Dominant Negative c-Jun Inhibits Platelet-Derived Growth Factor-Directed Migration by Vascular Smooth Muscle Cells

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Abstract. The mitogen–activated protein (MAP) kinase pathways has been shown to be necessary for mitogen-stimulated proliferation, but its role in cell migration has not been fully understood. In this study, we investigated the possible contribution of signaling pathways through c-Jun in platelet-derived growth factor (PDGF)-BB directed cell migration in rat aortic vascular smooth muscle cells (VSMCs) infected with a recombinant adenovirus containing the dominant-negative c-Jun (Ad-DN-c-Jun). DN-c-Jun protein was expressed dose-dependently in VSMCs infected with Ad-DN-c-Jun. Expression of DN-c-Jun significantly inhibited VSMC migration induced by PDGF-BB. Our results provide the first evidence that signaling pathways through c-Jun participates in cell migration induced by PDGF-BB in addition to other MAP kinase pathways in VSMCs.

Keywords: cell migration, vascular smooth muscle cell, gene transfer
using DN-c-Jun to inhibit activity of endogenous c-Jun in VSMCs.

VSMCs were prepared from thoracic aortas of 5- to 6-week-old male Sprangue-Dawley rats (Clea Japan, Tokyo) by using the collagenase digestion method, and cultured in DMEM (Sigma Chemical Co., St. Louis, MO, USA) containing 10% fetal bovine serum (FBS). For all experiments, rat aortic VSMCs from passages 5 to 7 were used. At 80% to 90% confluence, cells were placed in DMEM containing 0.1% FBS for 24 h prior to migration experiments. Cells were harvested with trypsin, counted, centrifuged, and re-suspended at $10^6$ cell/mL in 0.1% FBS DMEM. Cell migration was assayed using a modified Boyden chamber (Neuro Probe, Inc., Cabin John, MD, USA) (5, 10). Cells were plated on the upper side of a collagen-treated polycarbonate membrane with pores of 8 μM in diameter separating the two chambers of a 6.5-mm transwell culture plate. It is well established that PDGF modulates cell motility. Human recombinant PDGF-BB (Sigma Chemical Co.) was added to the lower chamber to stimulate cell migration. After 4 h, cells on the upper face of the membrane were scraped using a cotton swab. Cells that migrated to the lower face of the membranes were fixed with 4% formaldehyde and stained. The number of migrated cells on the lower face of the filter was counted in three fields.

VSMC migration was increased dose-dependently by the application of PDGF-BB in concentrations ranging from 2 – 10 ng/mL for 4 h (Fig. 1). PDGF-BB of 10 ng/mL is known to exert the maximal rate of DNA synthesis in VSMCs (9), so stimulation of VSMCs was carried out by PDGF-BB at a dose of 10 ng/mL in the following experiments.

To inhibit activity of endogenous c-Jun, we constructed Ad-DN-c-Jun and infected rat aortic VSMCs as previously described (9). The DN-c-Jun (TAM67) was generated by removing the transactivational domain of amino acids 3 to 122 of wild-type c-Jun by the polymerase chain reaction (Fig. 2A). Recombinant replication-defective E1 and E3 adenoviral vectors expressing the TAM67 gene (Ad-DN-c-Jun) were constructed by using an adenovirus expression vector kit (Takara Bioomedicals, Kyoto). A recombinant adenovirus containing bacterial β-galactosidase gene (Ad-LacZ) was also constructed as a negative control of Ad-DN-c-Jun. The titer of the virus was determined by limiting dilution in 293 cells and expressed as plaque-forming units. In vitro gene transfer to VSMCs was carried out by incubation with the adenovirus vector with a multiplicity of infection (MOI) of 10, 30, or 100 in DMEM containing 0.1% FBS for 1 h at 37°C and 5% CO₂/95% air. Then, VSMCs were made quiescent for 48 h before being assessed for the expression and effect of the transferred gene.

DN-c-Jun protein expressed dose-dependently in VSMCs infected with Ad-DN-c-Jun of 10, 30 and 100 MOI (Fig. 2B). Expression of DN-c-Jun protein in VSMCs was increased in a quiescent time dependent manner (Fig. 2C). The VSMCs were then made quiescent for 48 h, before being assessed for the expression and the effect of the transferred gene. To evaluate the possible contribution of c-Jun cascade via MKK (MAP kinase kinase) in cell migration, cell migration induced by PDGF-BB at 10 ng/mL was measured in VSMCs expressing DN-c-Jun. Gene transfer of Ad-DN-c-Jun of 30 and 100 MOI into VSMCs significantly decreased PDGF-BB-directed cell migration to 45% and 35% of non-infected control cells, respectively (Fig. 2D). The results indicate that over-expression of DN-c-Jun suppresses PDGF-BB-directed cell migration by inhibiting activity of endogenous c-Jun in VSMCs.

The proto-oncogene c-Jun is implicated in the regulation of cell motility as well as of gene expression and of cell proliferation, transformation, differentiation, and apoptosis. JNK specific inhibitors decrease the basal motility of epithelial cells and suppress cell motility stimulated by a JNK agonist (11). Retinoic acid inhibits cardiac neural crest migration by blocking JNK activation (12). On the other hand, vascular endothelial growth factor is known to induce cell migration and a marked reorganization of the microfilament network,
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followed by stimulating MAP kinases, ERK and p38 kinase, but not JNK in human umbilical vein endothelial cells (8). It is also noted that ERK but not JNK is sufficient in stimulating cell motility, while ERK and JNK activity are necessary for AP-1 activation in epidermal growth factor-stimulated human epidermal keratinocytes (13). The role of c-Jun in cell motility responses is likely to depend on the cell type and the stimuli that the cell is receiving. Recent studies using enzyme inhibitors for MAP kinases also failed to demonstrate that PDGF activates the signaling pathway through c-Jun in VSMCs (14, 15). However, we have previously demonstrated that PDGF-BB increases AP-1 transcriptional activity and cell proliferation in rat aortic VSMCs but suppresses those in cells expressing DN-c-Jun protein, indicating that the involvement of c-Jun in PDGF-BB-induced cell proliferation in VSMCs infected with Ad-DN-c-Jun (9). We provide here the first evidence that c-Jun participates in cell migration induced by stimulation of the PDGF receptors by PDGF-BB, by the use of Ad-DN-c-Jun to inhibit activity of endogenous c-Jun in the quiescent VSMCs.

In the present study, we also showed that SB203580 and PD98059 partly inhibit PDGF-directed VSMC migration (data not shown). The result confirmed the notion that the p38 MAP kinase and ERK pathways regulate VSMC migration induced by growth factors. Taken together with these findings, the present results suggest that PDGF activates JNK and p38 pathways via MAP kinase in addition to the ERK pathway via MEK, mediating migration of VSMCs.

Acknowledgments

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References

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