[Frontiers in Bioscience 13, 2909-2915, January 1, 2008]

Vascular effects of thrombin: Involvement of NOR-1 in thrombin-induced mitogenic stimulus in vascular cells

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1. ABSTRACT

Neuron-derived orphan receptor-1 (NOR-1) is a nuclear receptor recently involved in the onset and development of atherosclerosis. NOR-1 is induced in a cell-specific manner by extracellular stimuli. NOR-1 is over-expressed in human atherosclerotic plaques and in porcine arteries subjected to angioplasty, is induced by growth factors in vascular cells and it has been involved in cell migration and proliferation. This article examines the mechanisms that regulate NOR-1 in vascular cells and the effects of NOR-1 knockdown on cell growth induced by mitogens, in particular thrombin. Mitogenic stimuli up-regulates NOR-1 in endothelial cells (ECs) through multiple pathways, including increase of cytosolic calcium, activation of protein kinase C, mitogen-activated protein kinase (MAPK) (ERK1/2 and p38 MAPK) and downstream activation of cAMP response element binding protein (CREB). Inhibition of protease-activated receptor-1 (PAR-1) abolished thrombin-induced NOR-1 up-regulation and DNA synthesis. NOR-1 knockdown reduces DNA synthesis and EC re-growth in an in vitro model of wound repair. NOR-1 could be regarded as a new target to prevent endothelial effects triggered by thrombin and other mitogens.

2. INTRODUCTION

Endothelial cell (EC) growth is critical in different processes including endothelial repair at sites of spontaneous or iatrogenic disruption and in the formation of new vessels (neovascularization). In these processes ECs migrate and proliferate as a result of the mitogenic stimulus triggered by growth factors and cytokines. This involves the coordinately regulation of multiple genes by a set of transcription factors that control cell cycle entry and other EC functions (1). Recently, we have identified NOR-1 as an early-response gene in VSMCs and ECs (2-5). NOR-1, together with Nur77 and Nurr1, form the NR4A subfamily of orphan nuclear receptors (NRs). NRs of this subfamily are transcription factors regulated in a cell-specific manner by extracellular stimuli (6). These genes have emerged as potentially relevant players in the complex network of proteins that regulate vascular cell activation in inflammation and atherogenesis (2-9).

In recent years different studies point to thrombin as a key multifunctional serine protease, that besides its well known role in the blood coagulation cascade, activates

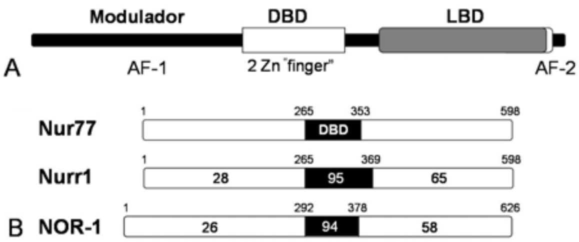

Figure 1. Structure of NRs and schematic representation of NR4A genes. (A) Typical modular structure of NRs composed by a variable N-terminal region containing the ligand-independent activation function-1 (AF-1); a central DNA-binding domain (DBD) and a variable linker region that connects to the C-terminus containing the ligand-binding domain (LBD). (B) Amino acid sequence alignment of NR4A genes and the percent of amino acid identity with the corresponding regions of Nur77.

PARs modulating vascular function (10). PAR-1, the predominant receptor of thrombin in vascular cells, elicits a variety of responses including regulation of vascular tone, cell migration and proliferation and angiogenesis (10). In this paper we analyze the molecular mechanisms underlying thrombin-induced EC activation and the role of PAR-1 and NOR-1 in this process.

3. NOR-1 IN THE VASCULAR WALL

3.1. Structure of NOR-1 and regulation of transcriptional activity

NOR-1 belongs to the NR4A subfamily of NRs that consists of three closely related members: Nur77, firstly identified as a gene induced by nerve growth factor (NGF) in the pheocromocytoma cell line PC12 (11); NOR-1, identified by Ohkura *et al.* (12) in forebrain neural cells undergoing apoptosis, and Nurr1 firstly characterized as a "brain-specific" transcription factor in dopaminergic neurons (13). These receptors share the typical modular structure of NRs composed by several functional domains, as follows: a variable N-terminal region, containing the ligand-independent activation function-1 (AF-1), responsible for interaction with other transcription factors; a central DNA-binding domain (DBD), and a variable linker region that connects the DBD to the C-terminus containing the ligand-binding domain (LBD) (Figure 1A). The most divergent domain in NR4A genes is the N-terminal domain, which could lead to significant qualitative or quantitative differences among them (Figure 1B). Although members of the NR4A subfamily have structural features of ligand-activated transcription factors, recent findings suggest that they do not require ligand binding for activation (14). The transcriptional activity of these genes is largely regulated by extracellular stimuli, which determine their expression level and modulates posttranslational modifications (6).

