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Nox1 Mediates Basic Fibroblast Growth Factor–Induced Migration of Vascular Smooth Muscle Cells

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Objectives—Basic fibroblast growth factor (bFGF) stimulates vascular smooth muscle cell (SMC) migration. We determined whether bFGF increases SMC reactive oxygen-species (ROS) and studied the role of ROS for SMC migration.

Methods and Results—bFGF rapidly increased rat SMC ROS formation and migration through pathways sensitive to inhibition of NADPH oxidases, PI3-kinase, protein kinase C, and Rac-1. SiRNA directed against the NADPH oxidase Nox4 impaired basal but not bFGF-induced ROS formation and did not affect migration. In contrast, siRNA against Nox1 blocked the agonist-induced ROS generation as well as the bFGF-induced migration. Agonist-induced migration was also attenuated in SMC derived from Nox1 y/− mice and transduction of Nox1 restored normal migration. Likewise, SMC outgrowth in response to bFGF was attenuated in aortic segments from Nox1 y/− mice as compared with Nox1 y/+ mice. bFGF activated JNK but not Src in a Nox1-dependent manner. Consequently, phosphorylation of the adaptor protein paxillin, which is central for migration and secretion of matrix-metalloproteinases, were dependent on Nox1 as well as JNK but not Src.

Conclusions—These data demonstrate that bFGF activates the Nox1-containing NADPH oxidase and that bFGF through a pathway involving ROS and JNK stimulates SMC migration. (***Arterioscler Thromb Vasc Biol.*** **2007;27:1736-1743.)**

Key Words: oxidative stress ■ superoxide ■ NADPH

Restenosis is the major clinical problem after percutaneous transluminal coronary angioplasty (PTCA) and coronary artery stent implantation and is characterized by vascular smooth muscle cell (VSMC) migration.[1] Several cytokines such as basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) that are known to promote SMC migration are released in the course of restenosis.[1] It is thought that PDGF elicits a chemotactic stimulus for SMC migration toward the intimal layer whereas bFGF does not evoke polarization of cells and thus directed migration. bFGF therefore rather increases the motility rate per se,[2] but this is sufficient to result in neointima formation in vivo[3] as well as intimal hyperplasia and angiogenesis.[4]

bFGF belongs to a class of at least 22 proteins with a molecular weight of 17 to 34 kDa which share a conserved sequence of 120 amino acids. FGFs mediate their biological effects by high affinity binding to its receptor with tyrosine kinase activity (FGFR1-FGFR4) but also to several low affinity receptors and heparin sulfate proteoglycans.[5] In the vasculature, bFGF is thought to mainly act via FGFR2 which autophosphorylates after ligand binding.

Migration is a complex process which involves several events inside as well as outside the cell. Small GTPases have a central function in controlling the cytoskeleton of cells thereby determining cell shape, motility, and migration.[6] Individual phases in the cell motility process are dependent on different GTPases: RhoA regulates the assembly of focal adhesions and actin stress fiber formation, whereas Rac-1 is needed for membrane ruffling.[7]

Many elements in the process leading to cell migration are considered to be redox-sensitive, such as the phosphorylation of receptor protein tyrosine kinases[8] or the regulation of the actin cytoskeleton.[9] However, it is not known whether bFGF alters the cellular redox balance.

The most significant sources of oxygen-derived free radicals (ROS) in the vascular system are NADPH oxidases.[10] Initially, such enzymes have been described in leukocytes. This classic NADPH oxidase consist of the two membrane-bound subunits Nox2 and p22phox and the cytosolic components p47phox, p67phox, p40phox, and Rac-1. The cytosolic subunits have to assemble with the membrane bound subunits to allow the transfer of electrons from NADPH to molecular oxygen by the Nox subunit. Recently, several isoforms of the NADPH oxidase have been reported,[10] which mainly differ in their large membrane-bound Nox subunit. Of the 5 proteins described until now, Nox1, Nox2, and Nox4 are involved in vascular ROS formation.[10] Whereas Nox4 is thought to be expressed in all layers of the vessel wall, Nox2 expression is usually restricted to endothelial cells[11] and fibroblasts, while Nox1 is found in SMC.[10] A vascular phenotype in NOX1-

