Aims

DJ-1/park7 is a ubiquitously expressed multifunctional protein that plays essential roles in a variety of cells. However, its function in the vascular system has not been determined. We investigated the protective roles of DJ-1/park7 in vascular disorders, especially in neointimal hyperplasia.

Methods and results

DJ-1/park7 was strongly expressed in the neointimal layer, in which its oxidized form was predominant. Treatment of vascular smooth muscle cells (VSMCs) from the mouse aorta with H2O2 increased the oxidation of DJ-1/park7 visualized on two-dimensional electrophoresis gels. The growth of VSMCs in FBS-containing media and the release of H2O2 were significantly increased in DJ-1/park7–/– knockout mice compared with DJ-1/park7+/+ wild-type mice. The expression of cyclin D1 and the phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 were greater in VSMCs from the DJ-1/park7–/– aorta than from the DJ-1/park7+/+ aorta. Both of these measures were inhibited by treatment with an ERK1/2 inhibitor or antioxidants and in DJ-1/park7-overexpressing cells. VSMC proliferation, cyclin D1 expression, and ERK1/2 phosphorylation in response to platelet-derived growth factor-BB were upregulated in DJ-1/park7–/– compared with DJ-1/park7+/+ mice. VSMCs of DJ-1/park7–/– mice exhibited higher levels of sprout outgrowth of aortic strips and neointimal plaque formation elicited by carotid artery ligation compared with those of DJ-1/park7+/+ mice.

Conclusion

These results indicate that DJ-1/park7 is involved in the growth of VSMCs, thereby inhibiting neointimal hyperplasia, and suggest that it might play protective roles in vascular remodelling.

Keywords

DJ-1/park7 • Vascular smooth muscle cell • Proliferation • Neointima plaque • Reactive oxygen species

1. Introduction

Many human disorders, including neurological diseases, cancer, diabetic complications, and ageing, are thought to be associated with an imbalance in redox regulation or with an excess production of reactive oxygen species (ROS).1 These molecules include superoxide anion (O2–), hydrogen peroxide (H2O2), hydroxyl radical (OH•), and nitric oxide (NO), and are associated with a wide variety of cell activities.2 Moreover, ROS play an important role in a number of activities involved in vascular remodelling.3 Extracellular stimuli are known to elevate ROS levels and to initiate the activation of mitogen-activated protein kinases (MAPKs),4 comprising the extracellular signal-regulated kinase (ERK) 1/2, p38 MAPK, and stress-activated protein kinase/c-jun N-terminal kinase.5 These MAPKs participate in cell growth and proliferation in response to various stimuli including platelet-derived growth factor (PDGF).6,7 It is well known that the proliferation of vascular smooth muscle cells (VSMCs) is closely associated with vascular disorders such as atherosclerosis and restenosis after angioplasty.8,9 Prolonged exposure of VSMCs to cytokines, growth factors, or oxidative stress results in cell proliferation, which exacerbates vascular disorders.8,10
Cell-cycle progression is tightly regulated at various biological checkpoints by cyclins, cyclin-dependent kinases (CDKs), and their inhibitors.\textsuperscript{11,12} Among the cyclin families, cyclin D1 regulates the G1-to-S phase cell-cycle transition. During the G1 phase, cyclin D1 is synthesized and bound to CDK4 and CDK6 in response to growth factors; its expression is regulated at the transcriptional level.\textsuperscript{13,14} It is known that cyclin D1 is involved in cell growth and proliferation through an ROS-mediated pathway. Thus, ROS have been implicated in the pathogenesis of disorders that are associated with the cell cycle.\textsuperscript{15} We and others have reported that the cell cycle and its related regulatory proteins in VSMCs are involved in cell proliferation and the formation of neointimal plaques.\textsuperscript{16,17} Therefore, identifying the causes of vascular proliferation is important if we are to understand the control of restenosis in blood vessels.

