AIF-1 Is an Actin-Polymerizing and Rac1-Activating Protein That Promotes Vascular Smooth Muscle Cell Migration

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Abstract—Development of vascular restenosis is a multifaceted process characterized by migration and proliferation of vascular smooth muscle cells (VSMCs), resulting in loss of lumen diameter. Characterization of proteins that mediate this process is essential in our understanding of the pathogenesis of arterial injury. Allograft inflammatory factor-1 (AIF-1) is a cytoplasmic, calcium-binding protein that is expressed in VSMCs by allograft and balloon angioplasty injury. AIF-1 is not present in cultured human VSMCs but is induced by cytokines, and overexpression of AIF-1 results in increased VSMC growth and cell-cycle gene expression. To characterize AIF-1 modulatory effects in primary human VSMCs, AIF-1–interacting proteins were identified by an AIF-1/glutathione S transferase fusion protein affinity assay. MALDI-TOF mass spectrophotometric amino analysis identified actin as an AIF-1 interacting protein. This interaction was verified by communoprecipitation. This is a functional interaction, because AIF-1 binds to and polymerizes F-actin in vitro. In unstimulated VSMCs, AIF-1 colocalizes with F-actin but translocates to lamellipodia on stimulation with platelet-derived growth factor. VSMCs stably transduced with AIF-1 retrovirus migrate 2.6-fold more rapidly (85.1 ± 2.9 μm/h vs 225.5 ± 16.6 μm/h; P < 0.001) in response to platelet-derived growth factor versus control cells. AIF-1 colocalizes with Rac1, and AIF-1–transduced VSMCs show a constitutive and enhanced activation of Rac1, providing a mechanism for the increased migration. These data indicate that AIF-1 binds and polymerizes F actin and also regulates Rac1 activity and VSMC migration. Considering the AIF-1 expression pattern in injured arteries, this suggests that AIF-1 may be involved in the cytoskeletal signaling network leading to vascular remodeling. (Circ Res. 2003;92:1107-1114.)

Key Words: allograft inflammatory factor-1 ■ smooth muscle cell ■ migration ■ Rac1

The pathogenesis of proliferative arteriopathies, which include restenosis after percutaneous transluminal coronary angioplasty and stenting as well as coronary artery vasculopathy subsequent to allograft rejection, originates with injury to the endothelium. Injured endothelial cells secrete growth and chemotactic factors, which recruit mononuclear cells that secrete inflammatory cytokines. This localized production of cytokines elicits activation of normally quiescent medial vascular smooth muscle cells (VSMCs).1 As part of the VSMC response to injury,2 they respond to these soluble factors by transforming from a differentiated contractile state to a dedifferentiated synthetic cell capable of migration, proliferation, and synthesis of cytokines and extracellular matrix. It has been suggested that the cytokine-induced activation of VSMCs is the most critical cellular event in the development of both cardiac allograft vasculopathy (CAV) and interventional restenosis.3,4 Accordingly, VSMC activation, migration, and proliferation represent points of therapeutic intervention to attenuate most vascular proliferative diseases. Within this context, the identification and functional characterization of genes that regulate these processes is a promising approach for the identification of targets to combat vascular proliferative disorders.5 AIF-1 is a 143 amino acid, cytoplasmic, calcium-binding protein.6 Studies from several diverse systems demonstrate AIF-1 expression in infiltrating macrophages in rat cardiac allografts,7 lesions of experimental autoimmune encephalomyelitis,8 the pancreas of prediabetic BB rats,9 and the allograft response of phylogenetically distant species as marine sponges.10 This evolutionarily conserved expression pattern implicates an important function for AIF-1 in inflammatory processes. A 44-amino acid segment of the AIF-1 protein contains an amidation signal and is flanked by a cluster of paired basic cleavage motif residues (KR-KKR), both of which are structural characteristics for peptide hormone precursor proteins.9