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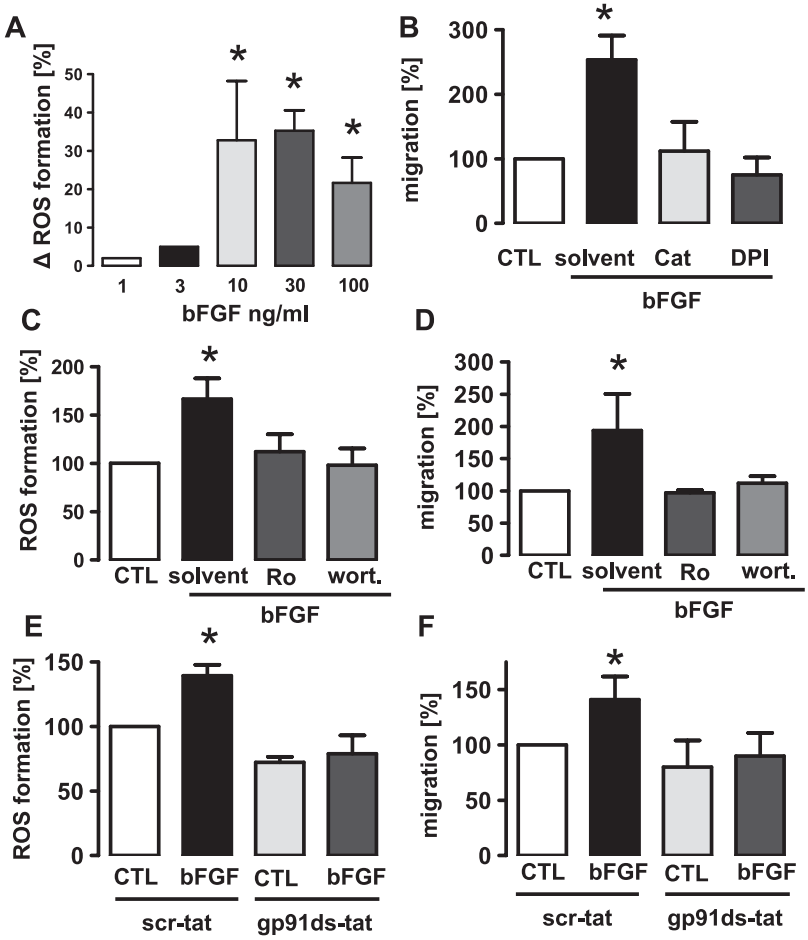

Figure 1. Involvement of NADPH oxidase–derived reactive oxygen species in bFGF-induced migration. Determination of ROS generation in SMC by the CM-DCHF oxidation assay (A, C, E) and SMC migration as determined by scratch wound assay (B, D, F) in the presence of absence of PEG-catalase (500 U/mL, Cat), the flavine inhibitor diphenylene iodonium (DPI, 10 μmol/L), the protein kinase C inhibitor Ro318220 (Ro, 0,3 μmol/L), the PI3-kinase inhibitor wortmannin (wort., 20 nmol/L), the NADPH oxidase peptide based inhibitor gp91ds-tat (100 μmol/L), or the inactive control peptide scr-tat (100 μmol/L). n $\geq$7, *P<0.05 vs the individual CTL.

deficient mice was independently reported by 2 groups.[10,12,13] NOX1-deficient mice showed markedly attenuate hypertension in response to angiotensin II (Ang II). Also, the Ang II–induced ROS generation was blunted in NOX1-deficient SMCs in vitro and in vivo.

Homologues have also been cloned for the cytosolic subunits p47phox and p67phox and were termed Noxo1 and Noxa1, and we recently reported that Noxa1 substitutes p67phox, which is absent in smooth muscle cells.[14]

In the present study we investigated whether bFGF increases the ROS formation in SMCs and determined whether ROS formation mediates bFGF-induced migration. Moreover, we set out to uncover the enzymatic sources involved in bFGF-induced ROS formation.

Materials and Methods

For detailed methods, please see the supplemental materials (available online at http://atvb.ahajournals.org).

Animals and Cell Culture

Nox1 y/+ mice were bred from a strain provided by one of the coauthors (K.-H.K.).[12]

RSMCs were isolated from the aorta of SD rats, and MSMCs isolated from the aortas of Nox1 y/− and Nox1 y/+ mice were used.

Function Assay in Culture Cells

Scratch wound assays were performed in cells serum deprived overnight as detailed in the online supplement. As an alternative the electric cell-substrate impedance sensing (ECIS) system (Applied Biophysics, model 1600R) was used with a method detailed in the supplement. Outgrowth assay from mouse aortic rings were performed in MCDB medium in the presence of 2% FCS over 7 days.

siRNA Transfection

SiRNAs were synthesized by Eurogentec (Seraing) and were transfected using the Gene-Eraser reagent (Stragene).

Lentiviral-Based Expression of Nox1 in Nox1 Y/− MSMCs

The human Nox1cDNA, a generous gift from T. Leto (National Institutes of Health, Bethesda, MD), was cloned into the Lenti6-V5-D-Topo vector and transduction was achieved using a lentiviral system (Invitrogen).

Gelatinase Assay

Gelatinase activity was determined by standard zymography.

ROS Production

H_2O_2 formation was determined by Amplex Red assay as well as luminol/horse radish peroxide chemiluminescence. ROS formation was also assessed by DCHF oxidation.