3.2. Regulation of NOR-1 by extracellular stimuli in vascular cells

3.2.1. Regulation of NOR-1 in VSMC

NOR-1 is an immediate-early response gene strongly induced by growth factors in VSMCs (2-4). Several growth factors commonly involved in atherogenesis, including platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and thrombin significantly induce NOR-1 in human VSMCs. In addition, NOR-1 mRNA levels are up-regulated by compounds that activate specific signalling pathways, such as phorbol-12-myristate-13-acetate (PMA; a PKC activator), A23187 (a calcium ionophore), forskolin (an adenylyl cyclase activator) and the cAMP analogue 8-Br-cAMP. However, the strongest inducers are serum and native low-density lipoproteins (LDL) (2-4), agents that contain bioactive molecules able to activate several cell signalling pathways leading to NOR-1 up-regulation. LDL-induced NOR-1 expression is mediated by early intracellular events, including increase of $[Ca^{2+}]_i$ and activation of PKC and MAPK pathways (ERK1/2 and p38 MAPK) (4), via a pertussis toxin (PTX)-sensitive mechanism independent of the classic LDL receptor (3).

3.2.2. Regulation of NOR-1 in endothelial cells

NOR-1 is strikingly induced by growth factors in ECs. In fact, it has been identified as one of the most strongly induced genes by vascular endothelial growth factor (VEGF) in these cells (8). This effect is produced via VEGF receptor-2 (VEGFR-2), also known as kinase insert domain-containing receptor (KDR), the main receptor mediating VEGF actions in ECs. NOR-1 up-regulation by VEGF is sensitive to ERK1/2, $[Ca^{2+}]_i$, PKC and calcineurin inhibition (5,8). By contrast, VEGF-induced expression of NOR-1 is insensitive to rapamycin and to LY2940002, an inhibitor of phosphatidyl-inositol-3-kinase (PI3K) (8). The signalling mechanisms mediating VEGF regulation of NOR-1 diverge from those responsible for expression of other VEGF-induced genes at level of calcineurin. Interestingly, cyclosporine A, an immunosuppressor drug that acts as a specific inhibitor of calcineurin and potently blocks angiogenesis, inhibited the expression of the three NR4A genes (8). The transcriptional activation of NOR-1 promoted by VEGF seems to be mediated by the nuclear factor of activated T cells (NFAT), a transcription factor that seems to be critical in Ca^{2+}/calcineurin-dependent vascular functions (15), and by CREB (5).

3.3. Modulation of NOR-1 transcription by CREB in vascular cells

The transcription of NOR-1 is highly dependent on CREB. NOR-1 promoter contains three CRE elements near its transcriptional start site that are critical for its transcriptional activation as we and others have extensively analyzed in VSMC and ECs (2-5,9). In these cells CREB activation seems to be a common output for the signal transduction pathways involved in NOR-1 induction. Stimuli that activate CREB, via phosphorylation in Ser^{133}, such as serum, PDGF, thrombin, LDL or VEGF strongly induce NOR-1 (2-5,9). By contrast, compounds that interfere with signalling pathways upstream of CREB, such as PKC inhibitors or calcium chelators, inhibit CREB phosphoylation and prevent NOR-1 up-regulation. CREB-mediated NOR-1 up-regulation is in agreement with reports that associate CREB activation with proliferation of vascular cells (16,17), likely as a result of its active role

