Smooth muscle cell (SMC) interactions with collagen mediate cell migration during the pathogenesis of atherosclerossis and restenosis. Discoidin domain receptors (DDRs) have been identified as novel collagen receptors. We used aortic SMCs from wild-type and DDR1−/− mice to evaluate the function of the DDR1 in regulating migration. DDR1−/− SMCs exhibited impaired attachment to and migration toward a type I collagen substrate. Matrix metalloproteinase-2 (MMP-2) and MMP-9 activities were concomitantly reduced in these cells. Transfection of a full-length cDNA for DDR1b rescued these deficits, whereas kinase-dead mutants of DDR1 restored attachment but not migration and MMP production. These results suggest that active DDR1 kinase is a central mediator of SMC migration.

Interactions between smooth muscle cells (SMCs) and collagen are important in cell migration during the pathogenesis of atherosclerosis and restenosis. The synthesis of several collagens, including fibrillar type I and III and short-chain type VIII, is upregulated after injury.1,2 These collagens act as chemotactic factors for SMCs, and migration through the extracellular matrix is facilitated when type VIII collagen triggers matrix metalloproteinase (MMP) synthesis.3,4

The discoidin domain receptors (DDRs) are two novel collagen receptors distinguished by an extracellular domain homologous to discoidin-I in Dictyostelium discoideum and by a catalytic tyrosine kinase domain in the cytoplasmic tail. The activation of DDR2 by its ligand, collagen type II, occurs via the generation of a latent to active gelatinase (Figure 1A), which can be assayed in zymogram gels. Smoother migration of SMCs toward 200 nmol/L type I collagen was impaired in DDR1−/− mice (Figure 1B). Transfection with DDR1b or with the kinase-truncated (K529*) or kinase-dead (K618A) DDR1 rescued the ability of the SMCs to migrate toward collagen (Figure 1B).

Migration of SMCs toward 200 nmol/L type I collagen was impaired in DDR1−/− mice compared with wild-type littermates (Figure 1C), transfection with DDR1b restored the ability of the DDR1−/− SMCs to migrate toward collagen. By contrast, migration of cells transfected with the kinase-truncated (K529*) or the kinase-dead DDR1a (K618A) was lower than wild-type SMCs (Figure 1C).

Zymogram gels were used to identify and assess gelatinase activity (Figure 2). DDR1−/− SMCs produced 4 major bands of gelatinolytic activity (lane 1): MMP-9 latent (102 kDa), MMP-9 active (92 kDa), MMP-2 latent (72 kDa), and MMP-2 active (62 kDa). DDR1−/− SMCs produced much less MMP activity compared with wild-type SMCs (lane 2). The DDR1−/− SMCs transfected with DDR1b produced all the MMPs at levels equivalent to wild-type SMCs (lane 3). By contrast, transfection of kinase-truncated (K529*) or kinase-dead (K618A) DDR1 was not rescued (lane 4). MMP-9 activity was reduced by transfection of DDR1b (lane 5) and DDR1−/− SMCs transfected with DDR1b produced all MMPs at levels equivalent to wild-type control (lane 3). By contrast, transfection of kinase-truncated (K529*) or kinase-dead (K618A) DDR1 did not rescue MMP production by the DDR1−/− SMCs.

Materials and Methods

SMCs were isolated from the aortas of DDR1−/− mice and wild-type littermates (mixture of 129/Sv and ICR strains). Knockout cells were transfected with the full-length cDNA of DDR1b, with a truncated form of DDR1b that lack the catalytic domain (K529*) or with a kinase-dead form of DDR1a containing a lysine to alanine mutation at position 618 in the catalytic domain (K618A).7 SM attachment was measured by toluidine blue staining; chemotaxis chamber migration assays and gelatin zymograms were performed using methods we have described.13 To localize the gelatinolytic activity in SMC cultures, the cells were overlaid with FITC-labeled DQ gelatin that is intermolecularly quenched (Enzchek; Molecular Probes). Gelatinase-catalyzed hydrolysis relieves the fluorescence quenching, and the cells were visualized using confocal microscopy.

An expanded Materials and Methods section can be found in the online data supplement available at http://www.circresaha.org.

Results and Discussion

A Western blot of cell lysates probed with a polyclonal antibody against human DDR1 detected a single band with a molecular mass of 125 kDa in DDR1−/− SMCs (Figure 1A, lane 1) but not in DDR1−/− SMCs (lane 2). Transfection of full-length DDR1b cDNA into DDR1−/− cells restored the expression of DDR1b (lane 4). DDR1−/− SMCs transfected with kinase-dead DDR1a (K618A) expressed an equivalent amount of the receptor (lane 3). There were no differences in expression of the DDR2 or β1 integrins between wild-type and DDR1−/− SMCs (not shown).

