Mast Cell and Neutrophil Peptidases Attack an Inactivation Segment in Hepatocyte Growth Factor to Generate NK4-like Antagonists*

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Hepatocyte growth factor (HGF) is a plasminogen-like protein with an α chain linked to a trypsin-like β chain without peptidase activity. The interaction of HGF with c-met, a receptor tyrosine kinase expressed by many cells, is important in cell growth, migration, and formation of endothelial and epithelial tubes. Stimulation of c-met requires two-chain, disulfide-linked HGF. Portions of an α chain containing an N-terminal segment and four kringle domains (NK4) antagonize HGF activity. Until now, no physiological pathway for generating NK4 was known. Here we show that chymases, which are chymotryptic peptidases secreted by mast cells, hydrolyze HGF, thereby abolishing scatter factor activity while generating an NK4-like antagonist of HGF scatter factor activity. Thus, chymase interferes with HGF directly by destroying active protein and indirectly by generating an antagonist. The site of hydrolysis, Leu480, lies in the α chain on the N-terminal side of the cysteine linking the α and β chains. This site appears to be specific for HGF because chymase does not hydrolyze other plasminogen-like proteins, such as macrophage-stimulating protein and plasminogen itself. Mast cell/neutrophil cathepsin G and neutrophil elastase generate similar fragments of HGF by cleaving near the chymase site. Mast cell and neutrophil peptidases are secreted during tissue injury, infection, ischemia, and allergic inflammation, where they may oppose HGF effects on epithelial repair. Thus, HGF possesses an “inactivation segment” that serves as an Achilles' heel attacked by inflammatory proteases. This work reveals a potential physiological pathway for inactivation of HGF and generation of NK4-like antagonists.

HGF is a mitogen, motogen, and morphogen for epithelial and mesenchymal cells (1). It is also known as scatter factor because it disperses epithelial cells in culture (2). Genetic deletion in mice suggests that HGF is critical for embryonic development (3). Adult mesenchymal cells also produce HGF, which is thought to regulate tissue regeneration and repair after injury (4, 5), epithelial to mesenchymal transitions, and formation of vessels and other tube-like structures (6). HGF production is inducible. Circulating levels rise markedly in several types of tissue injury, as in liver damage (7), arterial thrombosis (8), or acute rejection of a transplanted lung (9). When used as a drug, HGF stimulates lung regrowth after pneumonectomy (10), diminishes lung fibrosis (11) and allergic airway remodeling (12), and opposes myocardial ischemia-reperfusion injury (13) and pulmonary hypertension-associated vascular remodeling (14).

Mature HGF is a disulfide-linked, heterodimeric protein related to plasminogen, with α and β chains originating from an inactive, single-chain precursor (see Fig. 1). Although HGF is proteolytically incompetent because of mutations in the peptidase domain, it retains a serine peptidase-like mode of activation by cleavage at Arg494 in the precursor, changing conformation to allow productive binding, dimerization, and activation of its receptor, c-met (15). Mature α and β chains of HGF each can bind to c-met, although individually they have little receptor activating ability (15). A fragment of the α chain comprised of the N-terminal hairpin and four kringle domains (NK4) antagonizes c-met-mediated HGF effects, such as scatter factor activity, and also inhibits angiogenesis by independent mechanisms (16). NK4 is composed of the first 447 residues (pyrGlu22−Val479 of HGF) of the α chain. NK4 was generated originally by fragmentation with pancreatic elastase, which hydrolyzes HGF at Val479 (17). Subsequently, recombinant NK4 or NK4-expressing vectors were used to inhibit invasion, metastasis, and angiogenesis in tumor models (reviewed in Ref. 16).

HGF may influence development of mast cells, which like HGF itself are implicated in responses to injury, tissue repair, remodeling, and angiogenesis. Murine mast cell precursors express c-met during in vitro differentiation (18) and respond to HGF with β1 integrin-mediated migration. By releasing heparin, mast cells may raise circulating HGF after thrombotic events, like myocardial infarction (19). Heparin also may be a cofactor in HGF-induced angiogenesis (reviewed in Ref. 8). Thus, HGF may influence mast cell behavior and vice versa. In this respect, it is notable that secreted products of in vitro differentiated mast cells ablate HGF activity (18).