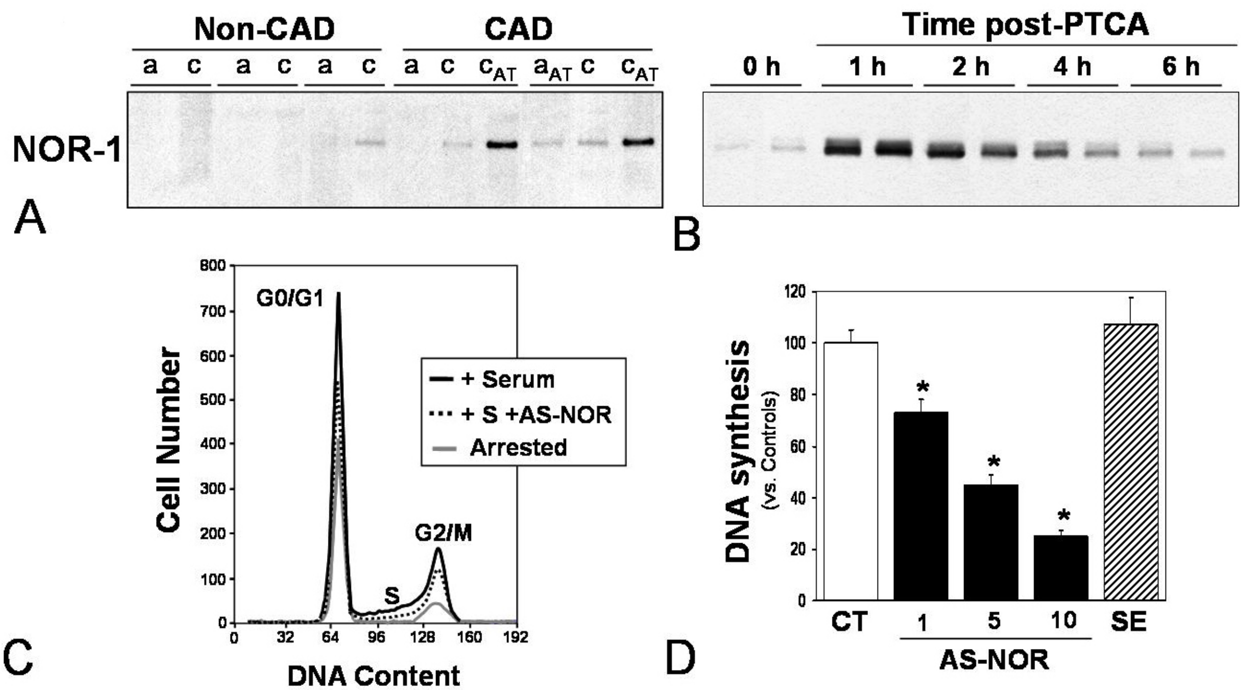

Figure 2. NOR-1 in the vascular wall. (A) Expression of NOR-1 in human arteries from patients with and without coronary artery disease (CAD). Human aorta and coronary arteries were obtained from explanted hearts, from patients with dilated idiopathic cardiomyopathy (Non-CAD) or CAD. Coronary arteries were examined under low magnification and classified in two categories: areas with atherosclerotic lesions and non-atherosclerotic areas. NOR-1 mRNA levels were analyzed by RT-PCR using primers described previously (reference 2) that amplify a PCR product 1081 bp in length. [intima/media from aorta (a) or coronary arteries (c) without atherosclerotic lesions; intima/media from atherosclerotic aorta (a_{AT}) or atherosclerotic coronary arteries (c_{AT})]; (B) Up-regulation of NOR-1 in porcine coronary arteries subjected to percutaneous transluminal coronary angioplasty (PTCA). Coronary lesions were performed by three inflations of 8 atmospheres of a 4.0 × 20.0 mm balloon catheter. Time intervals of study from angioplasty to animal sacrifice were 1, 2, 4 and 6 h (*n* =20). This angioplasty procedure resulted in over-stretching of the artery, causing severe injury. NOR-1 mRNA levels were analyzed by RT-PCR. (C) Representative flow cytometry analysis profiles from arrested human VSMC and cells stimulated with 10% human serum in the absence or presence of antisense ODNs against NOR-1 (AS-NOR, 10 μM) (n=3 experiments performed in quadruplicate). (D) DNA synthesis ([^{3}H]thymidine incorporation) in cells stimulated with human serum in the absence (CT) or presence of increasing concentrations of AS-NOR (μM). The effect of the corresponding sense ODNs (SE) is shown (n=4 experiments performed in triplicate). Results are expressed as mean±SEM. Multiple groups were compared by the one factor ANOVA, followed by Fisher PLSD to asses specific group differences. $p<0.05$: *, *vs.* controls (CT, cells treated with serum alone). For a more detailed description of methodology see reference 2.

regulating the expression of several cyclins and cyclin-dependent kinases involved in cell cycle progression.

3.4. Pathophysiological roles of NOR-1 in the vascular wall

NOR-1 as well as Nur77 and Nurr1 have been involved in different cell processes including apoptosis (18), cell differentiation (19) and proliferation (2-5,9). Interestingly, all three genes have been described in human atherosclerotic lesions (2,7), but conflicting results have been reported regarding their role in vascular cell proliferation. Indeed, recent results suggest that NR4A genes could play opposite roles in vascular cell proliferation. NOR-1 inhibition or genetic ablation of NOR-1 reduces the proliferation of vascular cells (2-5,9) while Nur77 over-expression inhibited cell proliferation (7). Recently, we showed that NOR-1 is up-regulated in active human coronary atherosclerotic lesions (Figure 2A) and is strongly induced in porcine arteries (both coronaries and carotids) subjected to angioplasty, a mechanical injury process that induces the expression of genes associated with VSMC activation and proliferation such as c-*fos*, c-*myc* or c-*myb* (20) (Figure 2B). In addition, NOR-1 inhibition by antisense oligonucleotides (ODNs) or small interference RNA (siRNA) significantly prevented proliferation and wound healing induced by growth factors (serum) in VSMC (Figure 2C and D) (2) and by cytokines (VEGF) in ECs (5). Finally, inhibition of VSMC proliferation by HMG-CoA reductase inhibitors (statins) is associated with the inhibition of NOR-1 up-regulation by a RhoA/Rho-associated kinase [ROCK]-dependent mechanism (4).

