Inhibition of Protein Geranylgeranylation Causes a Superinduction of Nitric-oxide Synthase-2 by Interleukin-1β in Vascular Smooth Muscle Cells*

(Received for publication, January 29, 1997, and in revised form, March 13, 1997)

Jonathan D. Finder‡, Jennifer L. Litz‡, Michelle A. Blaskovich§, Terence F. McGuire§, Yimin Qian¶, Andrew D. Hamilton†, Paul Davies§, and Saïd M. Sebti§

From the Departments of ‡Pharmacology, ‡Pediatrics, and §Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

Recently, we have designed farnesyltransferase and geranylgeranyltransferase I inhibitors (FTI-277 and GGTI-298) that selectively block protein farnesylation and geranylgeranylation, respectively. In this study, we describe the opposing effects of these inhibitors on interleukin-1β (IL-1β)-stimulated induction of nitric-oxide synthase-2 (NOS-2) in rat pulmonary artery smooth muscle cells (RPASMC) and rat hepatocytes. Pretreatment of cells with GGTI-298 caused a superinduction of NOS-2 by IL-1β. RPASMC treated with GGTI-298 (10 μM) prior to IL-1β (10 ng/ml) expressed levels of NOS-2 protein five times higher than those exposed to IL-1β alone. This superinduction of NOS-2 protein by pretreatment with GGTI-298 resulted in nitrite concentrations in the medium that were 5-fold higher at 10 ng/ml IL-1β and 10-fold higher at 1 ng/ml IL-1β. Furthermore, NOS-2 mRNA levels in RPASMC were also increased 6- and 14-fold (at 10 and 1 ng/ml IL-1β, respectively) when the cells were pretreated with GGTI-298. In contrast, treatment of cells with the inhibitor of protein farnesylation, FTI-277 (10 μM), blocked IL-1β-induced NOS-2 expression at mRNA and protein levels. Pretreatment with lovastatin, an inhibitor of protein prenylation, resulted in superinduction of NOS-2. This superinduction was reversed by geranylgeraniol, but not by farnesol, further confirming that inhibition of geranylgeranylation, not farnesylation, is responsible for enhanced NOS-2 expression. The results demonstrate that a farnesylated protein(s) mediates IL-1β induction of NOS-2, whereas a geranylgeranylated protein(s) represses this induction.

Nitrergic oxide (NO) is a radical with important physiologic effects that include modulation of vasoreactivity, prevention of thrombosis, and neurotransmission (1). NO is the product of an enzymatic reaction catalyzed by nitric-oxide synthase (NOS).1 Mammalian cells express three isozymes of NOS, neuronal (NOS-1), endothelial (NOS-3), and inducible (NOS-2) (reviewed in Ref. 2). NOS-1 and NOS-3 are constitutively active, whereas NOS-2 is not present unless the cell is triggered to produce it. The induction of NOS-2 in cells exposed to cytokines and lipopolysaccharides is implicated in the pathophysiology of shock (3).

IL-1β is an important cytokine that is released early in inflammation and shock (4). As a single agent, IL-1β is capable of inducing NOS-2 at levels comparable to those induced by a mixture of cytokines and lipopolysaccharides (5). IL-1β achieves its effects through signaling pathways that have not been fully defined (6), but that appear to include the mitogen-activated protein (MAP) kinase pathway since IL-1β activates MAP kinase itself (7–10), at least in some systems, via the kinase (MAP kinase kinase) immediately preceding it (10–12). This effect on MAP kinase kinase implicates the small GTP-binding protein Ras since this triggers activation of the pathway by interacting with Raf, a serine/threonine kinase lying immediately upstream of MAP kinase kinase (13). IL-1β is also capable of activating stress-activated protein kinase (14, 15) and p38 kinase (16), two kinases that occupy positions in pathways distinct from MAP kinase, but that are also potentially under the control of small G proteins such as Ras, Rho, and Rac (17).