The present work focuses on major inflammatory cell peptidases, including the human mast cell chymotryptic peptidase, chymase, a mouse enzyme, mast cell protease (MCP)4, with similar tissue distribution and enzymatic properties (20–22), and human neutrophil elastase and cathepsin G. Cathepsin G possesses trypsin-like, chymotryptic, and metalloprotease activity (23) and is expressed by mast cells as well as by neutrophils. Human chymase and cathepsin G are expressed in a subset of mast cells inhabiting the dermis and certain other microenvironments, such as airway submucosa (24, 25). These enzymes accumulate in secretory granules and are released by other mediators by antigen-bound IgE or other stimuli, such as neuropeptides, anaphylatoxins, and bacterial surface proteins. Elastase and cathepsin G are released by activated neutrophils, where association with the cell surface or with high local concentrations in quently released granules affords protection from circulating antipeptidases (26, 27). Thus, these enzymes are secreted in...
Various types of inflammation. The present work reveals that chymase, cathepsin G, and neutrophil elastase, despite divergent cleavage specificities, hydrolyze HGF in a short but vulnerable inactivation segment, ablating bioactivity and generating an antagonistic fragment.

**MATERIALS AND METHODS**

**Mast Cell and Leukocyte Peptidases**—Recombinant human prochymase was expressed, activated, and purified as described (21). The concentration of active chymase was determined with the substrate succinyl-L-Ala-Ala-Pro-Phe-4-nitroanilide as described (21). Chymase MCP4 was purified from mouse ears and assayed as described (21). Mast cell β-tryptase was purified from human lung (28). Human neutrophil elastase and cathepsin G were purchased from Elastin Products (Owensville, MO). The molar ratios of elastase and cathepsin G were calculated from protein content and assumed to be fully active.

**Incubation of Peptidases with HGF, Macrophage-stimulating Protein (MSP), and Plasminogen**—HGF and related kringel-rich, plasminogen-like proteins were incubated with peptidases. Human HGF was kindly provided by Genentech (South San Francisco, CA) or was purchased from Peprotech (Rocky Hill, NJ). These preparations are recombinant material expressed in Chinese hamster ovary and insect cells, respectively. Recombinant human MSP was from R&D Systems (Minneapolis, MN). Plasma-derived human plasminogen, porcine pancreatic elastase, and bovine chymotrypsin were from Sigma. Incubations with proteases were carried out in phosphate-buffered saline, pH 7.4, with or without heparin (10 μg/1 μg of HGF; bovine lung, Sigma, catalog number H4898, 150 units/mg), and were stopped by adding SDS-PAGE sample buffer and heating to 70 °C for 10 min.

**Electrophoresis, Blotting, and N-terminal Sequencing**—Results of incubation of HGF, MSP, and plasminogen with chymases were monitored by reducing and non-reducing gradient SDS-PAGE (Invitrogen). Coomassie Blue-stained gels were scanned and analyzed by densitometry. Chymase-generated fragments separated by non-reducing SDS-PAGE were transferred to polyvinylidene fluoride membrane. Blotted bands of interest were excised and subjected to N-terminal sequencing by Midwest Analytical (St. Louis, MO).