Results

Migration of RSMCs Depends on ROS Generated by a NADPH Oxidase

Basic FGF increased the ROS-formation in RSMCs (Figure 1A). For the following experiments a concentration of 30 ng/mL was used for bFGF which also stimulated SMC migration within 4 hours (Figure 1B, 1D, and 1F).

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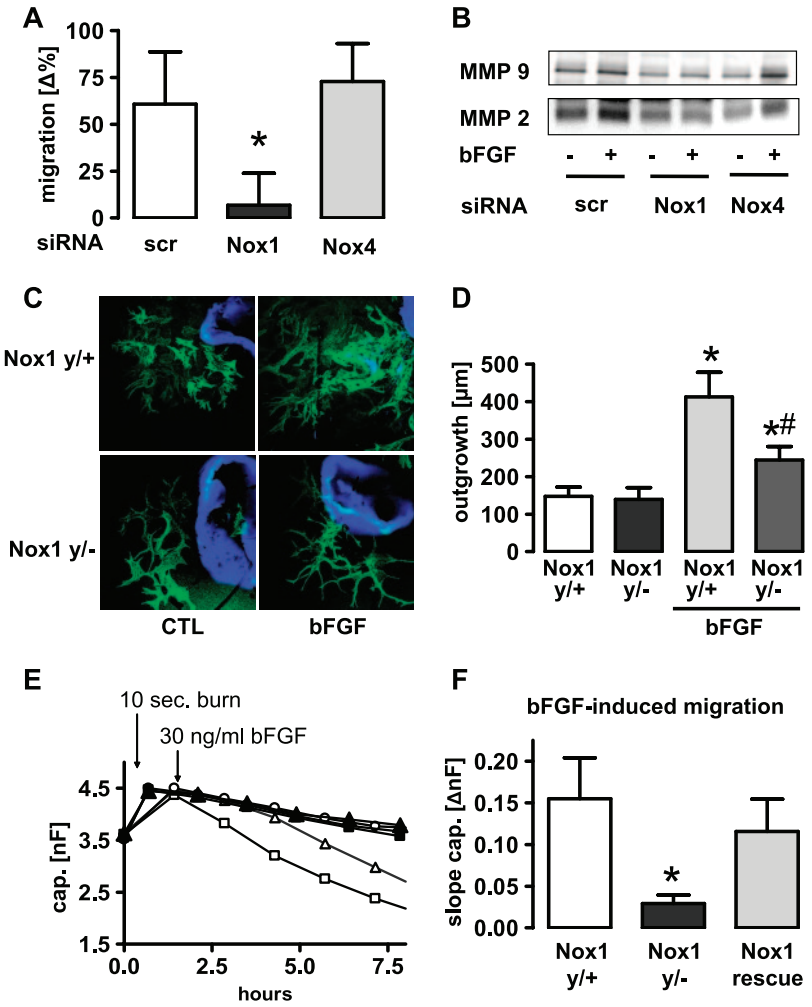

Figure 2. Role of Nox1 and Nox4 for bFGF-induced migration. A, Cells were transfected with siRNA directed against Nox1 or Nox4 or the control siRNA. Migration in response to bFGF (30 ng/mL, 4 hours) was determined by scratch wound assays and expressed relative to the migration in the absence of bFGF. *$P<0.05$ vs scr, n=8, paired *t* test with Bonferroni correction. B, Gelatinase assays of supernatants of RSMCs transfected with the siRNAs indicated with and without stimulation of the cells with bFGF (30 ng/mL); exemplary zymography of 5 similar experiments. C and D, Outgrowth of SMCs from aortic rings of Nox1 y/+ and Nox1 y/− mice within 7 days in the absence (CTL) or presence of bFGF (30 ng/mL). C, Representative image of the α-SM-actin (green) positive SMCs, ToPro3 served as nuclear marker staining the aortic ring and (D) statistical analysis of the outgrowth distances of all rings. *$P<0.05$ vs unstimulated control, #$P<0.05$ Nox1 y/+ vs Nox1 y/− by ANOVA, n=9. E and F, Effect of Nox1 knock down on SMC migration. Representative tracing (E) and statistical analysis (F) of the migration in the presence or absence of bFGF of SMC from aortas of Nox y/+ mice and Nox1 y/− and of SMC from Nox1 y/− mice with lentiviral-base transduction with Nox1 (Nox1 rescue). At the first arrow, a current was applied, detaching the cells on the 250-μm diameter electrode and causing the capacity to rise to that of an open electrode. Over time the capacity decreases as healthy cells migrate onto the electrode. Boxes indicate Nox1 y/+ SMCs (■□), circles symbolize Nox1 y/− SMCs (●○), and triangles denote Nox1 y/− cells stably transduced with Nox1 (Nox1 rescue, ▲△). Open symbols correspond to bFGF-treated cells, closed symbols to cells without bFGF. n=5, *$P<0.05$ vs Nox1 y/+.