Modulating the function of these small G proteins has recently become possible with inhibitors of prenyltransferases, the enzymes responsible for attaching prenyl lipids to the proteins as the first and most critical step in post-translational processing (18). The enzymes are farnesyltransferase (FTase) (19, 20), which attaches the 15-carbon farnesyl, and geranylgeranylationtransferase I (GGTase I) (21, 22), which attaches the 20-carbon geranylgeranyl. Among proteins that are farnesylated are H-Ras and nuclear lamins; among those that are geranylgeranylated are Rap1A, RhoA, and Rac1. Some proteins that can be both farnesylated and geranylgeranylated include RhoB and Ki-Ras 4B (23). Because farnesylation and/or geranylgeranylation of small G proteins is critical for their function, we (24–28) and others (29–34) have designed potent and highly selective inhibitors of FTase. More recently, we have designed GGTase I inhibitors (35–37). FTase inhibitors have been shown to block the processing of oncogenic Ras, to reverse transformation, to induce the accumulation in the cytoplasm of inactive Ras-Raf complexes, and to inhibit murine and human tumor growth in vivo (24–34). Furthermore, FTase inhibitors are not toxic in vivo (25, 30), are less effective at inhibiting normal cell growth (29, 31), and do not inhibit growth factor (epidermal growth factor or platelet-derived growth factor) activation of MAP kinase (37–39). Much less is known about GGTase I inhibitors, but recently, we have shown that these agents antagonize the signaling of oncogenic Ki-Ras 4B (35), decrease levels of tyrosine phosphorylation of the platelet-derived

* This work was supported by National Institutes of Health Grants CA-67771 (to S. M. S.) and HD-28836-04 (to J. D. F.) and by a grant-in-aid from the American Heart Association, Pennsylvania Chapter (to P. D.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Drug Discovery Program, H. Lee Moffitt Cancer Center and Dept. of Biochemistry and Molecular Biology, University of South Florida, 12902 Magnolia Dr., Tampa, FL 33612. Tel.: 813-979-6734; Fax: 813-979-6748.

1 The abbreviations used are: NOS, nitric-oxide synthase; IL-1β, interleukin-1β; MAP, mitogen-activated protein; FTase, farnesyltransferase; GGTase I, geranylgeranylationtransferase I; RPASMC, rat pulmonary artery smooth muscle cells; PBS, phosphate-buffered saline.
growth factor receptor (37), and block fibroblasts in the G1 phase of the cell cycle (36). Very little is known about the effects of FTase and GGTase I inhibitors in cells other than fibroblasts. Recently, Singh et al. (40) showed that an inhibitor of FTase blocked IL-1β-stimulated induction of NOS-2 and activation of MAP kinase in cardiac myocytes. Here we demonstrate that an inhibitor of GGTase I causes superinduction of IL-1β-stimulated NOS-2 in smooth muscle cells and hepatocytes.

EXPERIMENTAL PROCEDURES

Cell Culture—The primary culture of rat pulmonary artery smooth muscle cells (RPASMC) has been described in detail previously (5). Briefly, explants of pulmonary artery were obtained by dissection following pentobarbital euthanasia of male Harlan Sprague Dawley rats and placed in tissue culture flasks containing growth medium (1:1 Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium, 10% fetal bovine serum, and penicillin/streptomycin). Cells were allowed to migrate out of the explants for 5–7 days before removal of the explants and subsequent subpassaging by trypsinization. For the studies reported here, cells were trypsinized, replated at ~10^6 cells/75-cm² flask, and grown under standard conditions of 37°C, 5% CO2, and 100% humidity. Cells were used through the fifth passage.

