125FAK. Before elucidating the PDGF isoform, we examined whether the factor Xa-induced mitogenesis is associated with the release of endogenous PDGF in RASMCs. Unexpectedly, on the basis of experimental results obtained with neutralizing antibody and with PDGF receptor inhibitor, we have found that the factor Xa-induced RASMC mitogenesis is independent of the autocrine release of PDGF. Further supporting data obtained from immunoassay provided clear evidence indicating that no PDGF isoform is released from the factor Xa-stimulated RASMCs. This finding is in contrast to the observations of Ko et al. (25) and Herbert et al. (26), who report that factor Xa-induced mitogenesis depends on PDGF release in human and rat aortic SMCs. Bretschneider et al. (35), however, recently suggested that factor Xa-induced signaling and mitogenic activity in human venous SMCs are independent of PDGF.

It was then possible for us to speculate on the presence of a novel autocrine pathway that is associated with the factor Xa-induced mitogenesis of RASMCs. In the cultured medium of factor Xa-stimulated RASMCs, we found an autocrine product that was identified to be epiregulin, an EGF-related growth factor (29, 38). Epiregulin was purified to homogeneity from the cultured medium of factor Xa-stimulated RASMCs through a four-step procedure involving affinity, gel filtration, and reversed phase HPLC chromatography. Heparin-Sepharose affinity column has been widely used to purify PDGF and a number of growth factors that bind to heparin. However, it was interesting to note that epiregulin, a growth factor, does not bind to heparin-Sepharose even in the absence of NaCl. In this purification of epiregulin from the cultured medium, any detectable mitogenic activities retained in the heparin-Sepharose column were not found. This observation rules out the presence of heparin-binding growth factors that are related to the mitogenic response to factor Xa.

It has been established that epiregulin directly binds to both EGF receptor (ErbB-1) and ErbB-4 but not to ErbB-2 and ErbB-3 (39). Epiregulin stimulates not only the homodimers of both ErbB-1 and ErbB-4 but also activates all possible heterodimeric ErbB complexes (40). Because ErbB-4 is not expressed in RASMCs, EGF receptor, either as homodimers or as heterodimeric complexes with ErbB-2 or ErbB-3, appears to serve as the principal mitogenic receptor for epiregulin (32). Although epiregulin affinity for ErbB homo-heterodimers is markedly lower than EGF, epiregulin transmits more potent mitogenic signals than EGF (40). This discrepancy between binding affinity and bioactivity is attributed to a mechanism that prevents receptor down-regulation and results in a weak but prolonged intracellular signaling (40). Thus, factor Xa can effectively amplify its mitogenic signal in RASMCs through the autocrine production of epiregulin, which acts as an efficient and potent mitogen.
A recent article demonstrated that epiregulin is more potent and more effective than EGF or transforming growth factor-α in promoting wound repair (41). In the process of wound healing, the blood coagulation pathway is initially triggered, leading to the formation of factor Xa and thrombin. After blood clotting in the injured sites, the coagulation factors may be involved in wound repair by inducing the local production of potent mitogens, including epiregulin, in neighboring cells.

The mitogenic effect of factor Xa on RASMCs was markedly, but not completely, suppressed by an antibody directed against epiregulin or by AS-ODN to epiregulin. The observed incomplete mitogenic inhibition by anti-epiregulin antibody or by AS-ODN in factor Xa-induced RASMCs may be due to the presence of other growth factors that are rapidly degraded during the cell culture. However, this does not appear to be the case since the cultured medium containing rTAP and anti-epiregulin antibody was not able to affect RASMC mitogenesis.

There are several reports demonstrating that factor Xa can activate both protease-activated receptor-1 and protease-activated receptor-2 in vascular cells (37, 42–44). Protease-activated receptors are seven-transmembrane G protein-coupled receptors that most likely elicit signaling responses according to the classic paradigm established for other G protein-coupled receptors. Therefore, another possible explanation is that factor Xa may partially exert its mitogenic effect on RASMCs directly via intracellular signaling pathways.