We have previously reported the acute and transient expression of AIF-1 in medial and intimal VSMCs in several models of arterial injury in rat and swine.5,11 AIF-1 is not present in normal arteries but is expressed in neointimal VSMCs in injured arteries.11 AIF-1 is not expressed in unstimulated cultured human VSMCs but is strongly induced in response to inflammatory cytokines and T lymphocyte–conditioned media.11 Expression of AIF-1 in human cardiac allografts correlates with rejection, and the amount of AIF-1...
expressed correlates with the severity of rejection.12 Furthermore, AIF-1 is expressed in coronary arteries with clinical CAV, and persistent expression of AIF-1 in the cardiac allograft is associated with development of CAV.12 Overexpression of AIF-1 in a rat VSMC line leads to a more rapid growth and deregulated expression of cell-cycle proteins, suggesting that one function of AIF-1 might be in the regulation of VSMC proliferation.11,13 Despite these studies, little is known about the mechanism of these growth effects, particularly with respect to molecular interactions of AIF-1 in the activated VSMC. In this study, we present data showing a functional interaction with actin and enhanced migration of VSMCs that overexpress AIF-1 and suggest that this enhanced migratory activity is attributable to the AIF-1-induced activation of the Rac1 GTPase.

Materials and Methods

Cells and Culture

Human coronary VSMCs were obtained as cryopreserved secondary culture from Cascade Corporation (Portland, Ore) and subcultured in growth medium as described previously.11 Cells from passage 3 to 6 were used in the described studies. The growth media was changed every other day until cells approached confluence. Platelet-derived growth factor (PDGF) AB, 40 ng/mL, was purchased from Sigma.

Retroviral Construction and Stable Transduction

AIF-1 retrovirus (RV) was constructed using a kit from Clontech according to the manufacturer’s instructions. Briefly, the protein coding region of the AIF-1 cDNA was cloned into the pGEX vector. The AIF-1 protein coding region was cloned into the pGEX vector (Pharmacyclics, Inc). The affinity pull-down assay was performed as described.15 Briefly, extracts from human VSMCs in 35 S methionine media were transfected into an ectotrophic 293-derived packaging cell line.14 Media was then collected from these cells and used to infect an amphotropic second packaging cell line, PT-67, according to manufacturer’s instructions. This supernatant containing recombinant high-titer virus was then used to infect human VSMCs in 24-hour exposures of viral supernatant in the presence of 8 μg/mL Polybrene (approximately 40% to 60% stable transduction was achieved). Stably transduced hVSMC G418-resistant cells were pooled from each transduction rather than individual clones to avoid the effects of clonal variation.

Actin Interaction and Identification

The AIF-1 protein coding region was cloned into the pGEX vector and expressed in Escherichia coli as a glutathione S transferase (GST) fusion protein according to manufacturer’s protocol (Pharmacia, Inc). The affinity pull-down assay was performed as described.15 Briefly, extracts from human VSMCs in 35 S methionine media were first precleared with GST Sepharose beads and then incubated with GST Sepharose beads (lane 3) or with AIF-1/GST Sepharose beads (lane 4). Pellets were washed, and interacting proteins were separated by SDS-PAGE and identified by autoradiography. Interacting proteins from unlabeled extracts were excised from the gel and identified by MALDI-TOF mass spectrometry by the protein chemistry laboratory at the University of Pennsylvania. Actin interaction was also determined by HA-tag coimmunoprecipitation.16 VSMCs stably transduced with AIF-1 or AIF-1–HA RV were cultured in the presence of 15% FCS or 1% FCS and 30 μg/mL heparin to induce the synthetic or more quiescent phenotype.17 Coimmunoprecipitations using anti–HA-tagged Sepharose (Boehringer Mannheim) were performed according to manufacturer’s recommendations. After washing, actin isoforms were identified by Western blot using anti-α or anti-β actin monoclonal antibodies (Sigma). The α actin antibody was used for the VSMCs treated with 1% FCS and heparin, and the β actin antibody was used for the 15% FCS-treated VSMCs.

Com Immunoprecipitation of endogenously expressed AIF-1 was performed by adding 500 μL of ice-cold coimmunoprecipitation buffer (50 mmol/L HEPES-KOH, pH 7.5, 150 mmol/L NaCl, 0.1% Triton X-100, 5% glycerol, and 0.5 mg/mL of the protease inhibitors aprotinin, leupeptin, and soybean trypsin inhibitor) to approximately 4×10^6 human VSMCs grown in T lymphocyte–conditioned media for 72 hours to induce endogenous AIF-1 expression and 35 S methionine to label actin.11 Extracts were precleared by brief mixing with 20 μL protein A/G conjugate (Santa Cruz Inc) and centrifugation. Supernatants were incubated with 5 μg AIF-1 or irrelevant antibody control (5 μg goat anti-rabbit antibody) and 20 μL protein A/G conjugate for 7 hours at 4°C. Pellets were washed 3 times, interacting proteins were separated by SDS-PAGE, and actin was detected by autoradiography of the labeled protein and by Western blot.