Hepatocytes were isolated from male Harlan Sprague Dawley rats using the in situ collagenase perfusion technique of Geller et al. (41). After isolation, hepatocytes were plated on gelatin-coated Petri dishes (Corning Inc.) and grown in Williams’ medium E (Life Technologies, Inc.) with the addition of l-arginine (0.50 mM), insulin (1 µM), HEPES (15 mM), l-glutamine, penicillin/streptomycin, and 10% low endotoxin fetal calf serum. Cells were incubated in growth medium for 24 h, and then serum-free Williams’ medium E with the above additives was used for all experimental conditions.

Induction of NOS-2—This was achieved by treating cells with recombiant human IL-1β (R&D Systems, Minneapolis, MN) at the concentrations indicated. After 24 h, the medium was harvested for determination of nitrite concentration, and the cells for assessment of NOS-2 RNA and protein levels.

Treatment of Cells with GGTI-298 and FTI-277—RPASMC were grown to 50% confluence (generally, 24–48 h after plating). Fresh medium was added containing either FTI-277 or GGTI-298 at the concentrations indicated. The cells were allowed to grow with inhibitors for 24 h. Stimulation of cells with IL-1β took place in basal medium (Dulbecco’s modified Eagle’s medium and F-12 medium) and in medium containing 10% fetal calf serum. Cells were incubated in growth medium for 24 h, and then serum-free Williams’ medium E with the above additives was used for 24 h.

Western Blotting for NOS-2—Cell monolayers were lysed in ice-cold buffer containing 50 mM Tris (pH 8.0), 110 mM NaCl, 5 mM EDTA, 1% Triton X-100, and the protease inhibitors antipain, pepstatin, leupeptin, chymostatin, and phenylmethylsulfonyl fluoride. The lysate was transferred to a conical tube. Protein concentration was determined using the Bradford assay (Bio-Rad). Whole cell lysate was boiled for 10 min. The absorbance at 550 nm was measured, and nitrite was transferred to a conical tube. Protein concentration was determined using sodium nitrite as a standard.

Measurement of Nitrite—To quantify the amount of NO released from cells, nitrite, its stable product in aqueous solution, was measured by the Griess reaction. Nitrite concentration was measured by mixing an aliquot of cell supernatant with an equal volume of Griess reagent (1 part 0.1% naphthylethylenediamine dihydrochloride to 1 part 1% sulphanilamide in 5% phosphoric acid) and incubating at room temperature for 10 min. The absorbance at 550 nm was measured, and nitrite concentration was determined using sodium nitrite as a standard.

FIG. 1. Effect of FTI-277 and GGTI-298 on processing of Ras and Rap1A (A) and on IL-1β-stimulated nitrite formation (B). A. Cells were treated on each of 2 successive days with either FTI-277 or GGTI-298 and then harvested and subjected to Western analysis to demonstrate inhibition of processing by a band shift from the processed (p) to the unprocessed (u) protein. In lane 1, cells were untreated; in lanes 2–5, cells were treated with FTI-277 at 0.1, 1, 5, and 10 µM; and in lanes 6–9, cells were treated with GGTI-298 at the same concentrations. B. Cells were pretreated for 24 h with FTI-277 (10 µM) or GGTI-298 (10 µM) or were left untreated. Fresh medium with or without FTI-277 or GGTI-298 and containing IL-1β at 0, 1, 3, or 10 ng/ml was then added. After a further 24 h, the medium was harvested and assayed for nitrites. The curves show the response to IL-1β of untreated cells (●) or cells treated with FTI-277 (○) or GGTI-298 (□). The data are representative of two (A) and four (B) independent experiments.
FTI-277 and GGTI-298 Inhibit Selectively the Processing of Ras and Rap1A, Respectively, in RPASMC—Recently, we have designed and synthesized small organic molecules that mimic the carboxyl-terminal tetrapeptide CAAX of proteins that are farnesylated or geranylgeranylated. These peptidomimetics are potent and selective inhibitors of FTase and GGTase I in smooth muscle cells and are therefore efficacious in inhibiting cellular processing of farnesylated and geranylgeranylated proteins, respectively.

