Microsomal Prostaglandin E₂ Synthase-1 Modulates the Response to Vascular Injury

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Background—Microsomal (m) prostaglandin (PG) E₂ synthase (S)-1 catalyzes the formation of PGE₂ from PGH₂, a cyclooxygenase product that is derived from arachidonic acid. Previous studies in mice suggest that targeting mPGES-1 may be less likely to cause hypertension or thrombosis than cyclooxygenase-2–selective inhibition or deletion in vivo. Indeed, deletion of mPGES-1 retards atherogenesis and angiotensin II–induced aortic aneurysm formation. The role of mPGES-1 in the response to vascular injury is unknown.

Methods and Results—Mice were subjected to wire injury of the femoral artery. Both neointimal area and vascular stenosis were significantly reduced 4 weeks after injury in mPGES-1 knockout mice compared with wild-type controls (65.6±5.7 versus 37.7±5.1×10³ pixel area and 70.5±13.4% versus 47.7±17.4%, respectively; P<0.01). Induction of tenascin-C, a proproliferative and promigratory extracellular matrix protein, after injury was attenuated in the knockouts. Consistent with in vivo rediversion of PG biosynthesis, mPGES-1–deleted vascular smooth muscle cells generated less PGE₂ but more PGI₂ and expressed reduced tenasin-C compared with wild-type cells. Both suppression of PGE₂ and augmentation of PGI₂ attenuate tenascin-C expression and vascular smooth muscle cell proliferation and migration in vitro.

Conclusions—Deletion of mPGES-1 in mice attenuates neointimal hyperplasia after vascular injury, in part by regulating tenasin-C expression. This raises for consideration the therapeutic potential of mPGES-1 inhibitors as adjuvant therapy for percutaneous coronary intervention. (Circulation. 2011;123:631-639.)

Key Words: injury ■ percutaneous transluminal coronary angioplasty ■ prostacyclin ■ prostaglandins

A rachidonic acid is metabolized by cyclooxygenases (COXs) to an endoperoxide intermediate, prostaglandin (PG) H₂, which is subsequently converted by terminal isomerases to specific PGs. Traditional nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen relieve pain and fever by inhibiting COX-1 and COX-2. Some traditional NSAIDs such as diclofenac and meloxicam are selective inhibitors of COX-2. Purposefully designed NSAIDs selective for inhibition of COX-2 such as celecoxib predispose patients to myocardial infarction and stroke.1–3 Diverse lines of evidence indicate that this is attributable to suppression of COX-2–derived prostacyclin (PGI₂).4

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Microsomal (m) PGE synthase (S)-1, which catalyzes the isomerization of PGH₂ into PGE₂, has emerged as an alternative drug target to COX-2.5–7 Two other PGE synthases have been identified, mPGES-2² and cytosolic PGES.³⁰ mPGES-1, however, is the dominant source of PGE₂ biosynthesis, at least under basal conditions and in inflammatory syndromes in mice.¹¹ Targeting of mPGES-1 may be less likely to cause hypertension or thrombosis than COX-2–selective inhibitors in vivo.¹⁴,¹² Although this distinction may not be absolute,³³ deletion of mPGES-1 also retards atherogenesis¹⁴ and attenuates angiotensin II–induced abdominal aortic aneurysm formation in low density lipoprotein receptor null (LDLR⁻/⁻) mice.¹⁵ COXs and PGs differentially modulate the response to vascular injury. For example, wire-induced vascular proliferation is enhanced in mice that are genetically deficient in the PGI₂ receptor (IP), whereas deletion of the thromboxane A₂ receptor depresses this response.¹⁶ Despite the association of purposefully designed COX-2 inhibitors with a cardiovascular hazard, there are conflicting reports of the impact of disrupting this pathway on vascular remodeling. For example, pharmacological suppression of COX-2–derived PGI₂ pro-
motes adverse vascular remodeling in a flow-induced injury model, an effect replicated by deletion of the IP. However, celecoxib, with presumed inhibition of the same pathway, reduces neointimal hyperplasia in balloon-injured carotid arteries in rats and rabbits. Furthermore, a small controlled clinical trial suggests that celecoxib reduces in-stent late luminal loss at 6 months in patients with coronary artery disease who are receiving aspirin plus clopidogrel. Although Wu et al found that celecoxib, but not mPGES-1 deletion increased mortality in mice after experimental myocardial infarction, Degousee et al observed delayed adverse ventricular remodeling in mPGES-1 knockouts (KOs).