DJ-1/park7, also known as CAP1, was first found as a novel oncogene and identified as being responsible for an autosomal recessive early-onset form of Parkinson’s disease (PD).\textsuperscript{18,19} It is a ubiquitous expressed multifunctional protein that plays essential roles in biological activity in a variety of cells, and is involved in transcriptional regulation, antioxidative stress, and cancer formation.\textsuperscript{20} It is known that ROS are a common cause of the pathogenesis of neuronal and vascular diseases and that hypertension and atherosclerosis are associated with an increased risk of developing PD.\textsuperscript{2,20} These results imply that ROS-regulating proteins such as DJ-1/park7 might play key roles in both neuronal and vascular disorders. Recently, we identified DJ-1/park7 as an oxidative stress-responsive protein in VSMCs, using proteomic analysis.\textsuperscript{21} The expression of renal DJ-1/park7 was regulated by the activation of the dopamine receptor, leading to hypertension.\textsuperscript{22} Although these reports collectively suggest that DJ-1/park7 might modulate the function of the vascular system, its role in VSMCs has not been investigated. Therefore, in this study, we attempted to investigate the roles of DJ-1/park7 in the pathophysiology of VSMCs, especially in cell growth and proliferation, and to explore the potential protective roles of DJ-1/park7 in vascular disorders such as atherosclerosis and restenosis after angioplasty.

2. Methods

See Supplementary material for an expanded version of this section.

2.1 Animals

Male DJ-1/park7-homozygous knockout (DJ-1/park7\textsuperscript{−−}; B6.Cg-Park7tm1shn/J) mice (n = 28) and wild-type (DJ-1/park7\textsuperscript{+−}) mice (n = 28) with the same genetic background were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). This investigation was performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996) and was approved by the Animal Subjects Committee and by the Institutional Guidelines of Konkuk University, Korea.

2.2 Preparation of VSMCs

Animals were euthanized using CO\textsubscript{2} gas and bled by cutting the carotid arteries. VSMCs were enzymatically isolated from thoracic aorta of male DJ-1/park7\textsuperscript{−−} mice and wild DJ-1/park7\textsuperscript{+−} mice (6–8 weeks old, 20 g, n = 6) by collagenase and elastase treatment.

2.3 Cell growth and proliferation assays

To determine VSMC growth and proliferation, a 2,3-bis [2-methyloxy-4-nitro-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT) assay was employed using a WelCount\textsuperscript{TM} cell proliferation assay kit (WelGENE, Daegu, Korea).

2.4 Overexpression of DJ-1/park7 protein

A pcDNA3.1-V5 DEST-IHO3149 expression vector containing the whole coding sequence of DJ-1/park7 was constructed using a pcDNA3.1/nV5-DEST\textsuperscript{TM} Gateway\textsuperscript{TM} Vector Pack (Invitrogen, Carlsbad, CA, USA). VSMCs were transfected using Basic Nucleofector Kits with the Amaza Nucleofector (Amaza Biosystems GmbH, Cologne, Germany), according to the manufacturer’s instructions.

2.5 Measurement of intracellular H\textsubscript{2}O\textsubscript{2}

The generation of intracellular H\textsubscript{2}O\textsubscript{2} in VSMCs was measured using both the green fluorescence probe 6-carboxy-2,7′-dichlorofluoroscein diacetate (DCF-DA; Molecular Probes, Eugene, OR, USA) and flurometric analysis.

2.6 Immunohistochemical staining, western blotting, and ex vivo aortic sprout assay

Analyses were performed with methods as used in previous reports.\textsuperscript{23–25}

2.7 Mini two-dimensional electrophoresis-based western blotting

For western blotting using mini two-dimensional electrophoresis (2-DE) gels, protein samples from mouse aortas treated with H\textsubscript{2}O\textsubscript{2} at 300 \textmu M, a concentration that induces oxidative stress-related responses and protein modification in VSMC, were separated with mini immobilized pH gradient (IPG) strips (7 cm; pH 3–10, nonlinear).\textsuperscript{23,24}

2.8 Carotid artery ligation

Carotid artery ligation modelling was performed by the modification of a documented protocol.\textsuperscript{26} Briefly, male DJ-1/park7\textsuperscript{−−} and DJ-1/park7\textsuperscript{+−}/+ mice at 10–12 weeks of age were anesthetized by intramuscular injection of zotilet (1.6 mg/kg body weight; Verbac Laboratories, Carros, France) and rompun (1.2 mg/kg; Bayer Korea, Seoul, Korea). The adequacy of anaesthesia was monitored from the disappearance of pedal withdrawal reflex response to foot pinch. After the skin at the neck was opened, the common carotid artery was exposed and ligated with a 6–0 silk suture proximal from the carotid bifurcation. The animals were sacrificed by CO\textsubscript{2} inhalation at 3 weeks after carotid artery ligation for each experiment.