RESULTS AND DISCUSSION

Inhibition of Protein Geranylgeranylation Causes Superinduction of NOS-2 Protein and Nitrite Levels by IL-1β in RPASMC and Rat Hepatocytes—To determine the role of geranylgeranylated proteins in the signal transduction pathways that mediate IL-1β induction of NOS-2, we treated RPASMC and rat hepatocytes with the GGTase I inhibitor GGTI-298 prior to treatment with IL-1β as described under “Experimental Procedures.” Fig. 1B shows that in the absence of inhibitors, IL-1β (0–10 ng/ml) induced a concentration-dependent, but modest, increase in the medium levels of nitrite from 1 μM basal levels to 12 μM after treatment with 10 ng/ml IL-1β. Pretreatment with GGTI-298 (10 μM) caused a dramatic increase in IL-1β-induced nitrite formation (from 4 to 52 μM). The medium of cells treated with 1 ng/ml IL-1β alone accumulated nitrite levels of 4 μM, whereas the medium of cells treated with GGTI-298 prior to IL-1β accumulated levels of nitrites that were more than 10-fold higher (42 μM) (Fig. 1B).
The dramatic increase in the level of nitrite brought about by GGTI-298 could be due to direct activation of NOS-2 enzymatic activity or superinduction of NOS-2 protein. To determine the effects of GGTI-298 on NOS-2 protein levels, RPASMC were treated with GGTI-298 for 24 h and then stimulated with IL-1β for a further 24 h as described under “Experimental Procedures.” The cells were harvested, and the lysates were analyzed by Western blotting using a specific anti-NOS-2 antibody. Fig. 2A shows that in the absence of GGTI-298, significant induction of NOS-2 protein levels in RPASMC occurred only at 10 ng/ml IL-1β, whereas in the presence of inhibitor, NOS-2 was induced at concentrations as low as 1 ng/ml. In RPASMC treated with 10 ng/ml IL-1β, GGTI-298 enhanced the ability of IL-1β to induce NOS-2 protein by 5-fold. A similar effect was demonstrated in hepatocytes (Fig. 2B).

We next used an alternative method to demonstrate the involvement of geranylgeranylated proteins in the regulation of NOS-2 induction by IL-1β. Recently, we have shown that treatment of NIH-3T3 cells with lovastatin inhibited the processing of both farnesylated and geranylgeranylated proteins, with a much more pronounced effect on geranylgeranylated proteins, but that treatment of these cells with a combination of lovastatin and either geranylgeranyl or farnesol restored protein geranylgeranylation or farnesylation, respectively (43). Applying this strategy, we observed that RPASMC treated with lovastatin alone were much more responsive to IL-1β in inducing NOS-2 (Fig. 3). This lovastatin-mediated superinduction of IL-1β-stimulated NOS-2 was reversed by geranylgeraniol, but not by farnesol. Lovastatin inhibition of the processing of Ras and of the geranylgeranylated protein Rap1A in RPASMC was reversed with farnesol and geranylgeraniol, respectively (Fig. 3). The above results confirmed that inhibition of protein geranylgeranylation is responsible for the hypersensitivity of RPASMC to IL-1β induction of NOS-2. The results also suggest that inhibition of protein farnesylation is not involved in the superinduction of NOS-2 by IL-1β.

FTI-277 Blocks IL-1β Induction of NOS-2 in RPASMC and Rat Hepatocytes—The above results demonstrated that inhibition of protein geranylgeranylation causes a superinduction of NOS-2 by IL-1β. We next determined the consequences of inhibiting protein farnesylation. RPASMC were pretreated for 24 h with FTI-277 prior to a 24-h treatment with various concentrations of IL-1β (0–10 ng/ml). The cells were then harvested, and the levels of NOS-2 protein as well as the cumulative levels of nitrates in the cell-conditioned medium were determined as described under “Experimental Procedures.” Fig. 4A shows that pretreatment of RPASMC with FTI-277 (10 μM) blocked IL-1β induction of NOS-2 protein. Hepatocytes were treated similarly, but with slightly higher IL-1β levels (0–50 ng/ml). Fig. 4B shows a similar effect in the hepatocytes, although the effect was not as complete.