4. MODULATION OF ENDOTHELIAL FUNCTION BY THROMBIN

4.1. Endothelial functions modulated by thrombin via PAR-1

Thrombin is a multifunctional serine protease that activates blood platelets and elicits multiple effects on a

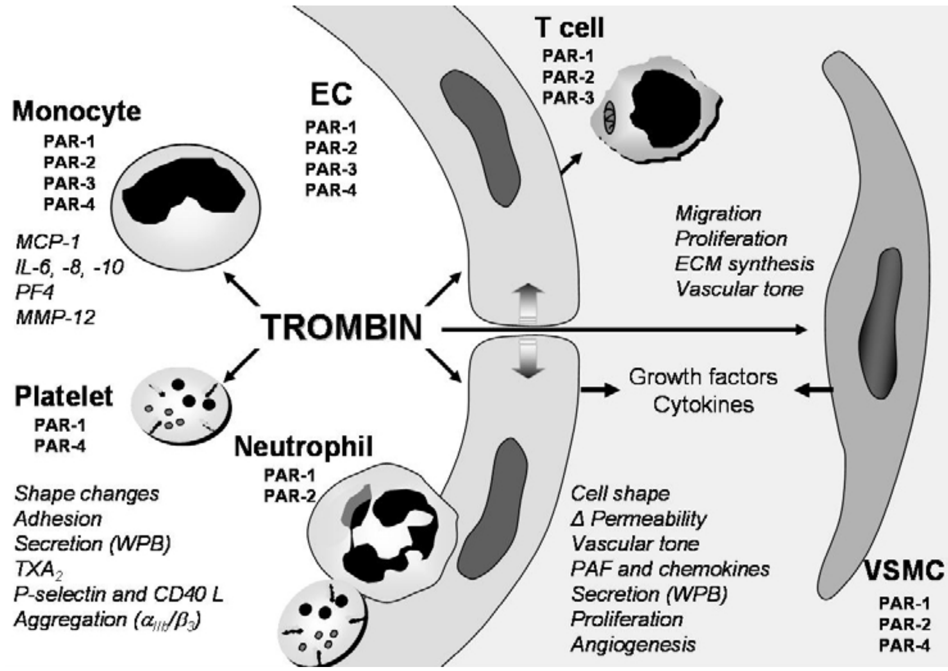

Figure 3. Thrombin's cellular effects in the vascular wall. Thrombin activates PARs and elicits multiple effects in cells involved in atherogenesis. The presence of PARs (PAR-1, -2, -3 and -4) in platelets, ECs, VSMC, monocytes and T cells as well as the main effects of thrombin on these cells are indicated.

variety of cell types among them ECs (10,21) (Figure 3). Cellular effects of thrombin are mediated by PARs, members of the G protein-coupled receptors (GPCRs) that carry their own ligand which remain cryptic until unmasked by proteolytic cleavage. Four PARs have been described: PAR-1, -2, -3 and -4. Thrombin seems to be the main physiological activator of PAR-1 and PAR-4, but it can also activate PAR-3, that functions as a cofactor for PAR-4, and is not considered to directly elicit intracellular signalling (22). Finally, although PAR-2 is not directly activated by thrombin it has been reported that it can be transactivated by thrombin-cleaved PAR-1 (23). In healthy arteries PARs are mainly expressed in ECs, in particular PAR-1, the main receptor involved in thrombin-mediated effect in ECs. PAR-1 participates in the regulation of vascular tone, vascular permeability, endothelial secretory activity, EC proliferation and angiogenesis (10,21). Indeed, the effect of thrombin on different stages of angiogenesis has focused the interest of many investigators in the last years. Thrombin inhibits in a dose-dependent manner EC adhesion, an early event in the angiogenic cascade, through a PAR-1 dependent mechanism (24). In addition, thrombin activates progelatinase A (matrix metalloproteinase-2 [MMP-2]), which contributes to the local dissolution of basement membrane facilitating migration of activated ECs (25). PAR-1 has also been involved in thrombin-induced VEGF up-regulation and EC proliferation (26,27). Furthermore, different studies have reported that thrombin is able to up-regulate hypoxia-inducible factor 1 alpha (HIF-1α) in vascular cells under non-hypoxic conditions in a reactive oxygen species-dependent manner (27,28). To modulate these endothelial functions thrombin alters the expression of an array of genes. Indeed, thrombin up-regulates the expression of genes related to haemostasis (tissue factor [TF], plasminogen activator inhibitor-1 [PAI-1]), cell growth and angiogenesis (angiopoietin, VEGF, PDGF), cell adhesion (vascular cell adhesion molecule-1 [VCAM-1], intercellular adhesion molecule-1 [ICAM-1], E-selectin) and different cytokines and chemokines (interleukin-6 [IL-6] and IL-8, monocyte chemoattractant protein-1 [MCP-1]) (Figure 3) (10,21,24).