2.9 Statistical analysis

Data are expressed as the mean ± SEM. Statistical evaluation of the data was performed using Student’s t-test for comparisons between pairs of groups and ANOVA for multiple comparisons using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA, USA); P < 0.05 was considered to be statistically significant.

3. Results

3.1 DJ-1/park7 upregulation in the neointima and responses to ROS

We characterized the pattern of expression of DJ-1/park7 in mouse neointimal plaque tissues after 3 weeks of carotid artery ligation. The neointima exhibited greater DJ-1/park7-positive fluorescence than in the adjacent medial layer (n = 8; Figure 1A and C) or in the nonligated control artery (n = 8; Figure 1A and B). The level of oxidized DJ-1/park7 was prominent in both the neointimal and medial layers, but it was greater in the neointimal layer than in the medial layer of ligated (n = 8; Figure 1A and C) or nonligated arteries.
To further evaluate the characteristics of DJ-1/park7 expression in response to exogenous oxidative stress, mice aortic smooth muscle strips were treated with H$_2$O$_2$ and proteins were then separated using the 2-DE technique. As shown in Figure 1D, treatment with 300 mM H$_2$O$_2$ for 30 min diminished the intensity of basic spots (the reduced form) of DJ-1/park7, whereas it increased that of acidic spots (the oxidized form) ($n=4$).

### 3.2 Elevated ROS generation in VSMCs from DJ-1/park7$^{-/-}$ mice

DJ-1/park7$^{-/-}$ and littermate DJ-1/park7$^{+/+}$ mice were subjected to PCR genotyping and western blot analysis. As shown in Figure 2A, PCR analysis of distal tips of tails from mice showed the absence of the gene encoding DJ-1/park7 in DJ-1/park7$^{-/-}$ mice and normal expression in DJ-1/park7$^{+/+}$ mice. Similar expression results were observed by western blotting analysis in aortic tissues ($n=9$; Figure 2A). We examined the levels of H$_2$O$_2$ produced in VSMCs from DJ-1/park7$^{-/-}$ and DJ-1/park7$^{+/+}$ aortas. The level of H$_2$O$_2$ was increased in VSMCs from DJ-1/park7$^{-/-}$ aorta compared with those from DJ-1/park7$^{+/+}$ aorta ($n=6–7$; Figure 2B and C).

### 3.3 Increased vascular responsiveness in DJ-1/park7$^{-/-}$ mice

To investigate the effects of DJ-1/park7 deficiency on vascular responses, we analysed the growth of VSMCs. Cell growth induced in medium with 10% FBS was time-dependently increased in VSMCs from DJ-1/park7$^{-/-}$ and DJ-1/park7$^{+/+}$ aortas and the response was greater in DJ-1/park7$^{-/-}$ mice than in DJ-1/park7$^{+/+}$ mice at each time point: 0, 12, 24, 36, and 48 h of the treatment ($n=8$; Figure 2D). PDGF-BB (10 ng/mL) stimulated proliferation in VSMCs from both DJ-1/park7$^{-/-}$ and DJ-1/park7$^{+/+}$ mice, but to a greater extent in the former cells ($n=8$; Figure 2E).

**Figure 1** DJ-1/park7 expression and its oxidation in response to oxidative stress and in neointimal plaque. (A) DJ-1/park7 expression and oxidation in the neointimal plaque of carotid arteries. Immunohistochemical staining for total and oxidized DJ-1/park7 was performed on histological cross-sections from mouse carotid arteries. Red: DJ-1/park7-positive cells (DJ-1/park7); green: oxidized (at C106) DJ-1/park7-positive cells (Ox-DJ-1/park7). I, intima; M, media. (B and C) Average fluorescence intensity of total and oxidative forms of DJ-1/park7. Nonligated vs. ligated arteries (B) and intimal vs. medial layers in ligated arteries (C). Each data were obtained from average intensity analysis of the images demonstrated in panel A. *$p<0.05$ ($n=8$). (D) Changes in the isoelectric point (pI) in IPG gels of the DJ-1/park7 protein from mouse aortic strips by H$_2$O$_2$ treatment. Strips were treated with H$_2$O$_2$ (300 µM) for 30 min. Arrows indicate the basic (reduced form, Re) and acidic (oxidized form, Ox) spots of DJ-1/park7 protein. $n=4$. *$p<0.05$; vehicle-control vs. H$_2$O$_2$-treated groups.
3.4 Changes in growth-associated signals in DJ-1/park7–/– VSMCs