The present study focused on the mechanism of mitogenic effect of factor Xa on RASMCs. We have shown here that the autocrine production of epiregulin, an EGF-related ligand, is induced in the factor Xa-stimulated mitogenic process of RASMCs. Further investigations will focus on the factor Xa-induced intracellular signaling pathway(s) leading to the epiregulin expression in RASMCs. This work will provide useful information for understanding the cellular function of factor Xa that is associated with RASMC mitogenesis.

**REFERENCES**

1. Luscher, T. F., Turina, M., and Braunwald, E. (1994) Coronary Artery Graft Disease: Mechanisms and Prevention, pp. 42–52, Springer, Heidelberg, Germany
2. Ross, R. (1986) *N. Engl. J. Med.* 314, 488–500
3. Ross, R. (1993) *Nature* 362, 801–809
4. Majesky, M. W., Lindner, V., Twardzik, D. R., Schwartz, S. M., and Reidy, M. A. (1991) *J. Clin. Invest.* 88, 904–910
5. Inoue, S., Ohno, A., Hase, T., Matsue, T., Hashimoto, M., Yamada, R., Ouchi, Y., Orimo, H., and Muroatsu, M. (1993) *Arterioscler. Thromb.* 13, 1359–1364
6. Li, Z., Moore, S., and Alavi, M. Z. (1995) *Exp. Mol. Pathol.* 63, 77–86
7. Itoh, T., Kawata, S., Miyagawa, J., Imai, Y., Tamura, S., Fukuda, K., Isozaki, K., Yamamori, K., Taniguchi, N., Higashiyama, S., and Matsuzawa, Y. (1996) *Arterioscler. Thromb. Vase. Biol.* 16, 1524–1531
8. Marmur, J. D., Rossakchina, M., Guha, A., Pyle, B., Friedrich, V., Mendlovitz, M., Nemerson, Y., and Taubman, M. B. (1993) *J. Clin. Invest.* 91, 2253–2259
9. Carmeliet, P., and Collen, D. (1998) *Int. J. Biochem. Cell Biol.* 30, 661–667
10. Kahan, C., Souwen, K., Meloche, S., and Vuylsteeg, J. (1992) *J. Biol. Chem.* 267, 13369–13375
11. Naldini, A., Carney, D. H., Bocci, V., Klimpel, K. D., Asuncion, M., Soares, L. E., and Klimpel, G. R. (1993) *Cell. Immunol.* 147, 367–377
12. Chakary, D. R., Erdmann, J. M., and Wilner, G. D. (1990) *J. Biol. Chem.* 265, 7729–7732
13. Bar-Shavit, R., Benezra, M., Eldor, A., Hy-Am, E., Fenton, J. W., Jr., Wilner, G. D., and Vlodavsky, I. (1990) *Cell Regul.* 1, 453–463
14. Herbert, J., Lamarche, I., and Dol, F. (1992) *FEBS Lett.* 301, 155–158
15. McNamara, C. A., Sarembock, I. J., Gimple, L. W., Fenton, J. W., Jr., Coughlin, G. K., and Owens, G. K. (1993) *J. Clin. Invest.* 91, 94–98
16. Bar-Shavit, R., Benezra, M., Sabbah, V., Bode, W., and Vlodavsky, I. (1992) *Am. J. Respir. Cell Mol. Biol.* 6, 123–130
17. Kamath, L., Meydani, A., Foss, F., and Kuliopulos, A. (2001) *Cancer Res.* 61, 5933–5940
18. Weiss, R. H., and Maduri, M. (1993) *J. Biol. Chem.* 268, 5724–5727
19. Stouffer, G. A., Sarembock, I. J., McNamara, C. A., Gimple, L. W., and Owens, G. K. (1993) *Am. J. Physiol.* 265, C806–C811
20. Nakano, T., Raines, E. W., Abraham, J. A., Wenzel, F. G., 4th, Higashiyama, S., Klagsbrun, M., and Ross, R. (1993) *J. Biol. Chem.* 268, 22941–22947
21. Bachhuber, B. G., Sarembock, I. J., Gimple, L. W., and Owens, G. K. (1997) *J. Vasc. Res.* 34, 41–48
22. Bono, F., Herault, J. P., Avril, C., Schaeffer, P., Lorneau, J. C., and Herbert, J. M. (1997) *J. Cell. Physiol.* 172, 36–43
23. Blanc-Brude, O. P., Chambers, R. C., Leoni, P., Bode, W., and Laurent, G. J. (2001) *Am. J. Physiol. Cell Physiol.* 281, C681–C689
24. Gasic, G. P., Arenas, C. P., Gasic, T. B., and Gasic, G. J. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 2317–2320
25. Ko, F. N., Yang, Y. C., Huang, S. C., and Ou, J. T. (1996) *J. Clin. Invest.* 98, 1493–1501
26. Herbert, J., Bono, F., Herault, J., Avril, C., Dol, F., Maes, A., and Schaeffer,
Epiregulin Dependence of Factor Xa-induced SMC Mitogenesis