**Purification and Sequencing of Peptidase-generated Fragments of HGF**—Unreduced fragments of HGF arising from incubation with human chymase were loaded onto a 2.1 × 250 mm C4 reverse-phase HPLC column (Alltech, Deerfield, IL) in 5% acetonitrile, 95% H₂O, 0.1% trifluoroacetic acid and eluted with a two-slope gradient of increasing concentrations of acetonitrile in H₂O, 0.1% trifluoroacetic acid. The eluted proteins were detected by monitoring of absorbance at 280 nm, collected, and analyzed by SDS-PAGE. A purified fragment was co-incubated with HGF in the scatter factor assays described below. In separate experiments, chymase-generated fragments of HGF were reduced in 40 mM dithiothreitol, alkylated by incubation in 80 mM iodoacetamide, then separated on the C4 column. The major early eluting peak was collected and further analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectroscopy (Voyager DE-STR, Applied Biosystems, Foster City, CA) at UCSF's Biomolecular Resource Center. The material was further probed by HPLC-linked tandem mass spectroscopy (QSTAR XL, Applied Biosystems). To characterize fragments of HGF generated by neutrophil elastase and cathepsin G, digested products were subjected directly to MALDI-TOF mass spectroscopy. Also, to compare the heparin-binding characteristics of HGF and products of chymase-mediated hydrolysis, partial digests were loaded onto a heparin HPLC column (TosoHaas, Montgomeryville, PA) and eluted with a linear gradient of 0.5–2 M NaCl in 10 mM bis-Tris-HCl, pH 6.1.

**Scatter Factor Activity**—To assess the bioactivity of chymase-hydrolyzed HGF, the scatter factor activity of cleaved protein was compared with that of native protein in Madin-Darby canine kidney (MDCK) cell assays (17). The antagonistic potential of an NK4-like fragment liberated by chymase was assessed in the same assay by incubating the purified fragment with mature, active HGF. Briefly, cells were plated on 6-well culture dishes at 17,000 cells/well 48 h before incubation with HGF. For inhibition studies, cells were preincubated with the NK4-like fragment for 30 min at 37 °C before addition of HGF. After incubation for 18 h, cells were fixed for 30 min in 4% paraformaldehyde and then washed in 70% ethanol and air-dried for light microscopy.

**RESULTS**

**Hydrolysis of HGF by Mast Cell Chymases**—As determined by reducing SDS-PAGE prior to incubation with peptidases, the HGF in this study is mature, two-chain HGF mixed with smaller amounts of single-chain pro-HGF. As shown in Fig. 2, human chymase and mouse MCP4 incubated at molar ratios of enzyme:HGF of 1:70 selectively hydrolyze HGF in a time- and heparin-dependant fashion, generating major bands of 30 and 45 kDa. N-terminal sequencing of an unreduced 30-kDa protein reveals two sequences of similar molarity: Val-Val-Asn-Gly-Ile, corresponding to HGF residues 495–499, which is the N terminus of the activated serine protease domain, and Asp-His-Pro-Val-Ile, corresponding to residues 481–485 in the proposed inactivation segment.
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Hydrolysis of HGF by Other Peptidases—As shown in Fig. 2, neutrophil elastase and cathepsin G hydrolyze HGF with a pattern similar to that of chymase and MCP4, including generation of an NK4-like fragment. Cathepsin G, which is less specific than chymase toward most substrates, was used in a molar ratio of enzyme:HGF of 1:38. Cathepsin G, which is weaker than chymase toward most substrates, was used in a molar ratio of 1:12, exhibits little activity, consistent with the lack of preferred aromatic residues in substrate specificity. Cathepsin G, which is less specific than chymase toward most substrates, was used in a molar ratio of enzyme:HGF of 1:38.

Failure of Chymase to Hydrolyze HGF Homologues—As shown in Fig. 2, chymase does not fragment human plasminogen and MSP, which are related members of the plasminogen subfamily of kringle-containing proteins with serine peptidase domains. Thus, human chymase sensitivity is specific to HGF among its close relatives. However, MCP4 can cleave human but not mouse plasminogen (not shown), consistent with somewhat differing substrate preferences between human chymase and MCP4.

Purification of an NK4-like Protein Generated by Chymase—Fig. 4 shows chromatographic separation and purification of the NK4-like fragment from the chymase digest. The size disparity of the two peaks is because of a large difference in the extinction coefficient between the two major fragments, which are more nearly equal on a molar basis. The peak 1 protein was used in scatter factor assays, as noted below. Not shown are the results of heparin affinity chromatography, which reveals that chymase-generated NK4-like protein binds much more strongly than the free β chain. This suggests that the heparin affinity of HGF derives principally from the NK4 portion of the α chain. The shared affinity of HGF and chymase for heparin may promote the formation of a ternary complex and accelerate chymase-mediated HGF inactivation and generation of NK4-like protein, as seen in Fig. 2.