In vitro actin binding was determined by differential centrifugation in a kit purchased from Cytoskeleton, Inc, according to manufacturer’s instructions. Briefly, 23 μmol/L of AIF-1 and actin were incubated together for 30 minutes in F-actin buffer containing ATP and Ca^2+ and then centrifuged at 150,000g for 1.5 hours. For polymerization analysis, AIF-1 and F-actin were centrifuged at 8000g, the speed at which only cross-linked actin will pellet. BSA, which does not bind actin, and α actin, an actin-binding and -polymerizing protein, were included in the kit and were used as negative and positive controls, respectively. Protein in the pellets and supernatants were analyzed by SDS-PAGE, Coomassie staining, and densitometry using NIH image software.

Western Blotting and Immunofluorescence

To prepare cell extracts, human VSMCs were washed with PBS and treated with 0.3 mL of ice-cold lysis buffer (50 mmol/L HEPES-KOH, pH 7.5, 150 mmol/L NaCl, 0.1% Triton X-100) containing protease inhibitors, centrifuged at 3000 rpm for 15 minutes at 4°C, and stored at −20°C as described.11 Equal protein concentrations of cell extracts were electrophoresed through polyacrylamide gel under reducing conditions and transferred to a nitrocellulose membrane. Membranes were blocked with 5% nonfat powdered milk in TBST buffer (0.1 mol/L Tris-HCL, pH 8.0, containing 1.5 mol/L NaCl and 0.5% Triton X-100). Membranes were incubated with a 1:2500 dilution of primary antibody and a 1:2000 dilution of goat anti-rabbit secondary antibody. AIF-1 antibody has been described,11 and Rac1 antibody was from BD Bioscience. Equal protein concentrations of cell extracts were determined by Bradford assay, and equal loading on gels was verified by Ponceau S staining of the membrane. The membrane was washed with TBST, and reactive proteins were visualized using the enhanced chemiluminescence method (Amer sham) according to the manufacturer’s instructions.

Primary human VSMCs grown on chambered slides were incubated with T lymphocyte–conditioned media for 72 hours to induce AIF-1 expression. Some were stimulated with PDGF for 30 minutes and then fixed in 10% formalin, blocked with 2% goat serum, and incubated with primary antibody for 1 hour at room temperature, followed by a 30-minute incubation with secondary antibody conjugated to AlexaFluor 568 (red) or AlexaFluor 488 (green) and counterstained with DAPI for nuclear staining. Some samples were stained with phalloidin-conjugated AlexaFluor 488 for 30 minutes, AIF-1 antibody was used at 1:2500, and Rac1 was used at 1:200 (BD Bioscience).

Migration and Chemotaxis

For analysis of chemotaxis, 6.5-mm-diameter Transwell Boyden chamber plates (Costar) with 8-μm polycarbonate membrane pore size were seeded with stably transduced VSMCs (20,000 cells per membrane) in medium containing 0.5% FCS. Forty nanograms of PDGF AB were placed in the lower chamber, and cells were incubated for 4 hours at 37°C, at which time cells were fixed and stained in Diff-Quick Cell Stain (American Hospital Supply). The upper layer was scraped free of cells. VSMCs that had migrated to the lower surface of the membrane were quantitated by counting 4 high-powered fields per membrane. Experiments were performed in triplicate from 3 independently transduced groups of VSMCs. For
directional migration, equal numbers of empty vector or AIF-1 RV stably transduced VSMCs were grown in growth media on glass slides to confluence, at which time monolayers were scraped with a cell scraper to create a 3-mm track devoid of cells in the center of the chamber. The wound tracks were immediately washed to remove any detached cells, and fresh media was added. At different times after wounding, cells were fixed and stained with hematoxylin.