4.2. NOR-1 is induced by thrombin and mediates endothelial cell growth

4.2.1. Thrombin induces NOR-1 in endothelial cells via PAR-1

Thrombin up-regulates NOR-1 expression with a similar potency to serum or VEGF. In human ECs thrombin induces NOR-1 expression in a dose- and time-dependent manner (29). The effect is observed at doses as low as 0.1 U/mL and it is maximal 1 hour after induction. The induction of NOR-1 by thrombin is dependent on PAR-1, thrombin receptor that mediates main biologic responses triggered by thrombin in vascular cells including cell proliferation (10,21). Indeed, similarly thrombin-receptor activator peptide-6 (TRAP-6), a PAR-1 agonist, increases NOR-1 mRNA levels in a time- and dose-dependent manner, while a PAR-1 blocking antibody (ATAP-2) (30) prevents such effect (29). NOR-1 up-regulation by thrombin is mediated by several intracellular pathways, including calcium mobilization and activation of PKC and MAPK pathways (ERK1/2 and p38 MAPKs). PTX significantly reduces the induction of NOR-1 produced by thrombin, according with the nature of PAR-1 as a GPCR that signals, at least in a part, through Galphai proteins. Thrombin-induced NOR-1 up-regulation is also reduced by

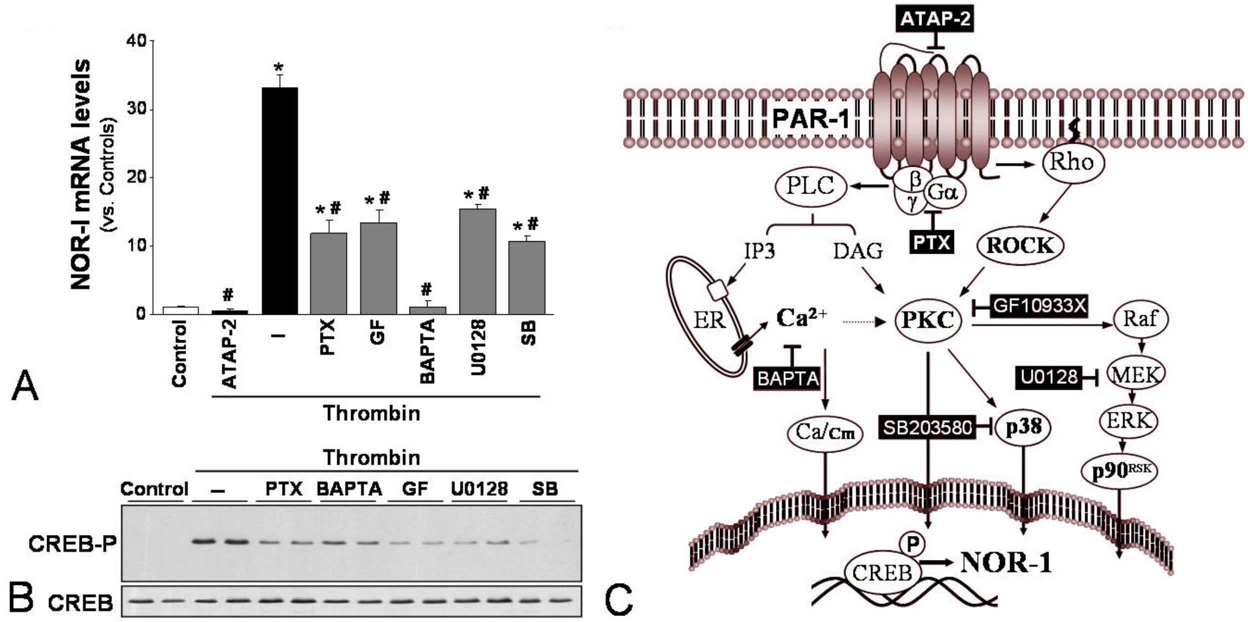

Figure 4. Signalling pathways involved in thrombin-induced NOR-1 expression in ECs. (A) NOR-1 expression, analyzed by real-time PCR, in human umbilical vein endothelial cells (HUVEC) induced with thrombin (10 U/mL) in the presence of different inhibitors: ATAP-2 (a protease-activated receptor-1 [PAR-1] blocking antibody; 20 μg/mL), PTX (pertussis toxin; an inhibitor of Galpha$_{i/0}$ proteins; 50 ng/mL), GF10933X (GF, a protein kinase C [PKC] inhibitor; 5 μM), BAPTA-AM (BAPTA, a calcium chelator; 10 μM), U0128 (a MAP kinase kinase (MEK) inhibitor; 10 μM) and SB203580 (SB, a p38 mitogen-activated protein kinase [MAPK] inhibitor; 5 μM). $p<0.05$: *, *vs.* control (arrested cells); #, *vs.* cells treated with thrombin alone. (B) Representative Western blot analysis showing the activation of cAMP response element binding protein (CREB) (CREB-P, phosphorylation in Ser^{133}) induced by thrombin and the inhibitory effect produced by inhibitors of different pathways (as indicated in A). Total CREB protein levels were used as a loading control (n=3 experiments performed in duplicate). (C) Schematic representation of signalling pathways involved in CREB activation and NOR-1 up-regulation by thrombin via PAR-1. The inhibition by specific agents (black boxes) is indicated. Calcium/calmodulin (CA/Cm), p38 MAPK (p38 in the picture) and p90^{RSK} are kinases potentially involved in CREB activation. For a more detailed description of methodology see reference 29.