DISCUSSION

This work reveals that mast cell chymase hydrolyzes HGF, ablating scatter factor activity and simultaneously generating an antagonistic fragment. The Leu⁴⁶⁰ hydrolysis site is selective in that related members of the plasminogen subfamily have little homology in this region and resist cleavage. The site of vulnerability is not in a classical kringle domain but in a region connecting K₄ to the "activation segment," which, as shown in Fig. 1, contains the Arg⁴⁹⁴ site of hydrolytic activa-
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FIGURE 3. Identification of cleavage sites and comparison of inactivation segments. A, MALDI-TOF mass spectrum of the major HPLC-purified peptides generated from chymase-cleaved HGF. Peaks representing mono-protonated peptides DHPVISC*AKTKQL (m/z = 1496.80) and DHPVISC*AKTKQLR (m/z = 1652.91) are noted. C*, carboxamidomethyl cysteine. The identity of these peptides was validated by tandem mass spectrometry. B, mass spectrum of neutrophil elastase-generated fragments of HGF, including peaks consistent withNLDPVISC*AKTKQL (m/z 1723.94) and NLDPVISC*AKTKQLR (m/z 1880.06). With results of SDS-PAGE, these findings suggest that the major site of HGF hydrolysis by chymase and neutrophil elastase is Leu480 and Val478, respectively. C, sequence alignment of seven mammalian HGFs and chicken HGF between residues 451 and 510, which includes the proposed protease-cleaved inactivation and activation segments bracketed by the end of the fourth kringle and beginning of the catalytic domain. Major sites of hydrolysis by elastase and chymase are shown by * and **, respectively. An arrow points to the site of hydrolysis of single-chain HGF by its activator to generate the active, two-chain form, in which the a chain is disulfide-linked to β chain via Cys487. Hydrolysis at any site between the two cysteines (vertical bars), which flank the 17-residue inactivation segment, unlinks a from β and generates a free, NK4-like fragment of a chain. Cleavage within the kringle domain itself does not generate a free fragment because of triple looping via Cys–Cys links. Note absolute conservation of the chymase cleavage site. D, compares sequence of human HGF, MSP, and plasminogen (PLG) in the same regions. Note that chymase and elastase hydrolysis sites in the HGF inactivation segment are not conserved in otherwise-homologous MSP and plasminogen.

We propose that Leu480 is part of a 17-residue “inactivation segment” comprised of residues 470–486, which are not connected to other parts of HGF by disulfide bridges. Conservation of Leu480 and surrounding residues in mammalian HGFs hints that changes in this segment are poorly tolerated, possibly because susceptibility to hydrolytic inactivation and release of an antagonistic fragment is important for regulating HGF function.