**Rac1 Activation**

The p21-activated kinase 1 protein (PAK) pull-down assay was performed as previously described. Briefly, stably transduced empty vector or AIF-1 RV human VSMCs were serum starved in 0.5% FCS overnight, challenged with 40 ng/mL PDGF for various times, and bound proteins were detected by Western blotting with Rac1 or Cdc42 antibodies and quantitated by densitometry of autoradiography.

The volume of lysate was adjusted to normalize for equal concentrations of proteins. Cell suspensions were incubated with GST-PAK Sepharose beads or with AIF-1/GST Sepharose beads as a negative control. The beads were washed, and interacting proteins were separated by SDS-PAGE and identified by autoradiography (Figure 1A). Trypsin digestion and MALDI-TOF mass spectroscopy identified the protein migrating at 42 kDa as smooth muscle cell actin. This interaction was verified immunologically, biochemically, and by colocalization in human VSMCs.

Using independent coimmunoprecipitation methods, human VSMCs stably transduced with AIF-1 with the hemagglutinin (HA) tag or untagged AIF-1 were incubated in 1% FCS and heparin-treated VSMCs, and the F-actin interaction is calcium-dependent, because 0.01 mmol/L EGTA reduces the amount of AIF-1 found in the pellet. To determine the effect of AIF-1 on actin dynamics, AIF-1 and F-actin were incubated and then centrifuged at low speed (8000g), which allows pelleting only of heavy, cross-linked F-actin. Controls

**Results**

**AIF-1 Interacts With and Polymerizes F-Actin**

AIF-1–interacting proteins were initially identified by fusion protein affinity pull-down experiments. Primary human VSMCs were labeled with 35S methionine, and extracts were precleared with GST protein and then incubated with an affinity resin consisting of a recombinant AIF-1-GST fusion protein coupled to glutathione Sepharose beads or GST Sepharose beads as a negative control. The beads were washed, and interacting proteins were separated by SDS-PAGE and identified by autoradiography (Figure 1A). Trypsin digestion and MALDI-TOF mass spectroscopy identified the protein migrating at 42 kDa as smooth muscle cell actin. This interaction was verified immunologically, biochemically, and by colocalization in human VSMCs.

Using independent coimmunoprecipitation methods, human VSMCs stably transduced with AIF-1 with the hemagglutinin (HA) tag or untagged AIF-1 were incubated in 1% FCS with heparin (30 μg/mL) for 6 days to induce quiescence and α actin expression or in the presence of 15% FCS to induce dedifferentiation and β actin expression. Extracts were incubated with anti–HA-Sepharose, and interacting proteins were subject to Western blot with antibody specific to anti-α or anti-β actin. Figure 1B verifies the protein microsequencing and also indicates that AIF-1 interacts with both α and β actin.

Endogenous AIF-1 expression was induced in human VSMCs by 72-hour incubation in T lymphocyte-conditioned media, overnight exposure to 35S methionine to label cellular proteins, and extracts incubated with AIF-1 antibody. Figure 1C shows that endogenously expressed AIF-1 coprecipitates with actin, indicating that interaction of these 2 proteins is not an artifact of AIF-1 overexpression.

AIF-1 actin binding and polymerizing activities were additionally investigated by differential sedimentation. Purified smooth muscle cell F-actin was incubated with recombinant AIF-1 protein, and F-actin was pelleted by high-speed (150 000g) centrifugation. Figure 2A shows that purified AIF-1 protein pellets with F-actin, indicating an interaction. Figure 2A also shows that the AIF-1–F-actin interaction is calcium-dependent, because 0.01 mmol/L EGTA reduces the amount of AIF-1 found in the pellet. To determine the effect of AIF-1 on actin dynamics, AIF-1 and F-actin were incubated and then centrifuged at low speed (8000g), which allows pelleting only of heavy, cross-linked F-actin. Controls
AIF-1 interaction with actin was additionally verified by colocalization in cultured human VSMCs. Endogenous AIF-1 expression was induced by incubation of VSMCs in T lymphocyte-conditioned media. In these cells, AIF-1 displays a cytoplasmic, fiber-like staining pattern and for the most part immunolocalizes with the thicker, bundled forms of F-actin (Figure 3). However, when these cells are stimulated with PDGF, most AIF-1 seems to translocate to and concentrate in the lamellipodia, and colocalization with F-actin is greatly reduced. These data also corroborate the previous 3 methods demonstrating an F-actin interaction, and the translocation to lamellipodia in response to PDGF suggests a role for AIF-1 in cell motility.