To assess how cell growth was induced by FBS and PDGF-BB in DJ-1/park7–/– mice, we compared cyclin D1 expression and MAPK phosphorylation in both VSMC sets. The expression of cyclin D1 was elevated in VSMCs cultured in 10% FBS-media in a time-dependent manner, but it was greater in those from DJ-1/park7–/– mice than from DJ-1/park7+/+ mice at each time point: 0, 12, and 24 h of culture (n = 6; Figure 3A). The phosphorylation of ERK1/2 induced by 10% FBS in DJ-1/park7–/– VSMCs was increased time-dependently, and the degree of this response at each time point was significantly greater than in the DJ-1/park7+/+ cells (n = 6; Figure 3B). Moreover, treatment with PDGF-BB (10 ng/mL) for 12 or 24 h elevated the expression of cyclin D1 and the phosphorylation of ERK1/2 in VSMCs from DJ-1/park7+/- and DJ-1/park7+/+ mice. These responses in DJ-1/park7+/- were greater than in DJ-1/park7+/+ mice (n = 4; Figure 3C and D).

To assess the role of cyclin D1-associated signalling in VSMC growth, the effects of inhibitors of ERK1/2 and ROS on cyclin D1 expression were examined. Cyclin D1 expression levels in VSMCs at 24 h of culture with 10% FBS were greater in DJ-1/park7–/– cells than in DJ-1/park7+/+ cells. This expression in both cells was inhibited by treatment with 30 μM PD98059, an ERK1/2 inhibitor (n = 6; Figure 4A). Moreover, the treatment of VSMCs with 3 mM tempol, an ROS inhibitor, attenuated the expression of cyclin D1 in VSMCs at 24 h (n = 6; Figure 4B). Similar results were also observed in cells treated with 3 mM tiron, an ROS inhibitor (n = 6; Figure 4B). Moreover, these ROS inhibitors suppressed the growth in response to 10% FBS in cells from DJ-1/park7–/– and DJ-1/park7+/+ mice (n = 8; Figure 4C).

3.5 Reversible effects of DJ-1/park7 overexpression in DJ-1/park7–/– VSMCs

We further tested the roles of DJ-1/park7 protein in vascular function, using a DJ-1/park7-overexpressing vector system. VSMCs from DJ-1/park7–/– mice were transfected with the DJ-1/park7 gene, using electroporation, and the level of DJ-1/park7 expression was assessed using western blotting. As shown in Figure 5A, the transfection of the DJ-1/park7 vector into VSMCs from DJ-1/park7–/– mouse aortas enhanced the level of protein expression. Cyclin D1 expression was reduced in DJ-1/park7-overexpressing cells compared with cells transfected with the control DJ-1/park7-free vector (n = 6; Figure 5B). Similar results were also observed for ERK1/2 phosphorylation levels (n = 6). Moreover, VSMC growth induced by 10% FBS at each
time point (0, 12, and 24 h) was decreased in DJ-1/park7-overexpressing cells compared with controls \((n = 8; \text{Figure } 5B)\).

### 3.6 Elevation of aortic sprout outgrowth and neointimal hyperplasia in DJ-1/park7–/– mice

The increased growth in VSMCs from DJ-1/park7–/– mice was evaluated by aortic sprout assays ex vivo.\(^2\)\(^3\)\(^2\)\(^4\) Aortic strips from both DJ-1/park7\(^{+/+}\) and DJ-1/park7–/– mice were embedded in Matrigel, and sprout outgrowth was assessed. In the quiescent state cultured with vehicle alone, the sprout growth of the aortic strips was greater in strips from DJ-1/park7–/– mice than in those from DJ-1/park7\(^{+/+}\) mice \((n = 8; \text{Figure } 6A)\). PDGF-BB (10 ng/mL) induced an increase in the sprout growth of the aortic strips that was greater in those from DJ-1/park7–/– mice than from DJ-1/park7\(^{+/+}\) mice. To investigate the effect of DJ-1/park7 on the neointimal formation in carotid arteries, we used a model of ligation-induced neointimal plaque
formation. As shown in Figure 6B, the intima-to-media ratio was also significantly elevated in the carotid arteries of DJ-1/park7–/– mice compared with DJ-1/park7+/+ mice ($n=14$). Furthermore, proliferating cell nuclear antigen (PCNA)-positive staining in cross-sections of the carotid neointima was higher in tissues from DJ-1/park7–/– mice than in those from DJ-1/park7+/+ mice ($n=4$; see Supplementary material online, Figure SI).