The proposed inactivation segment is one of few regions in which proteolysis will divide HGF into free, inactive fragments. This is because most of HGF, including kringle and peptidase domains, is fortified by overlapping Cys–Cys links. Hydrolysis at most sites will produce nicking without separation of fragments. The peptides between kringles are theoretically vulnerable but appear to be protease-insensitive likely because they are short and inaccessible to bulky peptidases. The observation that elastases cleave human HGF at a site just two residues away from the site cleaved by chymase, along with the finding that cathepsin G cleaves in the same vicinity, invites speculation that these sites lie in an interdomain region in which neutral and hydrophobic residues are exposed. Crystal-derived structures of HGF do not address this point because the inactivation peptide is not included in existing structures. Identification of Leu at the site of hydrolysis itself argues for an unusual degree of exposure, because Leu is not favored at the scissile bond in short peptides cleaved by chymotryptic peptidases generally and chymase particularly. A profiling of the substrate preferences of human chymase with a combinatorial peptide library found the cleavage of substrates with P1 Leu at the site of hydrolysis to be far less likely than when the P1 residue is Phe or Tyr (30). Profiling with selected 4-nitroanilide and angiotensin-like peptides similarly suggests preferences for P1 aromatic residues (31, 32). Notwithstanding target preferences suggested by screening short peptides, at least two other proteins, procollagenase (33) and procollagen 1α (34), are cleaved by human chymase at P1 Leu. These proteins and HGF evidently present Leu-containing segments in a more cleavable conformation than in short peptides with little secondary structure. It bears noting that combinatorial screening suggests that the residues occupying the P4–P2 positions (Ile-Val-Asn) of HGF adjacent to the site hydrolyzed by chymase are favorable when present in short peptides (30). This suggests that P1 side chain accessibility, combined with P4–P2 side chains interacting favorably with the extended substrate-binding site, contribute to the ability of chymase to hydrolyze at Leu480. The inability of mast cell tryptase to hydrolyze HGF is consistent with the lack of basic residues preferred by tryptic enzymes in the inactivation segment.

The present data suggest an explanation for the observation that a product of in vitro differentiated mouse mast cells degrades the HGF α chain (18). Our data indicate that a particular mouse chymase, MCP4, hydrolyzes HGF in a pattern similar to that generated by the complex
mixture of biomolecules released by degranulating mast cells. Thus, MCP4 may be the principal HGF-degrading enzyme of mouse mast cells. In comparison to the large fragment of α chain produced by human chymase, MCP4 also yields one or more smaller bands, which appear to derive from the larger band. This finding suggests hydrolysis by MCP4 at sites less susceptible to human enzyme. Furthermore, MCP4 (but not human chymase) generates angiotatin-like fragments from human plasminogen (not shown). This is not physiologically significant because of the species mismatch and because mouse plasminogen is not cleaved. Although human chymase and mouse MCP4 are similar in physical and enzymatic properties, the present data indicate that they can also differ in hydrolyzing protein targets. Imperfect matching of mouse and human substrate preferences is not surprising given previously established differences, as in hydrolysis of angiotensin (21, 35). There are no human phylogenetic equivalents to MCP4 and other β-chymases, which may be rodent-specific. The mouse chymase with closest overall structural and phylogenetic similarity to human chymase is MCP5 α-chymase (36). However, an amino acid change in mouse and rat MCP-5 alters the specificity from chymotryptic to elastolytic with little ability to cleave peptide substrates with P1 Phe, Tyr, or Leu (37, 38). Overall, the present work supports the proposal that MCP4 β-chymase is the closest functional equivalent of human α-chymase (22).

Other investigations have established that a human mast cell line (HMC-1) and in vitro differentiated mouse mast cells express HGF receptor c-met and respond to HGF (18, 39). In HMC-1 cells, c-met expression is inducible and exposure to HGF suppresses release of tumor necrosis factor-α (39). In bone marrow-derived mouse mast cells, HGF promotes developmental stage- and integrin-dependant migration (18). Thus, secreted mast cell chymase and cathepsin G may decrease cytokine release and migration responses to HGF in the vicinity of a degranulating mast cell. Perhaps more significant are the implications of mast cell and neutrophil peptidase-mediated destruction of HGF for the behavior of other cells. Given what is known of the roles of HGF in pathobiology, inactivation by inflammatory peptidases could limit proliferation, migration, or differentiation of c-met-bearing cells in acute response to infection, injury, or allergen exposure. More specifically, selective hydrolysis may oppose HGF effects on epithelial repair in pneumonia, allergic airway inflammation (12), and ischemia-reperfusion injury (13). By interrupting control of epithelial to mesenchymal transformation by HGF, proteolytic inactivation may promote lung and airway fibrosis (11). Mast cell secretion of chymase and cathepsin G may modulate the rise of plasma HGF in clot-related disorders such as myocardial infarction. This rise has been linked to clot-stimulated degranulation of mast cells, which release heparin, to which HGF binds, facilitating its role in reparative angiogenesis (8, 19). Like HGF, chymase and cathepsin G bind to heparin. Indeed, they associate tightly with heparin in secretory granules and remain bound upon secretion. The mutual attraction of HGF and peptidase to heparin may encourage formation of a ternary complex and selective degradation.