Incubation of the cells with polyethylene glycol (PEG)-catalase (500 U/mL) blocked the bFGF-induced migration (Figure 1B). To identify the enzymatic sources of ROS, several inhibitors were used. DPI (10 μmol/L) a relatively unspecific flavoprotein inhibitor completely prevented the bFGF-induced migration of RSMCs (Figure 1B). NADPH oxidases are flavoproteins, and at least the Nox1- and Nox2-containing NADPH oxidases are activated by protein kinase C (PKC) and PI-3-kinase. Accordingly, inhibition of PKC by Ro318220 (0.3 μmol/L) and PI3-kinase by wortmannin (20 nmol/L) blocked the ROS formation and migration of RSMC in response to bFGF (Figure 1C and 1D).

The above results point toward a possible role of NADPH oxidases in the process of bFGF-induced migration. To further prove the involvement of these enzymes, we used the peptide-based inhibitor gp91ds-tat (100μmol/L; synthesized by Eurogentec).[15] Treatment with the gp91ds-tat but not with the scrambled control peptide scr-tat abrogated ROS formation as well as migration in response to bFGF (Figure 1E and 1F).

Nox1 but not Nox4 Mediates bFGF-Induced Migration

Gp91ds-tat is probably not isoform-selective for the different NADPH oxidases. To identify the Nox protein involved in bFGF-induced signaling in RSMCs a siRNA approach was used. Nox1 siRNA completely prevented the bFGF-induced migration, whereas Nox4 siRNA had no effect (Figure 2A). Matrix metalloproteinases (MMPs) are required for migration in vivo as they digest the matrix surrounding the cells. Interestingly, secretion and activation of MMPs in response to bFGF was attenuated in cells treated with Nox1 siRNA but not affected by Nox4 siRNA (Figure 2B).

To ascertain the physiological relevance of these observations and to exclude unspecific effects of the siRNA approach, experiments were performed in tissue obtained from Nox1 y/− mice and their wild-type littermates. Indeed, the outgrowth of SMCs in response to bFGF was impaired in aortic segments from Nox1 y/− as compared with segments from Nox1 y/+ mice (Figure 2C and 2D). Accordingly, the bFGF-induced migration of cultured SMCs from aortas of Nox1 y/− mice was attenuated compared with SMCs of aortas from Nox1 y/+ litter mates. Importantly, stable transduction of Nox1 into SMCs from aortas of Nox1 y/− using a lentiviral approach restored the migratory response to bFGF (Figure 2E and 2F).

BFGF-Induced ROS Production Depends on Nox1

In SMCs knock-down of Nox4 reduced the basal ROS production, which was not the case when cells were transfected with Nox1 siRNA or control siRNA (Figure 3A and

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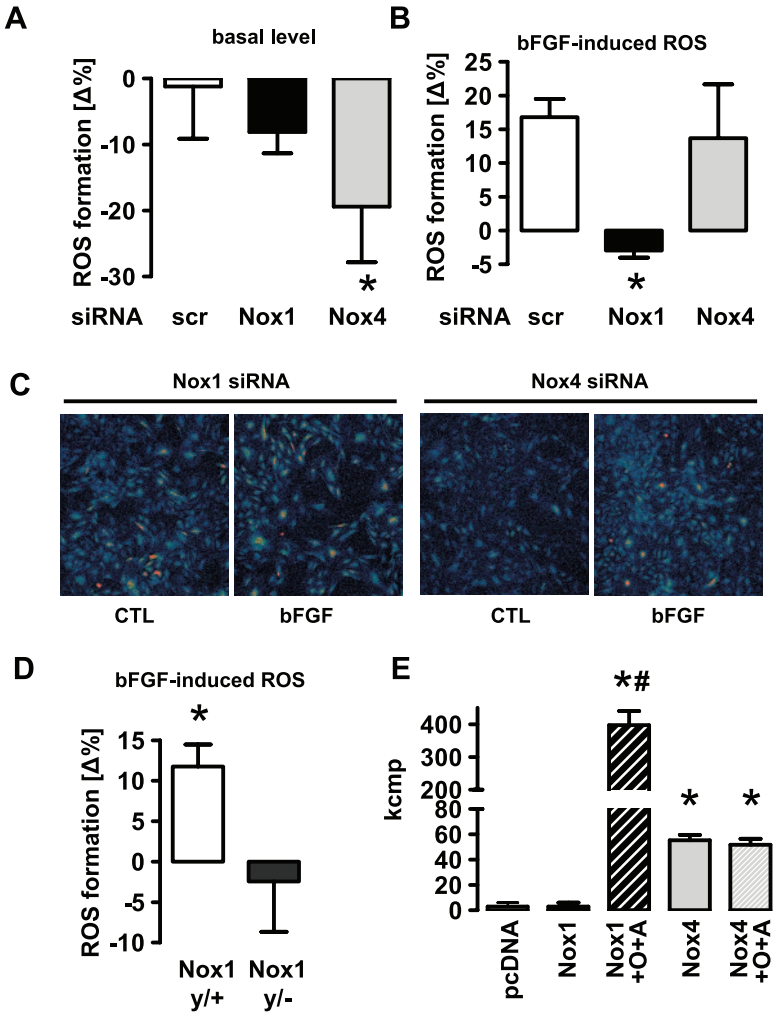