P. (1998) J. Clin. Invest. 101, 993–1000
27. Senden, N. H., Jeunhomme, T. M., Heemskerk, J. W., Wagenvoord, R., van’t Veer, C., Hemker, H. C., and Buurman, W. A. (1996) J. Immunol. 161, 4318–4324
28. Papapetropoulos, A., Piccardoni, P., Cirino, G., Bucci, M., Sorrentino, R., Cicala, C., Johnson, K., Zachariou, V., Sessa, W. C., and Altiere, D. C. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 4738–4742
29. Toyoda, H., Komurasaki, T., Uchida, D., Takayama, Y., Isobe, T., Okuyama, T., and Hanada, K. (1995) J. Biol. Chem. 270, 7495–7500
30. Shirakata, Y., Komurasaki, T., Toyoda, H., Hanakawa, Y., Yamasaki, K., Tokumaru, S., Sayama, K., and Hashimoto, K. (2000) J. Biol. Chem. 275, 5748–5753
31. Zhu, Z., Kleeff, J., Friess, H., Wang, L., Zimmermann, A., Yarden, Y., Buchler, M. W., and Kore, M. (2000) Biochem. Biophys. Res. Commun. 273, 1019–1024
32. Taylor, D. S., Cheng, X., Pawlowski, J. E., Wallace, A. R., Ferrer, P., and Molley, C. J. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1633–1638
33. Chamley-Campbell, J. H., Campbell, G. R., and Ross, R. (1979) Physiol. Rev. 59, 1–61
34. Kovalenko, M., Gazit, A., Bohmer, A., Rorsman, C., Ronnstrand, L., Heldin, C. H., Waltenberger, J., Bohmer, F. D., and Levitzki, A. (1994) Cancer Res. 54, 6106–6114
35. Bretschneider, E., Braun, M., Fischer, A., Wittkoch, M., Glusa, K., and Schror, K. (2000) Thromb. Haemostasis 84, 499–505
36. Jiang, B., Yamamura, S., Nelson, P. R., Mureebe, L., and Kent, K. C. (1996) Surgery 120, 427–431
37. Koo, B. H., Chung, K. H., Hwang, K. C., and Kim, D. S. (2002) FEBS Lett. 523, 85–89
38. Toyoda, H., Komurasaki, T., Ikeda, Y., Yoshimoto, M., and Morimoto, S. (1995) FEBS Lett. 377, 403–407
39. Komurasaki, T., Toyoda, H., Uchida, D., and Morimoto, S. (1997) Oncogene 15, 2841–2848
40. Shelly, M., Pinkas-Kramarski, R., Guarino, B. C., Waterman, H., Wang, L. M., Lyass, L., Alimandi, M., Kuo, A., Baccus, S. S., Pierce, J. H., Andrews, G. C., and Yarden, Y. (1998) J. Biol. Chem. 273, 10496–10505
41. Draper, B. K., Komurasaki, T., Davidson, M. K., and Nanney, L. B. (2003) J. Cell. Biochem. 89, 1126–1137
42. Bono, F., Schaefer, P., Herault, J. P., Michaux, C., Nestor, A. L., Guillemot, J. C., and Herbert, J. M. (2000) Arterioscler. Thromb. Vasc. Biol. 20, 107–112
43. Camerer, E., Huang, W., and Coughlin, S. R. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 5255–5260
44. Riewald, M., Kravchenko, V. V., Petrovan, R. J., O’Brien, P. J., Brass, L. F., Ulevitch, R. J., and Ruf, W. (2001) Blood 97, 3109–3116