GGTI-298 Enhances, whereas FTI-277 Blocks, the Ability of IL-1β to Induce NOS-2 mRNA—We next determined whether the effects of GGTI-298 and FTI-277 on NOS-2 protein and nitrite levels were due to alterations at the mRNA level. RPASMC were treated with vehicle, IL-1β, FTI-277, GGTI-298, and IL-1β with either FTI-277 or GGTI-298, and the levels of NOS-2 mRNA were determined by Northern blotting as described under “Experimental Procedures.” Stimulation of cells with IL-1β showed a modest induction of NOS-2 mRNA (Fig. 5, lanes 1–3), whereas treatment of cells with FTI-277 or GGTI-298 alone had no detectable effect (lanes 4 and 7). Treatment of cells with FTI-277 blocked the ability of IL-1β to induce NOS-2 mRNA (compare lanes 1–3 with lanes 4–6). In contrast, GGTI-298 enhanced the ability of IL-1β to induce NOS-2 by 6- and 14-fold at 10 and 1 ng/ml IL-1β, respectively (compare lanes 1–3 with lanes 7–9). Cyclopentbin mRNA levels were not affected by the treatments and were similar in all lanes of Fig. 5.

The data presented in this report clearly demonstrate that IL-1β induction of NOS-2 in RPASMC and rat hepatocytes is under the tight control of farnesylated and geranylgeranylated proteins. Whereas inhibition of protein farnesylation blocked NOS-2 expression, inhibition of protein geranylgeranylation had the opposite effect of dramatically enhancing NOS-2 expression. The results suggest that a farnesylated protein mediates IL-1β induction of NOS-2, whereas a geranylgeranylated protein represses this induction. A potential candidate for mediating IL-1β induction of NOS-2 is the Ras protein. This suggestion is consistent with the recent report by Singh et al. (40) that showed that IL-1β activates the MAP kinase pathway and induces NOS-2 in cardiac myocytes and that this can be blocked by the FTase inhibitor BZA-5B. Therefore, Ras proteins that are farnesylated and are known to activate the MAP kinase pathway are good candidates for mediating IL-1β induction of NOS-2. Among geranylgeranylated proteins that can serve to antagonize IL-1β induction of NOS-2 are the small G proteins Rho and Rac. Although the involvement of these proteins in growth factor signaling is now well documented, their role in cytokine signaling is less clear. It is plausible that cytokines such as IL-1β could activate both the Ras/MAP kinase (7–10) and the Rho/Rac/stress-activated protein kinase (14, 15) pathways. Integration of the MAP kinase and stress-activated protein kinase pathways in smooth muscle cells and hepatocytes would result in the net outcome of induction of NOS-2 expression. Inhibition of the stress-activated protein kinase pathway that depends on the geranylgeranylated RhoA and Rac proteins would tip the equilibrium in the direction of releasing the repressing pathways and superinducing NOS-2. On the other hand, inhibition of the MAP kinase pathways that depend on farnesylated proteins such as Ras would block NOS-2 induction. Regardless of the mechanism by which inhibition of protein geranylgeranylation causes superinduction of NOS-2, the consequences of this novel finding are of great therapeutic potential. We are presently evaluating the potential of GGTI-298 to reverse in animal models intimal hyperplasia associated with restenosis and atherosclerosis. Our ultimate goal is to prevent local hyperplasia that compromises the success of angioplasty and surgical bypass for obstructive vascular lesions.

Acknowledgments—We thank Dr. Timothy Billiar and Debbie Williams for providing the hepatocyte cultures.