AIF-1 Overexpression Enhances VSMC Motility

Based on its actin interaction and localization to lamellipodia in response to migratory stimuli, we next investigated the effects of AIF-1 overexpression on VSMC chemotaxis. Human VSMCs were stably transduced with empty vector or AIF-1 RV and then seeded into Boyden chambers. Differences in chemotaxis were quantitated by counting migrated cells. Figure 4A shows that human VSMCs stably transduced with AIF-1 migrate 2.6-fold faster (85.1 ± 2.9 versus 225.5 ± 16.6; P<0.001) than control cells, indicating a positive effect of AIF-1 expression on cell movement. To additionally demonstrate a function for AIF-1 expression in VSMC motility, directional migration of VSMC monolayers after mechanical wounding was performed. Equal numbers of confluent, stably transduced AIF-1 or empty RV VSMCs were scraped to create a 3-mm-wide wound track devoid of cells, and the time needed to fill the wound was compared. The data presented in Figure 5 demonstrate that AIF-1 RV cells migrate into the wound at a more rapid rate than do empty vector–transduced cells.

AIF-1 Overexpression Enhances Rac1 Activity

Actin reorganization and cell movement in response to PDGF is regulated by the coordinated activation of small GTPases, including Rac1. VSMCs stably transduced with either empty vector or AIF-1 RV were examined for differences in Rac1 activity by the GST-PAK Sepharose pull-down.
assay. The results of this experiment are presented in Figure 6 and point to several conclusions. First, compared with empty vector–transduced VSMCs, AIF-1–containing cells show enhanced Rac1 activation in response to PDGF. Second, a constitutively elevated level of Rac1 activation is present in AIF-1–transduced VSMCs, even in the absence of chemotactic stimuli (Figure 6B). Third, this is an activation of Rac1 GTPase activity, not an increased expression, because levels of Rac1 in empty vector and AIF-1 cells, as measured by Western blot of cellular extracts, are similar. Although Cdc42, a GTPase protein also involved in motility, is expressed to a low degree in these cells, no change in Cdc42 activation was observed (data not shown).

In response to chemotactic stimuli, Rac1 is found in the lamellipodia, where it is involved in actin remodeling at the leading and trailing edges of cells. A direct interaction between AIF-1 and Rac1 was investigated by both the GST pull-down and HA tag coimmunoprecipitation assays. Although no interaction was detected (data not shown), Figure 7 demonstrates a colocalization between endogenously expressed AIF-1 and Rac1 in PDGF-stimulated VSMC lamellipodia.

Discussion

Previous studies have shown that AIF-1 is expressed in neointimal cells in various types of arterial injury and plays a role in VSMC activation. However, the role of AIF-1 in arterial injury and the mechanism by which AIF-1 mediates these effects are less clear. It is often possible to deduce the function of a protein by identification of its binding partners. Using pull-down and coimmunoprecipitation assays, we demonstrate that AIF-1 interacts with both α and β isoforms of actin in primary human VSMCs. This is not an artifact of AIF-1 overexpression, because endogenously expressed AIF-1 also coprecipitates with actin. This is the first reported molecular interaction of AIF-1 and has initiated a series of experiments to determine the functional significance of this interaction.

In vitro, AIF-1 possesses a calcium-sensitive F-actin–binding activity. Similar to other actin-binding proteins, such as α actinin and fimbrin, the AIF-1 protein contains an EF-hand calcium-binding sequence and binds calcium. Because the AIF-1 protein sequence has an imperfect EF-hand, its calcium binding is weak and more likely functions to

Figure 4. AIF-1 expression enhances VSMC chemotaxis. Stably transduced human VSMCs were seeded onto Boyden chamber membranes and exposed to 40 ng/mL PDGF for 4 hours. VSMCs that had migrated to the lower surface of the membrane were quantitated by counting 4 high-powered fields per membrane. Values are means from 3 experiments performed in triplicate from 3 independent retrovirally transduced groups of VSMCs. Errors are reported as standard deviation.