Figure 3. Role of Nox1 and Nox4 for bFGF-induced ROS formation. A-C, Effect of control siRNA, Nox1, or Nox4 siRNA on the basal (A) and bFGF-induced (B, 30 ng/mL, 60 minutes) ROS-formation in RSMCs using the CM-DCHF oxidation assay. n=5, *$P<0.05$ vs Scr, paired *t* test with Bonferroni correction. C, Representative microscopic pictures. D, Effect of bFGF (30 ng/mL) on the H_2O_2 formation determined by HRP-catalyzed oxidation of amplex red of isolated SMCs from aortas of Nox1 y/+ and Nox1 y/− mice. n=5, *$P<0.05$ with vs without bFGF, unpaired *t* test. E, Hek293 cells were transiently transfected with rat Nox1, Noxo1 (O), Noxa1 (A), Nox4, or empty vector (pcDNA) as indicated. ROS production was measured by luminol (200 μmol/L) and HRP-(1 U/mL)-driven chemiluminescence. kcpm=1000× counts per minute. n=8, *$P<0.05$ vs pcDNA, #$P<0.05$ without vs with Noxa1 and Noxo1, ANOVA.

3C). In contrast Nox1 siRNA prevented bFGF-induced ROS formation which in turn remained unaffected by Nox4 siRNA (Figure 3B and 3C). Accordingly, only SMCs isolated from Nox1 y/+ but not Nox1 y/− mice exhibited a bFGF-inducible ROS formation (Figure 3D). These data suggest that Nox4 but not Nox1 is constitutively active. Indeed, transfection of Hek293 cells with rat Nox1 had no effect on radical formation whereas overexpression of rat Nox4 alone resulted in an increased ROS production. Coexpression of the Nox1-activating proteins Noxa1 and Noxo1 in combination with Nox1 resulted in an excessive ROS production but had no stimulating effect on Nox4-dependent ROS generation (Figure 3E).

BFGF-Induced Migration Depends on Rac-1

The involvement of Rac-1 was studied as this GTPase is required for NADPH oxidase activation. Rac-1 has also been suggested to be redox-sensitive and is involved in migration per se by altering the conformation of the cytoskeleton. BFGF strongly activated Rac-1. Inhibition of Rac-1 by clostridium difficile toxin B completely blocked the bFGF-induced migration and ROS production. Nevertheless, activation of Rac-1 by bFGF was unaffected by Nox1 and Nox4 siRNA, suggesting that Rac-1 is upstream of the oxidase in bFGF-induced signaling and that redox-dependent activation of Rac-1 does not contribute to the effects observed (Figure 4).

BFGF-Induced Migration Requires Nox1-Mediated Activation of the c-Jun–NH2-Terimal Kinase (JNK)

Finally, we aimed to identify signal transduction molecules mediating the redox-dependency of the bFGF-induced migration. Src could be a classic candidate molecule for this process, as Src is known to be redox sensitive and involved in migration through the activation of the focal adhesion kinase. Different from Ang II, however, bFGF did not activate Src, and inhibition of this tyrosine kinase using PP2 (10 μmol/L) did not prevent the bFGF-induced migration (Figure 5A and 5B).

In contrast, the redox-sensitive MAP kinase JNK was strongly activated when SMCs were treated with bFGF (Figure 5A and 5C), and preincubation of the cells with catalase, DPI, or gp91ds-tat attenuated the bFGF-stimulated phosphorylation of JNK (data not shown). Importantly, knockdown of Nox1 but not of Nox4 resulted in an impaired bFGF-induced phosphorylation of JNK, whereas the phosphorylation of Erk1/2 remained unaffected (Figure 5C). JNK-dependent migration is thought to involve paxillin, and