BAPTA-AM (a calcium chelator), GF10933X (a PKC inhibitor), U0128 (a MEK inhibitor) and SB203580 (a p38 MAPK inhibitor) (Figure 4A). These pathways are similar to those involved in NOR-1 induction by growth factors in VSMC and by VEGF in ECs (2-5).

4.2.2. CREB activation is critical in NOR-1 induction by thrombin

The up-regulation of NOR-1 by thrombin is associated to the ability of this protease to promote CREB activation (29). Those inhibitors that reduced NOR-1 up-regulation induced by thrombin (PTX, BAPTA-AM, GF10933X, U0128 and SB203580) also significantly prevents CREB activation (phosphorylation in Ser133) (Figure 4B). In addition, TRAP-6 that mimics the effect of thrombin on NOR-1 expression also promotes CREB activation, while ATAP-2 prevents such effect. Finally, site-directed mutagenesis of the two CRE sites located at -79 and -53 bp respectively upstream the transcription star-site, or co-transfection with a CREB dominant-negative (CREB mutated in Ser133), abolish thrombin-induced NOR-1 promoter activity. The signalling pathways leading to NOR-1 up-regulation by thrombin in ECs are shown in figure 4C.

4.2.3. NOR-1 modulates thrombin-induced DNA synthesis and cell growth

NOR-1 seems to play a key role as a transcription factor involved in thrombin-induced EC mitogenesis and re-endothelization following a mechanical injury (29). Certainly, specific inhibition of NOR-1 expression with antisense ODNs (AS-NOR-1) or siRNA (siRNA/NOR-1) significantly prevents thrombin-induced DNA synthesis (Figure 5A). The inhibition of NOR-1 expression by these means is as efficient as the direct blockage of PAR-1 (using ATAP-2) preventing thrombin-induced EC DNA synthesis. In addition, inhibition of NOR-1 expression, transfecting ECs with siRNA/NOR-1, prevents thrombin-induced EC re-growth in an *in vitro* model of wound repair (Figure 5B). These results are in agreement with findings suggesting a prominent role of this orphan receptor in VSMC growth using similar approaches (2-5).

5. SUMMARY AND PERSPECTIVES

Thrombin induces NOR-1 expression in ECs through a mechanism dependent on PAR-1 that involves different signalling pathways leading to CREB activation. The direct inhibition of NOR-1 expression prevented endothelial cell growth induced by thrombin and other

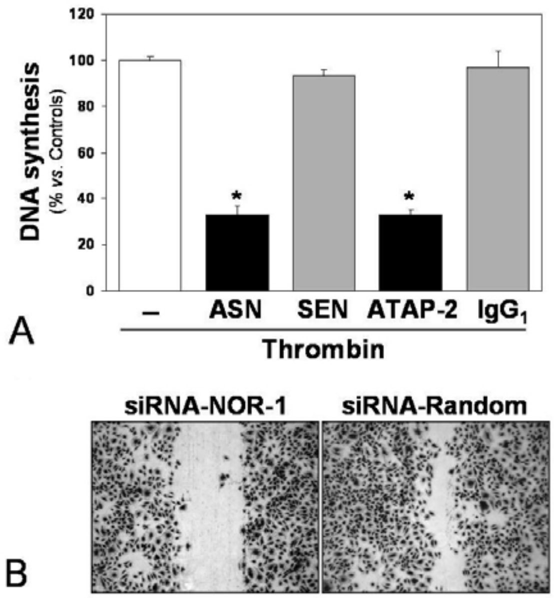

Figure 5. PAR-1 and NOR-1 are involved in thrombin-induced migration and proliferation in ECs. (A) DNA synthesis ([3H]thymidine incorporation) by ECs induced by thrombin was significantly inhibited by either antisense ODNs against NOR-1 (ASN) or antibodies that block PAR-1 (ATAP-2). No effects were observed in cells treated with sense ODNs (SEN) or unspecific IgG1. $p<0.05$: *, vs. controls (cells treated with thrombin alone). siRNA targeting NOR-1 (NR4A3 ID #41668, 1 mM) or Silencer™ Pre-designed siRNA from Ambion™ were used. (n=3 experiments performed in quadruplicate). (B) HUVEC transfected with siRNA against NOR-1 (siRNA-NOR-1) or control siRNA (siRNA-Random) were arrested, injured with a scraper and induced with thrombin (5 U/mL). Forty-eight hours later cells in the damage zone were quantified. siRNA-NOR-1 significantly inhibited endothelial re-growth in this in vitro model of would healing. (n=2 experiments performed in quintuple). For a more detailed description of methodology see reference 29.

mitogens, suggesting that NOR-1 could be a key CREB targeted transcription factor regulating the endothelial cell proliferative response. NOR-1 has emerged as a new player in the complex network of transcription factors that regulate EC growth. Since EC proliferation is a key event in the angiogenic response associated to pathologic processes such as atherosclerosis and cancer, NOR-1 could be regarded as a potential therapeutic target in strategies aimed to prevent cell proliferation.