4. Discussion

In the present study, we found that the DJ-1/park7 gene was expressed in VSMCs and was prominent in neointimal layers after carotid ligation, implying that it might not only participate in VSMC physiology, but also contribute to vascular disorders. We also found that the FBS-induced growth of DJ-1/park7–/– VSMCs was significantly greater than that of DJ-1/park7+/+ VSMCs and that this was reversed in DJ-1/park7-overexpressing VSMCs. These results were verified by the increases in neointimal plaque formation and ex vivo sprout growth in DJ-1/park7+/+ mice. Moreover, there were more PCNA-positive cells in neointimal layers in DJ-1/park7–/– mice than in DJ-1/park7+/+ mice. These results indicate that DJ-1/park7 is tightly involved in the VSMC growth that occurs during neointimal formation after carotid artery ligation. Furthermore, vascular responsiveness as measured by VSMC proliferation in response to PDGF-BB was greater in DJ-1/park7–/– mice than in DJ-1/park7+/+ mice. It is known that VSMC proliferation and migration are critical for the development of neointimal plaques. PDGF and activation of its receptors have been implicated in the cellular activation in a variety of cells, most notably VSMCs, and in neointimal plaque formation. These results imply that DJ-1/park7 might play crucial roles in vascular pathophysiology—in particular, neointimal plaque formation—by inhibiting VSMC responsiveness. This is the first report showing that DJ-1/park7 might be associated with VSMC functions in the vascular system. These possible new effects of DJ-1/park7 on vascular function could provide a basis for therapeutic methods aimed at inhibiting VSMC proliferation after angioplasty.

We also found that the generation of H$_2$O$_2$ was elevated in DJ-1/park7–/– VSMCs compared with those from DJ-1/park7+/+ mice, as noted in previous reports. The treatment of VSMCs with H$_2$O$_2$ showed an increase in the oxidation of DJ-1/park7 protein in 2-DE-based western blotting analyses. It is known that vascular ROS levels are increased following balloon angioplasty. Here, we showed that the oxidative form of DJ-1/park7 was increased in the neointimal layer of the ligated artery compared with the nonligated
control artery. This might have been caused by the excessive ROS produced after ligation. It is known that DJ-1/park7 scavenges ROS by rapid oxidation, which results in a change in the protein from the basic to the acidic form. From these results, it can be assumed that ROS are produced excessively by a variety of cells, including VSMCs migrating from the medial layer or proliferating within neointimal plaques, and that this induces oxidation of the DJ-1/park7 protein as an antioxidative protective measure. Furthermore, a role for DJ-1/park7 has been implicated in the response to oxidative stress.20,33 Thus, overexpression of DJ-1/park7 protected neuronal cells against oxidative stress-induced injury. However, although the level of DJ-1/park7 was elevated in the neointimal layer, plaque formation was not suppressed. From these results, we surmise that modifications of DJ-1/park7 render it unable to exert its antioxidative properties, especially against oxidation mediated by ROS generated in the neointima. Cell responses in this study were inhibited by treatments with ROS inhibitors. Elevated ROS levels stimulate the synthesis of many mitogenic factors that stimulate VSMC proliferation and neointimal plaque formation.35,36 Administration of antioxidants has been reported to diminish neointimal formation by suppressing VSMC proliferation.37 Therefore, we suggest that the increase in cell growth and proliferation seen in DJ-1/park7+/− VSMCs might be mediated by the increased ROS-related signalling, caused in turn by a deficiency in the antioxidative functions of DJ-1/park7 in these gene knockout mice.