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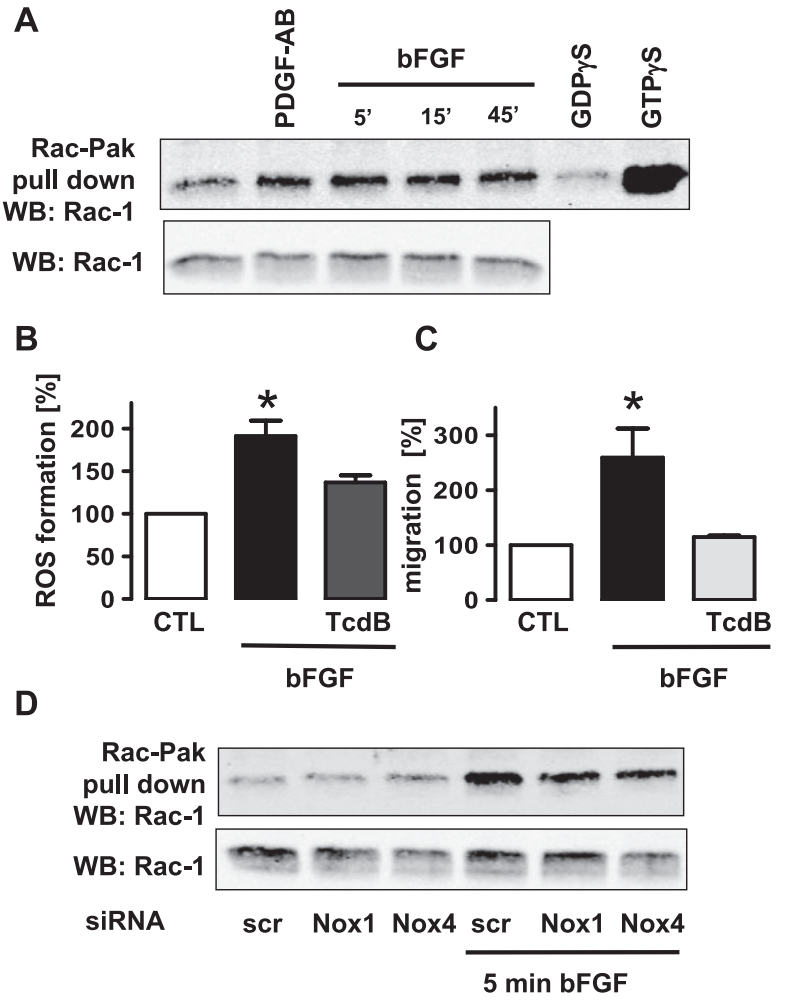

Figure 4. Role of Rac-1 for bFGF-induced migration. A, Rac-1 activation as determined by a Rac-Pak pull down assay from cells stimulated with or without PDGF (50 ng/mL) and bFGF (30 ng/mL) for the times indicated. GDPγS and GTPγS served as negative and positive controls respectively added to the lysis buffer. Exemplary blot of 3 similar experiments. B and C, Relative increases of ROS formation (B) and migration (C) in RSMCs treated with bFGF in the presence of absence of Clostridium difficile lethal toxin B (10 ng/mL, 4 hours). n=7, *$P<0.05$ vs CTL. D, Rac-1 activation as determined by a Rac-Pak pull down assay from cells stimulated with or without bFGF (30 ng/mL) after transfection with siRNA directed against Nox1 or Nox4 or scrambled control siRNA. Exemplary blot of 4 similar experiments.

indeed, the phosphorylation of the paxillin was impaired in SMCs from aortas of Nox1 y/− mice as compared with SMC from aortas of Nox1 y/+ mice. Stable transduction of Nox1 y/− cells with Nox1 restored the bFGF-induced phosphorylation of paxillin (Figure 5D). To demonstrate a role of JNK for bFGF-induced migration, the specific inhibitor SP600125 (10 μmol/L) was used. Inhibition of JNK by this compound blocked the bFGF-induced migration and activation of MMPs. (Figure 5E and 5F). In contrast, activation of MMPs was unaffected by the inhibition of Src (Figure 5F).

Discussion

In this study we demonstrate that bFGF-induced migration of rodent smooth muscle cells depends on ROS generated by the Nox1. ROS activate JNK which subsequently promotes migration through the phosphorylation of the adaptor protein paxillin and the release and activation of MMPs. We therefore for the first time demonstrate that bFGF signals through Nox1 and that this enzyme is a central mediator for SMC migration.

The signaling associated with bFGF-induced migration and ROS formation was similar to some extent to what has been previously published for Ang II and PDGF. Ang II activates the SMC NADPH oxidase through PKC, PI3-kinase, and Rac,[16] and similar observations were made for bFGF in the present study. Indeed, bFGF activates PKC in several cells,[17] and PKC in turn phosphorylates and activates the cytosolic subunit p47phox of the NAPDH oxidase. Inhibition of PKC blocked migration in the present study, although the overall function of PKC for migration is controversial and in SMCs PKC rather attenuates this process.[18] This may suggest that the effects of PKC inhibition in the present study are a consequence of the inhibition of NADPH oxidase and not of the events involved in migration per se.