DDR1−/− SMCs from wild-type mice attachment to plates coated with 100 nmol/L type I collagen resulted in an optical density (OD) of 1.25±0.11, which was significantly greater than the attachment of the DDR1−/− cells with an OD of 0.61±0.04 (P<0.0001) (Figure 1B). Transfection with DDR1b or with the kinase-truncated (K529*) or kinase-dead (K618A) DDR1 rescued the ability of the DDR1−/− SMCs to attach to collagen (Figure 1B).

Mice were fed a high-fat diet for 8 weeks, and balloon catheter injury of the rat carotid artery, and intimal thickening was lower than wild-type SMCs (Figure 1C).

Zymogram gels were used to identify and assess gelatinase activity (Figure 2). DDR1−/− SMCs produced 4 major bands of lytic activity (lane 1): MMP-9 latent (102 kDa), MMP-9 active (83 kDa), MMP-2 latent (72 kDa), and MMP-2 active (62 kDa). DDR1−/− SMCs produced much less MMP activity compared with wild-type SMCs (lane 2). The DDR1−/− SMCs transfected with DDR1b produced all the MMPs at levels equivalent to wild-type control (lane 3). By contrast, transfection of kinase-truncated (K529*) or kinase-dead (K618A) DDR1 did not rescue MMP production by the DDR1−/− SMCs.

DDR1 was expressed on migrating SMCs after balloon catheter injury of the rat carotid artery, and intimal thickening after arterial injury was reduced in the DDR1−/− mouse.9 In the present study, we have investigated the role of DDR1 in SMC migration by transfecting DDR1−/− SMCs with full-length or kinase-mutated DDR1 constructs to determine whether kinase activity is required for migration and MMP production.

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One limitation of SDS-PAGE gelatin zymography is that latent MMPs appear active because SDS present in the gels denatures the proenzyme and exposes the active site. To assess gelatinase activity produced by live cells without exposure to SDS, we developed a technique for in situ gelatin zymography. SMCs were overlaid with auto-quenched, heavily FITC-labeled gelatin; where MMPs were active, gelatin was degraded with an increase in fluorescence that was detectable using confocal microscopy. In DDR1−/−/H11001 SMCs in subconfluent cultures, we found that gelatinase activity was localized to the cell periphery, associated with the cell membrane (Figure 3; monolayer, WT). Much less gelatinase activity was seen in cultures of DDR1+/−/H11002 SMCs (KO). The DDR1b-transfected knockout SMCs appeared to have even greater gelatinase activity than wild-type SMCs (LXSN-DDR1b). Gelatinase activity was inhibited completely by the MMP inhibitor phenanthroline.

We also assessed gelatinase activity in migrating cells after scrape-wounding the monolayer. In DDR1−/− SMCs, intense gelatinase activity was evident at the wound edge, but it was dramatically reduced in the DDR1−/− SMCs (Figure 3). Rescue of the DDR1−/− cells by transfection with LXSN-DDR1b resulted in an increase in gelatinase activity to wild-type levels.

We have demonstrated significant deficiencies in adhesion to collagen, migration, and MMP production in SMCs lacking DDR1. Transfection and reexpression of full-length DDR1b in the DDR1−/− SMCs rescued all of these deficits. This rules out the possibility that alterations in another pathway led to the deficits in the DDR1-null SMCs and suggests that in the absence of DDR1 the other collagen receptors (α1β1, α1β1, or DDR2) cannot compensate for these functions.

Our results are in agreement with other studies showing that the DDRs play important roles regulating cell migration and differentiation. It was recently reported that DDR1a is highly expressed in leukocytes, where it functions to mediate cell adhesion to and invasion into three-dimensional collagen gels.11 DDR2 is important for the migration and invasion of hepatic stellate cells6 and skin fibroblasts12 via an MMP-2–mediated process. Furthermore, the DDRs play important roles regulating the assembly and differentiation of neurons, mammary cells, and...
myoblasts in vitro, processes that involve coordinated interactions with the matrix during proliferation, migration, and extension of cellular protrusions.3,13,14

MMPs clear a path for migration by degrading matrix,15 modify the matrix to provide a substrate more favorable for migration,16 and may generate neoepitopes for cell surface receptors.17 Synthesis of new type VIII collagen stimulates the production of MMP-2 and MMP-9 by SMCs.3 Our results suggest that the DDR1 plays an important role in modulating SMC production of MMP-2 and MMP-9. Furthermore, our results show that active DDR1 kinase is critical for mediating MMP production, whereas simple attachment can be mediated by the receptor without catalytic activity. This suggests that activation of intracellular signaling pathways is required for MMP production and invasion of SMCs.

In conclusion, we have demonstrated that DDR1 played an important role in mediating SMC migration in response to type I collagen.

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References


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