Here, we showed that the levels of cyclin D1 expression and ERK1/2 phosphorylation were increased in DJ-1/park7+/− VSMCs compared with DJ-1/park7+/+ VSMCs. These effects were reversed by the inhibition of ERK1/2 or of ROS production and in DJ-1/park7-overexpressing VSMCs. ERK1/2 has been identified as a common pathway for enhancing the growth and proliferation of VSMCs, and ROS enhancement of the activity of this kinase.38 ROS signalling can initiate the ERK1/2 pathway that activates growth-promoting transcription factors or cell-cycle regulators such as cyclin D1, thereby supporting cell proliferation.38,39 PDGF stimulation increases the expression of cyclins E1 and D1 and of related kinases.40 Cyclin D1 is implicated in the stimulation of cell growth and proliferation through an ROS-mediated pathway.41 We reported previously that MAPK activation was regulated by the production of ROS in VSMCs.17,41 These results indicated that the elevated expression of cyclin D1 in DJ-1/park7+/− VSMCs might be regulated via ROS-induced ERK1/2 activation. Furthermore, a recent finding showed that DJ-1/park7 expression could be linked to spleen tyrosine kinase (Syk) and that this kinase was a mediator acting in PDGF receptor activity and MAPK signalling in VSMCs.42,43 Syk is also activated by ROS in VSMCs, and this leads to the formation of neointimal plaques induced by endothelial cell injury.17 Given these results, it is likely that DJ-1/park7 binds to Syk and inhibits its kinase activity, and that this inhibition is reversed by the oxidation of DJ-1/park7 by ROS, eventually leading to the Syk activation-associated signalling associated with vascular pathology. Therefore, we suggest that functional interactions between Syk and DJ-1/park7 might play central roles in neointimal plaque formation.

Cardiovascular risk factors such as diabetes mellitus and central obesity have been associated with the pathogenesis of PD. Thus, hypertension is an important risk factor for PD, and optimal control of blood pressure may reduce its incidence.44 Moreover, neurological diseases, atherosclerosis, cancer, diabetic complications, and ageing are all thought to be regulated by an imbalance in cellular redox systems.45 Oxidative stress causes functional damage to DJ-1/park7 in individuals with PD,46 and also, as shown here, in the vascular neointima. Previously, we identified DJ-1/park7 as an oxidative stress-responsive protein in VSMCs.21 Therefore, we suggest that DJ-1/park7 can participate in oxidative stress-related pathogenesis in a variety of cellular systems and that DJ-1/park7 might act as a key protein performing key functions in the development of various diseases.

In summary, we have demonstrated here that the growth of VSMCs and the formation of neointimal plaques were increased in DJ-1/park7+/− mice compared with DJ-1/park7+/+ controls. Cyclin D1 expression and ERK1/2 phosphorylation in response to FBS or PDGF-BB were greater in DJ-1/park7+/− VSMCs than in those from DJ-1/park7+/+ mice and these effects were reversed by treatment with ROS inhibitors and in DJ-1/park7-overexpressing cells. These results indicate that DJ-1/park7 is involved in the growth of VSMCs via the ERK1/2-mediated cyclin D1 pathway, thereby inhibiting the formation of neointima. We suggest that DJ-1/park7 might play protective roles in modulating the risk of vascular remodelling.
**Supplementary material**

Supplementary material is available at Cardiovascular Research online.

**Conflict of interest:** none declared.

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**References**


**Figure 6** Elevated formation of aortic sprout outgrowth and neointimal plaques in DJ-1/park7–/– mice. (A) Aortic sprout outgrowth. Aortic strips (1 × 1 mm²) were embedded in Matrigel and treated with PDGF-BB (10 ng/mL) or vehicle-control (4 μM HCl). The results were observed on day 5. Upper panels: Representative images showing the outgrowth of aortic strip sprouts. Lower panels: Graphs of the results obtained from the upper panels. The sprout formation level in the vehicle-treated group of aortic strips from DJ-1/park7++ was expressed as 100% (n = 8). (B) Neointimal plaque formation. H&E staining was performed on histological cross-sections from the left carotid artery sampled 21 days after ligation. The upper panels are representative micrographs showing neointimal formation in the ligated carotid artery. The lower graph shows the intima-to-media ratio obtained from the upper panels (n = 14). *p < 0.05; DJ-1/park7++ vs. DJ-1/park7–/–. #p < 0.05; vehicle vs. PDGF-BB-treated groups. I, intima; M, media.