Our data also indicate that bFGF stimulates PI3-kinase, which is somewhat in contrast to a previous study.[19] We observed that inhibition of PI3 kinase using low, specific concentrations of wortmannin prevented the bFGF-induced ROS production and migration of SMCs. Indeed, activation of Rac can be dependent on PI3 kinase as some Rac-1 GEFs are activated by PIP3.[20] Rac-1 is not only required for membrane ruffling but is also an essential activator of the Nox2- as well as Nox1-containing NADPH oxidases. The activity of Nox4 in contrast seems to be independent of Rac-1.[21] Several lines of evidence point toward an important role of Rac-1 for bFGF-induced migration: we observed that bFGF within minutes increases Rac-1 activity and bFGF-induced lamellipodia formation is blocked by dominant-negative Rac-1.[22] Also in the present study, inhibition of Rac-1 using clostridium difficile toxin B blocked bFGF-

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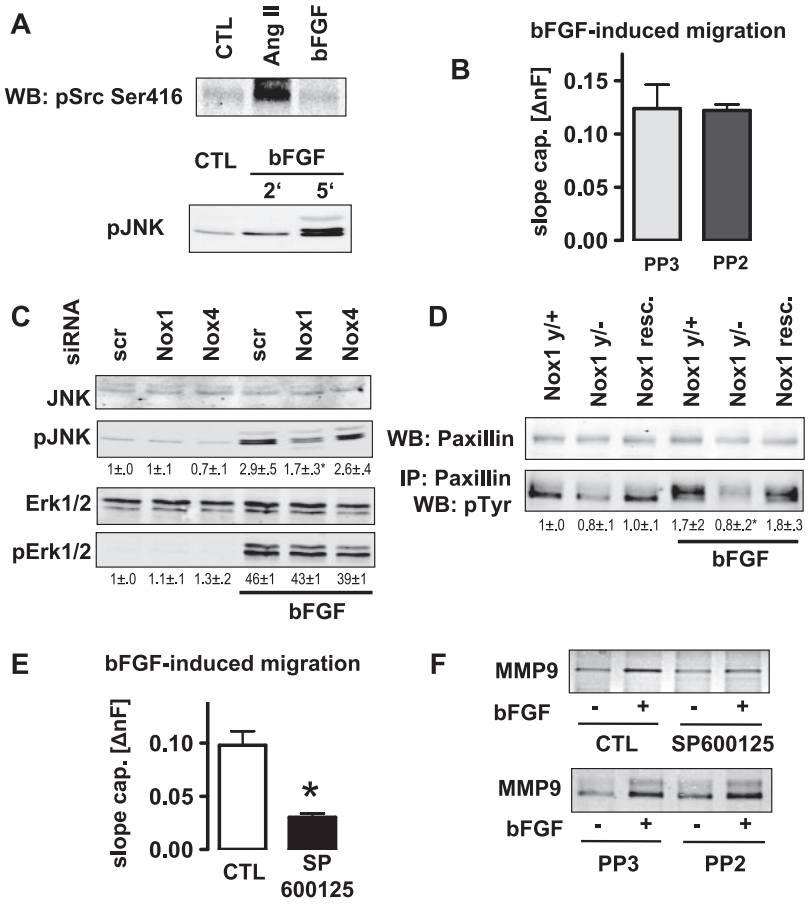

Figure 5. Mechanism of Nox1-dependent migration. A, Phosphorylation of serine 416 of Src and phosphorylation of threonine 183/tyrosine 187 of JNK (pJNK) in response to angiotensin II (Ang II, 100 nmol/L) and bFGF (30 ng/mL) respectively as determined by Western Blot using phospho-specific antibodies. B, Statistical analysis of the migration of RSMCs in response to bFGF in the presence of the Src inhibitor PP2 (10 μmol/L) or the inactive control compound PP3 (10 μmol/L). n=8, P=ns. C, Effect of scrambled siRNA and Nox1 or Nox4 siRNA on the bFGF-induced (30 ng/mL, 5 minutes) phosphorylation of JNK and extracellular signal-regulated kinase (ERK) as determined by Western blot analysis using the antibodies indicated. Exemplary Western blot of 5 similar experiments. Numbers below the blots indicate the means±SEM of the densitometric analysis relative to the abundance of the nonstimulated and nonphosphorylated protein. *P<0.05 vs scrambled siRNA. D, Phosphorylation of paxillin tyrosine residues detected by anti–phosphotyrosine Western blot from immunoprecipitates against paxillin of MSMCs from aorta of Nox1 y/+, Nox1 y/−, and MSMCs from Nox1 y/− mice stably transduced with Nox1 (Nox1 resc.) with and without stimulation with bFGF (30 ng/mL, 5 minutes). Exemplary Western blot of 5 similar experiments. Numbers below the blots indicate the means±SEM of the densitometric analysis relative to the abundance of the nonstimulated protein. *P<0.05 vs scrambled siRNA. E, Statistical analysis of the relative effect of bFGF (30 ng/mL) on the migration of RSMCs in the presence or absence of the JNK inhibitor SP600125 (10μmol/L) or DMSO (CTL). n=8, P<0.05 DMSO vs SP600125, paired t test. F, Gelatinase assays of supernatants of RSMC performed in the presence or absence of DMSO (CTL), SP600125 (10 μmol/L), PP3 (10 μmol/L), or PP2 (10 μmol/L) with and without stimulation of the cells with bFGF (30 ng/mL), exemplary zymographies of 5 similar experiments.