REFERENCES

1. Schmidt, H. H. H., and Walter, U. (1994) Cell 78, 919–925
2. Morris, S. M., Jr., and Billiar, T. R. (1994) Am. J. Physiol. 266, E829–E839
3. Stahoe, C. (1995) Neu Horizons 3, 5–32
4. Dinarello, C. (1994) FASEB J. 3, 1013–1025
5. Nakayama, D. K., Geller, D. A., Lowenstein, J. C., Chen, H. D., Davies, P., Pitt, B. R., Simmons, R. L., and Billiar, T. R. (1992) Am. J. Physiol. 268, H757–H765
6. O’Neill, L. A. J. (1995) Biochim. Biophys. Acta 1266, 31–44
7. Ahlers, A., Belka, C., Gestel, M., Lamping, N., Scott, C., Herrmann, F., and Brach, M. A. (1994) Mol. Pharmacol. 46, 1077–1083
8. Bird, T. A., Sleath, P., deBoos, P. C., Dower, S. K., and Virca, G. D. (1991) J. Biol. Chem. 266, 22661–22670
9. Gould, G. W., Cuenda, A., Thomson, F. J., and Cohen, P. (1995) Biochem. J. 311, 735–738
10. Guy, G. R., Chau, S. P., Wong, N. S., Ng, S. B., and Tan, Y. H. (1991) J. Biol. Chem. 266, 2534–2542
11. Guedon, P., Frenshney, N., Waller, R. J., Rawlinson, L., and Saklatvala, J. (1993) J. Biol. Chem. 268, 4236–4243
12. Saklatvala, J., Rawlinson, L. M., Marshall, C. J., and Kracht, M. (1993) FEBS Lett. 334, 189–192
13. Hallberg, B., Rayter, S. I., and Downward, J. (1994) J. Biol. Chem. 269, 3913–3916
14. Bird, T. A., Kyrakis, J. M., Tysialer, L., Gayle, M., Milne, A., and Virca, G. D. (1994) J. Biol. Chem. 269, 31836–31844
15. Kracht, M., Truong, O., Totty, N. F., Shirou, M., and Saklatvala, J. (1994) J. Biol. Chem. 269, 2017–2026
16. Frenshney, N. R., Rawlinson, L., Guedon, F., Jones, E., Cowley, S., and
17. Marshall, M. S. (1995) *FASEB J.* 9, 1311–1318
18. Gutierrez, L., Magee, A., Marshall, C., and Hancock, J. (1989) *EMBO J.* 8, 1093–1098
19. Reiss, Y., Goldstein, J., Seabra, M., Casey, P., and Brown, M. (1990) *Cell* 62, 81–88
20. Manne, V., Roberts, D., Tabin, C., O'Rourke, E., De Vigilio, M., Meyers, C., Ahmed, N., Kurz, B., Resh, M., Kung, H.-F., and Barbacid, M. (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 7541–7545
21. Moomaw, J., and Casey, P. (1992) *J. Biol. Chem.* 267, 17438–17443
22. James, G., Goldstein, J., and Brown, M. (1995) *FASEB J.* 9, 1311–1318
23. Casey, P. J., Moomaw, J. F., Zhang, F. L., Higgins, Y. B., and Thissen, J. A. (1994) *Recent Prog. Horm. Res.* 49, 215–238
24. Lerner, E. C., Qian, Y., Blaskovich, M. A., Fossum, R. D., Vogt, A., Sun, J., Cox, A. D., Der, C. J., Hamilton, A. D., and Sebti, S. M. (1995) *J. Biol. Chem.* 270, 26770–26773
25. Qian, Y., Vogt, A., Sebti, S. M., and Hamilton, A. D. (1996) *J. Med. Chem.* 39, 217–223
26. Bernhard, E. S., Kao, G., Cox, A. D., Sebti, S. M., Hamilton, A. D., Muschel, R. J., and McKenna, W. G. (1996) *Cancer Res.* 55, 4243–4247
27. Singh, K., Balligand, J. L., Fischer, T. A., Smith, T. W., and Kelly, R. A. (1996) *J. Biol. Chem.* 271, 1111–1117