The significance of in situ generation of NK4-like protein may lie in coupling of production of an antagonist with destruction of intrinsic activity. This combination may be more powerful than either effect by itself. There may be additional effects specific to the NK4-like fragment, for NK4 antagonizes HGF through competitive inhibition of binding to c-met and also has actions apparently unrelated to c-met, including inhibition of angiogenesis. The large α chain fragment generated by chymase is similar but not identical to NK4 in that NK4 lacks the C-terminal Asn-Leu dipeptide of the chymase-generated fragment. In its capacity to inhibit HGF scatter factor activity, the NK4-like fragment appears to be at least as potent as NK4, in that inhibition is achieved at ratios of antagonist to HGF much lower than reported for NK4 (17). Cells transcribing the HGF gene sometimes generate splice variants, resulting in truncated proteins containing N-terminal domain plus kringle 1 (NK1) or N-terminal domain plus kringle 1 and 2 (NK2) (40). These shortened proteins also can act as agonists of HGF. NK1 appears to require heparin to act as an agonist. When used as a drug, NK4 inhibits tumor growth and metastasis (16). Whether NK4-like protein produced endogenously by inflammatory peptidases has similar activity remains to be determined.

These findings suggest potential physiological pathways for generating NK4-like HGF antagonists in allergic and neutrophilic inflammation. The necessary ratios of enzyme to substrate are achievable in the vicinity of migrating neutrophils and degranulating mast cells, where local concentrations of enzyme are transiently high. These ratios also
can be reached on a more sustained basis in settings such as purulent bronchitis in cystic fibrosis, where disintegrating neutrophils release active peptidases and overwhelm local antipeptidase defenses.

In conclusion, this work reveals that serine peptidases released during allergic and neutrophilic inflammation, acting on HGF, couple inactivation with generation of an HGF antagonist. This is achieved by hydrolyzing an inactivation segment that is conserved in mammals and vulnerable to cleavage by peptidases of varying specificity. Regulation and dysregulation of HGF activity by inflammatory peptidases may be important in diverse types of inflammation.