Figure 5. AIF-1 expression enhances migration of VSMCs in response to wounding. Equal numbers of confluent human VSMCs stably transduced with either empty vector or AIF-1-RV were scraped with a cell scraper to create a wound track 3 mm wide. Appearance of wound margin immediately on scraping and washing (A and B), 24 hours after wounding (C and D), and 48 hours after wounding (E and F). Cells were stained with hematoxylin and are magnified ×40. This is representative of 3 similar experiments from independently transduced groups of VSMCs.
modify its tertiary structure rather than to sequester calcium directly. This would modify its interactions with other proteins and is likely the cause for the observed calcium-sensitive F-actin binding. The arrangement and polymerization state of actin in a particular compartment of the cell is based on the function of its associated binding proteins. These experiments also show that AIF-1 possesses an F-actin polymerizing activity. Several actin-binding proteins, including α-actinin and fimbrin, also function to regulate actin bundling.

Most endogenously expressed AIF-1 colocalizes with the larger bundles of F-actin. When these VSMCs are stimulated with PDGF, AIF-1 disassociates from actin fibers and translocates to the lamellipodia. Many actin binding and remodeling proteins have been identified that reside in lamellipodia, including fimbrin, α-actinin, and talin. It has been shown that several of these, including α-actinin and fimbrin, dissociate from F-actin in the presence of calcium ions. In addition to initiation of actin polymerization and cytoskeletal reorganization, PDGF initiates a rapid flux in cytosolic calcium concentration, which may also drive AIF-1 dissociation from actin filaments in PDGF-stimulated cells.

Actin polymerization is a requisite for leading-edge cellular protrusion during migration. Lamellipodia extrusion and cytoplasmic streaming is a function of the continuous remodeling of actin at the cell periphery and is necessary to drive cell locomotion. AIF-1 localization to the cell lamellipodia, together with its actin-modifying activity, suggests a function for this protein in PDGF-driven VSMC chemotaxis. Stable, AIF-1 RV–transduced VSMCs migrated 2.6-fold more rapidly in response to PDGF than did control cells, strongly implicating a role for AIF-1 in chemotaxis.

The Rho family of small GTPases controls organization of the actin skeleton and migration in response to chemotactic stimuli. Members of this family, such as Rac1 and Cdc42, modify peripheral F-actin organization and also regulate VSMC migration in response to PDGF. Based on AIF-1 effects on migration and localization to lamellipodia, we hypothesized a positive affect of AIF-1 on GTPase activation. One result of this experiment demonstrated an enhanced PDGF-driven Rac1 activation in VSMCs that over express AIF-1. A second observation from this experiment is that the high levels of constitutive Rac1 expression in serum reduced

![Figure 6. AIF-1 expression enhances Rac1 activity. A, Human VSMCs stably transduced with either empty vector or AIF-1-RV were serum-starved overnight and then stimulated with 40 ng/mL PDGF for the times indicated. Equal amounts of protein extracts were incubated with GST-PAK Sepharose beads, and activated PAK-bound Rac1 was identified by Western analysis and quantitated by densitometry of the corresponding bands. One tenth of the amount of extract was also blotted with Rac1 and AIF-1. Gel shown is representative of 3 experiments performed on 3 independently derived, stably transduced VSMCs. B, AIF-1 activates Rac1 activity in the absence of stimuli. Scanning densitometry of 3 experiments from independently derived stably transduced VSMCs shows activation of Rac1 in serum-starved VSMCs.](http://circres.ahajournals.org/)