28. Geller, D. A., Nussler, A. K., DiSilvio, M., Lowenstein, C. J., Shaprio, R. A., Wang, S. C., Simmons, R. L., and Billiar, T. R. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 522–526
29. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* 162, 156–159
30. McGuire, T. M., and Sebti, S. M. (1997) *Oncogene* 14, 305–312
31. James, G. L., Goldstein, J. L., Brown, M. S., Rawson, T. E., Somers, T. C., McDowell, R. S., Crowley, C. W., Lucas, B. K., Levinson, A. D., and Marsters, J. C., Jr. (1993) *Science* 260, 1937–1942
32. Garcia, A. M., Rowell, C., Ackermann, K., Kowalczyk, J., and Lewis, M. D. (1993) *J. Biol. Chem.* 268, 18415–18418
33. Patel, D. V., Gordon, E. M., Schmidt, R. J., Weller, H. N., Young, M. G., Zahler, R., Barbacid, M., Carboni, J. M., Gullo-Brown, J. L., Hunihan, L., Rice, C., Robinson, S., Seizinger, B. R., Tuomari, A. V., and Manne, V. (1995) *J. Med. Chem.* 38, 435–442
34. Bishop, W. R., Bond, R., Petrin, J., Wang, L., Patton, R., Doll, R., Njoroge, G., Catino, J., Schwartz, J., Windsor, W., Syto, R., Carr, D., James, L., and Kirschmeier, P. (1995) *J. Biol. Chem.* 270, 30611–30618
35. Lerner, E. C., Qian, Y., Hamilton, A. D., and Sebti, S. M. (1995) *J. Biol. Chem.* 270, 26770–26773
36. Vogt, A., Qian, Y., McGuire, T. F., Hamilton, A. D., and Sebti, S. M. (1996) *Oncogene* 15, 1991–1999
37. McGuire, T. F., Qian, Y., Hamilton, A. D., and Sebti, S. M. (1996) *J. Biol. Chem.* 271, 27402–27407
38. McGuire, T. F., Qian, Y., Blaskovich, M. A., Fossum, R. D., Sun, J., Marlowe, T., Corey, S. J., Wathen, S. P., Vogt, A., Hamilton, A. D., and Sebti, S. M. (1995) *Biochem. Biophys. Res. Commun.* 214, 305–312
39. James, G. L., Brown, M. S., Cobb, M. H., and Goldstein, J. L. (1994) *J. Biol. Chem.* 269, 27705–27714
40. Singh, K., Balligand, J. L., Fischer, T. A., Smith, T. W., and Kelly, R. A. (1996) *J. Biol. Chem.* 271, 1111–1117
41. Geller, D. A., Nussler, A. K., DiSilvio, M., Lowenstein, C. J., Shaprio, R. A., Wang, S. C., Simmons, R. L., and Billiar, T. R. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 522–526
42. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* 162, 156–159
43. McGuire, T. M., and Sebti, S. M. (1997) *Oncogene* 14, 305–312
Inhibition of Protein Geranylgeranylation Causes a Superinduction of Nitric-oxide Synthase-2 by Interleukin-1 β in Vascular Smooth Muscle Cells
Jonathan D. Finder, Jennifer L. Litz, Michelle A. Blaskovich, Terence F. McGuire, Yimin Qian, Andrew D. Hamilton, Paul Davies and Said M. Sebti

J. Biol. Chem. 1997, 272:13484-13488.
doi: 10.1074/jbc.272.21.13484

Access the most updated version of this article at http://www.jbc.org/content/272/21/13484

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 43 references, 24 of which can be accessed free at http://www.jbc.org/content/272/21/13484.full.html#ref-list-1