6. ACKNOWLEDGMENTS

This work was partially supported by grants from Red Temática de Investigación Cardiovascular (RECAVA) (RD06/0014/0027) and funds provided by Ministerio de Sanidad y Consumo-Instituto de Salud Carlos III (FIS PI061480) and the Ministerio de Educación y Ciencia (SAF 2006-07378 and SAF 2006-10091) and CSIC-Intramural 200620I054. M. Gentile is a recipient of a research fellowship (FI) from Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) de la Generalitat de Catalunya.

7. REFERENCES

1.Carmeliet P.: Angiogenesis in health and disease. *Nat Med*, 9, 653-660 (2003)
2. Martínez-González J., J. Rius , A. Castello, C. Cases-Langhoff & L. Badimon: Neuron-derived orphan receptor-1 (NOR-1) modulates vascular smooth muscle cell proliferation. *Circ Res*, 92, 96-103 (2003)
3. Rius J., J. Martínez-González , J. Crespo & L. Badimon: Involvement of NOR-1 in LDL-induced mitogenic stimulus in vascular smooth muscle cells: role of CREB. *Arterioscler Thromb Vasc Biol*, 24, 697-702 (2004)
4. Crespo J., J. Martínez-González, J. Rius & L. Badimon: Simvastatin inhibits NOR-1 expression induced by hyperlipemia by interfering with CREB activation. *Cardiovasc Res*, 67, 333-341 (2005)
5. Rius J., J. Martínez-González, J. Crespo, & L. Badimon: NOR-1 is involved in VEGF-induced endothelial cell growth. *Atherosclerosis*, 184, 276-282 (2006)
6. Martínez-González J. & L. Badimon: The NR4A subfamily of nuclear receptors: new early genes regulated by growth factors in vascular cells. *Cardiovasc Res*, 65, 609-618 (2005)
7. Arkenbout E. K., V. de Waard, M. van Bragt, T. A. van Achterberg, J. M. Grimbergen, B. Pichon, H. Pannekoek & C. J. de Vries : Protective function of transcription factor TR3 orphan receptor in atherogenesis: decreased lesion formation in carotid artery ligation model in TR3 transgenic mice. *Circulation* , 106, 1530-1535 (2002)
8. Liu D., H. Jia, D. I. Holmes, A. Stannard & I. Zachary: VEGF-regulated gene expression in endothelial cells. *Arterioscler Thromb Vasc Biol*, 23, 2002-2007 (2003)
9. Nomiyama T., T. Nakamachi, F. Gizard, E. B. Heywood, K. L. Jones, N. Ohkura, R. Kawamori, O. M. Conneely & D. Bruemmer: The NR4A orphan nuclear receptor NOR1 is induced by platelet-derived growth factor and mediates vascular smooth muscle cell proliferation. *J Biol Chem*, 281, 33467-33476 (2006)
10. Coughlin S. R.: PARs in hemostasis, thrombosis and vascular biology. *J Thromb Haemost*, 3, 1800-1814 (2005)
11. Milbrandt J.: Nerve growth factor induces a gene homologous to the glucocorticoid receptor gene. Neuron, 1, 183-8 (1988)
12. Ohkura N., M. Hijikuro, A. Yamamoto & K. Miki. Molecular cloning of a novel thyroid/steroid receptor superfamily gene from cultured rat neural cells. *Biochem Biophys Res Commun*, 205, 1959-1965 (1994)
13. Law S. W., O. M. Conneely, F. J. DeMayo & B. W. O'Malley: Identification of a new brain-specific transcription factor, Nurr1. *Mol Endocrinol*, 6, 2129-2135 (1992)
14. Wang Z., G. Benoit, J. Liu, S. Prasad, P. Aarnisalo, X. Liu, H. Xu, N. P. Walker & T. Perlmann: Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors. *Nature*, 423, 555-560 (2003)
15. Hill-Eubanks D. C., M. F. Gomez, A. S. Stevenson & M. T. Nelson: NFAT regulation in smooth muscle. *Trends Cardiovasc Med*, 13, 56-62 (2003)