induced migration of SMCs. As neither Nox1 nor Nox4 siRNA significantly blocked Rac-1 activation by bFGF, we conclude that Rac is upstream of the NAPDH oxidase but potentially might activate signaling pathways parallel to the NADPH oxidases. One of these could be the Rac-1–mediated activation of the p21-activated protein kinase-1 (Pak-1).[23] Pak-1 initiates the reorganization process of the cytoskeleton[24] by 2 mechanisms: phosphorylation which guides Pak-1 to the plasma membrane, and activation by small Rac-1. In particular the phosphorylation at Tyr423 is thought to be redox-dependent and is mediated by PDK-1, which can be activated by the redox-sensitive tyrosine kinase Src.[25] Therefore a role of Src in migration has been suggested for G protein–coupled receptor agonists such as Ang II.[26,27] In this signaling cascade, Src is required to transactivate receptor tyrosine kinases, such as the PDGF-receptor kinase.[28] Indeed, also in the present study Ang II activated Src. BFGF in contrast had no effect on Src activity and it might be speculated that Src is dispensable for bFGF-mediated signaling as the bFGF receptor has intrinsic tyrosine kinase activity. Accordingly, bFGF-induced migration as well as MMP activation were not affected by the Src inhibitor PP2.

Rac-1 has been observed to activate JNK.[29] Interestingly, also in the present study this redox-sensitive MAP kinase was strongly activated by bFGF. Nox1- but not Nox4 siRNA prevented activation of JNK, suggesting that the kinase acts downstream of Nox1 and bFGF-induced ROS formation. Importantly, also the bFGF-induced migration and MMP activation were prevented by inhibition of JNK and several studies linked JNK to migration. JNK phosphorylates paxillin promoting focal adhesion assembly.[30] Paxillin serves as an essential molecular adapter or scaffolding protein in migration providing multiple docking and phosphorylation sites for a large number of proteins[31] such as FAK[32] and JNK.[33] Also in the present study bFGF induced the phosphorylation of paxillin and this process was dependent on Nox1: in Nox1 y/− MSMCs paxillin was less phosphorylated and this was restored when Nox1 was reintroduced into the knockout cells.

Animal studies and preliminary studies in patients using antioxidants suggest a role of ROS during restenosis development in vivo.[34] From these studies it has to be concluded that ROS are not only "byproducts" but mediate essential steps in the course of agonist-induced migration. Because of the ephemeral nature of ROS it is however currently impossible to reliably quantify radical formation in living cells. ROS are decomposed or scavenged rapidly in vivo, and ROS concentrations involved in signaling are exceedingly low. Most of the assays used to date therefore provide only indices for ROS production or largely exaggerate the ROS signal through redox-cycling. Despite these difficulties, we ob-

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served that bFGF increases the Nox1- but not Nox4-mediated ROS production. Importantly, knockdown of Nox1 completely blocked the bFGF-induced migration whereas the Nox4 siRNA had no effect. It appears that Nox1 and Nox4 serve different functions in SMCs. Whereas Nox1 is involved in immediate agonist-induced signaling, as observed in the present study, Nox4 rather is responsible for long term processes such as differentiation.[35] A possible explanation of these diverse functions of the Nox proteins could be derived from the mode of activity: Nox4 seems to be constitutively active, acts independently from cytosolic subunits, and thus Nox4-dependent ROS formation is rather controlled by the expression level of the enzyme.[10] This is in contrast to Nox1, where acute activation and the subsequent release of ROS make this enzyme likely to be involved in short-term signaling. Indeed, in this study cells were exposed to bFGF for a short period (4 hours) only—not enough time to sufficiently induce ROS production by increasing Nox expression. Nevertheless, this signal extended beyond the acute phase: outgrowth of SMCs from aortic segments in response to bFGF was also dependent on Nox1.

In the present study we exclusively focused on SMCs derived from mice and rats. It is important to realize that the contribution of Nox1 in SMCs from rodents might be larger than in human cells. Nox1 expression in human aortic SMCs is lower than in rat aortic SMCs (Brandes, unpublished observation, 2006), and Nox2 has been shown to substitute for Nox1 in SMCs obtained from human gluteal biopsies.[36] Moreover, human SMCs potentially express Nox5, which is absent in mouse and rats.

In conclusion: We demonstrate that in rodents Nox1 is a crucial mediator of bFGF-induced signaling and essentially controls bFGF migration in cultured cells as well as smooth muscle cells in situ. Inhibition of Nox1 is an attractive approach to block processes linked to vascular diseases that involve SMC migration, such as restenosis.

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Disclosures

None.

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