![Figure 7. Endogenously expressed AIF-1 colocalizes with Rac1. Primary VSMCs were grown on glass coverslips in T cell–conditioned medium to induce AIF-1 expression and then stimulated with PDGF for 30 minutes. Cells were fixed and stained with AIF-1 antibody (red) and Rac1 (green) and counterstained with DAPI for nuclear staining and observed by fluorescence microscopy. Shown is representative of 3 independent groups of human VSMCs. Magnification is ×400.](http://circres.ahajournals.org/)
unstimulated VSMCs. Because these cells were incubated in low (0.5%) FCS, this implies that AIF-1 expression alone is sufficient to activate Rac1 above basal levels. A third result of this experiment shows that AIF-1 expression seems to have no effect on Cdc42 activation. Both Rac1 and Cdc42 function as molecular switches that cycle between inactive GDP-bound forms and active GTP-bound forms, which transduce signals from cell-surface receptors to corresponding cytoplasmic targets. However, there are distinct differences between the 2 GTPases. Rac1 plays a role in organization of lamellipodia, whereas Cdc42 is involved in development of microspikes or filopodia protrusion, implying that AIF-1 plays a role more in formation of lamellipodia and Rac1-mediated migration rather than Cdc42-mediated formation of membrane ruffling. Other reports suggest that Rac1 and Cdc42 participate in cell proliferation through different signaling pathways.

Scrape wounding of cell monolayers is a frequently utilized model for elucidating the mechanisms of cell migration. Because primary VSMCs display contact inhibition, unidirectional migration is limited to those cells adjacent to the wound and is polarized into the wound space. In the absence of PDGF, VSMCs stably transduced with AIF-1 RV migrate into the wound more rapidly than do empty vector–transduced VSMCs. This is likely mediated by the presence of higher than normal levels of activated Rac1 in these versus control cells, because it has been shown that inhibition of Rac1 activity in mouse embryo fibroblasts by transduction of a dominant-negative Rac1 inhibits movement of cells into the wound space.

PDGF receptor binding activates small GTPases in addition to Rac1, which also regulate actin polymerization. A large group of downstream effectors of GTPases, such as guanine nucleotide exchange factors (GEFs), specifically activate or inactivate these GTPases, regulating the exchange of GDP for GTP. The large number of these proteins may provide tissue or receptor specificity. Other upstream receptor-mediated signaling events that activate GTPase activity remain unclear. The CRIB (Cdc42/Rac interactive binding) motif is present in many proteins, such as GEFs that interact with Rac1 and Cdc42. The AIF-1 protein does not contain a CRIB region nor other signatures that would elucidate a GTPase modifying activity. We were able to demonstrate colocalization but not a direct interaction between AIF-1 and Rac1. Together, this implies that an additional protein, such as a GEF, might be necessary for the AIF–1–mediated Rac1 activation. Alternatively, AIF-1 may act as a scaffold or adaptor to position Rac1 in proximity with its activating protein.

Although the precise mechanisms remain unclear, functional Rac1 is required for normal cell growth and cell-cycle progression. Through its involvement with Ras, Rac1 is thought to have a direct role in mitogenesis. Rac1, when microinjected into quiescent fibroblasts, stimulated cell-cycle progression through G1 and initiated DNA synthesis. Adenoviral expression of a dominant-negative form of Rac1 results in cytostatic growth arrest at the G1/M phase of the cell cycle. In general, it is accepted that the multitude of cytoskeletal alterations needed for cell activation and division require the active participation of Rac1. We have previously shown that constitutive overexpression of AIF-1 in a rat VSMC line results in increased growth of those cells even in reduced serum. In these studies, it was shown that cell-cycle protein expression was dysregulated in cells that overexpress AIF-1. Consequently, the activation of Rac1 GTPase activity by AIF-1 may be a proximal step that results in enhanced growth and subsequent aberrant cell-cycle progression.

Vascular remodeling in response to injury requires a rearrangement in the VSMC cytoskeleton and includes migration of activated VSMCs from the tunica media into the intimal layer of the arterial wall. Much of this remodeling is mediated by small GTPases, which are implicated in vascular disorders such as remodeling, regulation of blood pressure, and wound healing. Immunohistochemical analysis of angioplasty-injured swine coronary arteries shows that neointimal cells closest to the lumen stain positive for AIF-1. AIF-1 is constitutively expressed in normally motile cells such as monocytes and macrophages but only expressed in VSMCs in response to inflammatory stimuli. The data presented in this report indicate that AIF-1 binds to and participates in F-actin rearrangement. On growth factor stimulation, AIF-1 translocates to lamellipodia, where it participates in Rac1 activation, leading to enhanced motility. Our hypothesis is that AIF-1 expression contributes to the development of intimal hyperplasia in injured arteries by enhancing migration of activated VSMCs and thus plays an important role in the development of vascular restenosis.

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References


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