16. Tokunou T., T. Ichiki, K. Takeda, Y. Funakoshi, N. Lino & A. Takeshita: CREB mediates thrombin-induced proliferation of vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*, 21, 1764-1769 (2001)
17. Klemm D. J., P. A. Watson , M. G. Frid, E. C. Dempsey, J. Schaack , L. A. Colton, A. Nesterova, K. R. Stenmark, & J. E. Reusch: cAMP response element-binding protein content is a molecular determinant of smooth muscle cell proliferation and migration. *J Biol Chem*, 276, 46132-46141 (2001)
18. Cheng L. E., F. K. Chan, D. Cado, & A. Winoto: Functional redundancy of the Nur77 and Nor-1 orphan steroid receptors in T-cell apoptosis. *EMBO J*, 16, 1865-1875 (1997)
19. Ponnio T. & O. M. Conneely: NOR-1 regulates hippocampal axon guidance, pyramidal cell survival, and seizure susceptibility. *Mol Cell Biol*, 24, 9070-9078 (2004)
20. Braun-Dullaeus R. C., M. J. Mann & V. J. Dzau : Cell cycle progression: new therapeutic target for vascular proliferative disease. *Circulation*, 98, 82-89 (1998)
21. Minami T., A. Sugiyama, S. Q. Wu, R. Abid, T. Kodama & W. C. Aird: Thrombin and phenotypic modulation of endothelium. *Arterioscler Thromb Vasc Biol*, 24, 41-53 (2004)
22. Nakanishi-Matsui M., Y. W. Zheng, D. J. Sulciner, E. J. Weiss & S. R. Coughlin: PAR3 is a coactivator for PAR4 activation by thrombin. *Nature*, 404, 609-613 (2000)
23. O'Brien P. J., N. Prevost, M. Molino, M. K. Hollinger, M. J. Woolkalis, D. S. Woulfe & L. F. Brass. Thrombin responses in human endothelial cells. Contributions from receptors other than PAR1 include the transactivation of PAR2 by thrombin-cleaved PAR1. *J Biol Chem*, 275, 13502-13509 (2000)
24. Tsopanoglou N. E. & M. E. Maragoudakis: On the mechanism of thrombin-induced angiogenesis: inhibition of attachment of endothelial cells on basement membrane components. *Angiogenesis*, 1, 192-200 (1998)
25. Maragoudakis M. E., N. Kraniti, E. Giannopoulou, K. Alexopoulos & J. Matsoukas: Modulation of angiogenesis and progelatinase a by thrombin receptor mimetics and antagonists. *Endothelium*, 8, 195-205 (2001)
26. Caunt M., Y. Q. Huang, P. C. Brooks & S. Karpatkin: Thrombin induces neoangiogenesis in the chick chorioallantonic membrane. *J Thromb Haemost*, 1, 2097-2102 (2003)
27. Dupuy E., A. Habib, M. Lebret, R. Yang, S. Levy-toledano, S. Tobelem, & G. Tobelem: Thrombin induces angiogenesis and VEGF expression in human endothelial cells: possible relevance of HIF-1alpha. *J Thromb Haemost*, 1, 1096-1102 (2003)
28. BelAiba R. S., T. Djordjevic, S. Bonello, D. Flugel, J. Hess, T. Kietzmann & A. Görlach: Redox-sensitive regulation of HIF pathway under non-hypoxic conditions in pulmonary artery smooth muscle cells. *Biol Chem*, 385, 249-257 (2004)
29. Martorell L., J. Martínez-González, J. Crespo, O. Calvayrac & L. Badimon: NOR-1 is induced by thrombin and mediates vascular endothelial cell growth. *J Thromb Haemost*, 5, 1766-1773 (2007)
30. Brass L. F., R. R. Vassallo, E. Belmonte, M. Ahuja, K. Cichowski & J.A. Hoxie: Structure and function of the human platelet thrombin receptor. Studies using monoclonal antibodies directed against a defined domain within the receptor N terminus. *J Biol Chem*, 267, 13795-13798 (1992)

Abbreviations: NOR-1: neuron-derived orphan receptor-1; VSMCs: vascular smooth muscle cells; PKC: protein kinase C; MAPK: mitogen-activated protein kinase; ERK: extracellular-regulated kinase; CREB: cAMP response element binding protein; PAR: protease-activated receptor; NR: nuclear receptor; NGF: nerve growth factor; AF-1: activation function-1; DBD: DNA-binding domain; LBD: ligand-binding domain; PDGF: platelet-derived growth factor; EGF: epidermal growth factor; PMA: phorbol-12-myristate-13-acetate; LDL: low-density lipoproteins; PTX: pertussis toxin; VEGF: vascular endothelial growth factor; VEGFR-2: VEGF receptor-2; KDR: kinase insert domain-containing receptor; PI3K: phosphatidyl-inositol-3-kinase; NFAT: nuclear factor of activated T cells; BAPTA-AM: 1,2-bis(2-aminophenoxy)ethano-*N,N,N',N'*-tetraacetic acid tetrakis acetoxymethyl ester; ODNs: oligonucleotides; siRNA: small interference RNA; ROCK: Rho-associated kinase; GPCR: G protein-coupled receptor; MMP: matrix metalloproteinase; TF: tissue factor; PAI-1: plasminogen activator inhibitor-1; VCAM-1: vascular cell adhesion molecule-1; ICAM-1: intercellular adhesion molecule-1; TRAP-6: thrombin-receptor activator peptide-6; MCP-1: monocyte chemoattractant protein-1; IL: interleukin; CAD: coronary artery disease; PTCA: percutaneous transluminal coronary angioplasty; PF4: platelet factor 4; WPB: Weibel-Palade bodies: TXA_2, thromboxane A_2: PAF: platelet activating factor. HUVEC: human umbilical vein endothelial cells

Key Words: NOR-1, Atherosclerosis, Transcription Factors, Thrombin, Endothelial Cells

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