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REFERENCES

1. Birchmeier, C., Birchmeier, W., Gherardi, E., and Vande Woude, G. F. (2003) Nat. Rev. Mol. Cell. Biol. 4, 915–925
2. Weidner, K. M., Arakaki, N., Hartmann, G., Vandekerckhove, J., Weingart, S., Rieder, H., Fonatsch, C., Tsubouchi, H., Hishida, T., Daikuhara, Y., et al. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 7001–7005
3. Uehara, Y., Minowa, O., Mori, C., Shiota, K., Kuno, J., Noda, T., and Kitamura, N. (1995) Nature 373, 702–705
4. Matsumoto, K., Kataoka, H., Date, K., and Nakamura, T. (1997) J. Biol. Chem. 272, 266, 1934–1941
5. Tchougounova, E., Pejler, G., and Abrink, M. (2003) J. Clin. Investig. 112, 22913–22920
6. Fehlner-Gardiner, C. C., Cao, H., Jackson-Boeters, L., Nakamura, T., Elliott, B. E., Uniyal, S., and Chan, B. M. (1999) Differentiation 65, 27–42
7. Birrer, M. I., Atkinson, W. L., Eble, J. N., and Ellis, P. J. (2000) Cancer. Biol. 28, 841–850
8. Pfeffer, S. R., and Losordo, D. W. (2000) Circulation 101, 1113–1116
9. Matsumoto, K., Miyamoto, T., Ohashi, N., Sasayama, S., and Matsumori, A. (2002) Circulation 106, 3133–3138
10. Sakamaki, Y., Matsumoto, K., Mizuno, S., Miyoshi, S., Matsuda, H., and Nakamura, T. (2000) J. Biol. Chem. 275, 11574–11578
11. Yaekashiwa, M., Nakayama, S., Ohnuma, K., Sakai, T., Abe, T., Satoh, K., Matsumoto, K., Nakamura, T., Takahashi, T., and Nukiwa, T. (1997) Am. J. Respir. Crit. Care Med. 156, 1937–1944
12. Ito, W., Kanehiro, A., Matsumoto, K., Shimura, H., Tanaka, M., and Nakamura, T. (2000) J. Biol. Chem. 275, 1509–1515
13. Yano, K., Nakao, K., Sayama, K., Harada, Y., Oono, Y., and Sawai, Y. (1999) Cancer. Biol. 28, 5921–5930
14. Date, K., Matsumoto, K., Shimura, H., Tanaka, M., and Nakamura, T. (1997) FEBS Lett. 420, 1–6
15. Hartmann, G., Naldini, L., Weidner, K. M., Sachs, M., Vigna, E., Comoglio, P. M., and Birchmeier, W. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 11574–11578
16. Matsunoto, K., and Nakamura, T. (2003) Cancer Sci. 94, 321–327
17. Date, K., Matsumoto, K., Shimura, H., Tanaka, M., and Nakamura, T. (1997) FEBS Lett. 420, 1–6
18. Fehringer-Gardiner, C. C., Cao, H., Jackson-Boeters, L., Nakamura, T., Elliott, B. E., Uniyal, S., and Chan, B. M. (1999) Differentiation 65, 27–42
19. Kino, S., Miyamoto, T., Ohashi, N., Sasayama, S., and Matsumori, A. (2002) Circulation 106, 3133–3138
20. Serafini, W. E., Sullivan, T. P., Conder, G. A., Ebrahimi, A., Marcham, P., Johnson, S. S., and Schwartz, L. B. (1989) J. Biol. Chem. 266, 1934–1941
21. Caughey, G. H., Raymond, W. W., and Wolters, P. J. (2000) Biochim. Biophys. Acta 1480, 245–257
22. Matsumoto, K., Kataoka, H., Date, K., and Nakamura, T. (1998) J. Biol. Chem. 273, 22913–22920
23. Raymond, W. W., Waugh Ruggles, S., Craik, C. S., and Caughey, G. H. (2003) J. Biol. Chem. 278, 43517–43524
24. Powers, J. C., Tanaka, T., Harper, J. W., Minematsu, Y., Parker, L., Lin, D., Crumley, K. V., Fraki, J. E., Schechter, N. M., Lazarus, G. G., and Fukumizu, A. (2002) J. Biol. Chem. 277, 2963–2968
25. Saarinen, J., Kallikainen, N., Welgus, H. G., and Kovanen, P. T. (1994) J. Biol. Chem. 269, 18134–18140
26. Kofford, M. W., Schwartz, L. B., Schechter, N. M., Yager, D. R., and Graham, M. F. (1997) J. Biol. Chem. 272, 1217–1231
27. Saito, K., Muto, T., Tomimoto, Y., Imajo, S., Maruoka, H., Tanaka, T., Yamashiro, K., and Fukuda, Y. (2003) Biochem. Biophys. Res. Commun. 302, 773–777
28. Caughey, G. H. (2002) Mol. Immunol. 38, 1353–1357
29. Kunori, Y., Koizumi, M., Masegi, T., Kasai, H., Kawabata, H., Yamazaki, Y., and Fukumizu, A. (2002) Eur. J. Biochem. 269, 5921–5930
30. Karlson, U., Pejler, G., Tomasi-Johansson, B., and Hellman, L. (2003) J. Biol. Chem. 278, 39625–39631
31. Yano, K., Nakao, K., Sayama, K., Harada, Y., Harada, Y., and Sawai, Y. (1999) Biochim. Biophys. Res. Commun. 259, 740–745
32. Chan, A. M., Rubin, J. S., Bottaro, D. P., Hirschfeld, D. W., Chedid, M., and Aaronson, S. A. (1991) Science 254, 1382–1385