Tenascin-C (TN-C) is a multifunctional extracellular matrix (ECM) glycoprotein that regulates cell differentiation, proliferation, survival, and migration during development and tissue remodeling. TN-C is highly expressed during embryonic branching morphogenesis and dissipated in adult lungs. TN-C is re-expressed during wound healing and regeneration, as well as under pathological conditions. In the latter setting, TN-C promotes pulmonary and systemic vascular neo-intimal hyperplasia by promoting vascular smooth muscle cell (VSMC) growth. In the case of pulmonary hypertension, TN-C regulates VSMC growth in part by inducing integrin-associated EGF receptor activation followed by regulation of downstream cell survival. A role for PGs in the regulation of TN-C expression is suggested by earlier studies. Inhaled iloprost, a prostacyclin analog, reverses TN-C expression and vascular remodeling in experimental pulmonary hypertension in rats. PGE2 induces expression of TN-C in stromal cells of the murine uterus. Matrix metalloproteinases upregulate TN-C by generating β3 integrin ligands in type I collagen, whereas mPGES-1 promotes vascular matrix metalloproteinase-2 activity in a vascular inflammatory condition, aortic aneurysm.

Here, we demonstrate that deletion of mPGES-1 in mice attenuates neointimal hyperplasia after vascular injury. Both suppression of PGE2 and redirection of the accumulated PGH2 substrate to PGI2 may contribute to impaired VSMC proliferation, dysregulated expression of TN-C expression, and impaired VSMC migration in the KOs. These studies indicate a potential utility of targeting mPGES-1 in percutaneous coronary intervention.

**Methods**

**Mice and the Vascular Injury Model**

mPGES-1 KOs and their wild-type (WT) controls were generated by corresponding homozygous breeders derived from mPGES-1/H/H mice, which are backcrossed to C57B6 for 7 generations from the original mPGES-1/H/H mice, which were a kind gift from Pfizer. The femoral artery wire injury was performed as previously described with modifications. Briefly, 1 side of the femoral artery was exposed by blunt dissection while mice were under anesthesia. The accompanying femoral nerve and femoral vein were carefully separated from the artery. The femoral artery was looped proximally and distally with 6–0 silk suture for temporary blood flow cessation during the procedure. A small branch between the rectus femoris and vastus medialis muscles was isolated; a transverse arteriotomy was performed in the branch; and a flexible angioplasty wire (0.35-mm diameter; Cook Inc, Bloomington, IN) was inserted into the femoral artery for 3 mm toward the iliac artery. The wire was left in place for 3 minutes to denote and dilate the artery. Then, the wire was removed, and the silk suture looped at the proximal portion of the muscular branch artery was secured. Blood flow in the femoral artery was restored by releasing the sutures, and the skin incision was closed with a 5–0 silk suture. The femoral artery on the other side was sham-operated and served as a control. Alzet osmotic minipumps (model 2002, Alza Scientific Products, Palo Alto, CA) were placed subcutaneously via a midback incision and loaded with...
Bromodeoxyuridine (BrdU; Sigma-Aldrich, St Louis, MO) to deliver 25 mg·kg⁻¹·d⁻¹. Twenty-eight days after injury, animals were euthanized and perfused with 0.9% NaCl followed by Prefer (Anatech, Battle Creek, MI). Femoral arteries were harvested and embedded in paraffin for morphometric or histological analysis. All animals were housed according to guidelines of the Institutional Animal Care and Use Committee of the University of Pennsylvania, and all experiments were approved by that committee.

**Biochemical, Molecular, and Cellular Methods**

PGs or metabolites were determined with ultraperformance liquid chromatography–tandem mass spectrometry as previously described. The ultraperformance liquid chromatography column used was a Hypersil GOLD, 200×2.1 mm, with a particle size of 3 μm (Thermo Scientific, West Palm Beach, FL), which allows distinction of the authentic peak of 2,3-dinor-6-keto PGF₁α from an endogenous peak of unknown identity at the specified ion transition. VSMCs were isolated from the aortas of WT, mPGES-1, or IP KO mice as previously described, and only VSMCs below passage 5 were used. Neutralized collagen solutions were applied to thin film of fibrillar type I collagen, which allows cell behavior after seeding onto thin film of fibrillar type I collagen, to be monitored. Such prepared collagen films have increased rigidity, which can cause cells to assume a proliferative phenotype, mimicking an injured state in vivo. Migration studies and TN-C staining were carried out 60 hours after siRNA transfection with overnight serum starvation.

**Measurements of Cell Motility**

Cell motility was studied by posthoc analysis of movies that record cell behavior after seeding onto thin film of fibrillar type I collagen, prepared as described previously. Neutralized collagen solutions were applied to flat-bottom, non–tissue-cultured–treated polystyrene plates (BD Biosciences, San Jose, CA) and then placed at 37°C overnight to initiate fibrillogenesis. After incubation, the gelled collagen film (monolayer) was rinsed with PBS (~10 times) and deionized water (~10 times). Samples were then dried under a stream of filtered N₂ and immediately placed back into a PBS solution. Such prepared collagen films have increased rigidity, which can cause cells to assume a proliferative phenotype, mimicking an injured state in vivo. Twenty-four hours before the experiments, cells were treated with complete medium containing 2% FBS. Before seeding, cells were trypsinized with 0.25% trypsin-EDTA (Gibco BRL, Invitrogen Corp) and seeded in 0.5 mL of 2% FBS medium at a density of 2×10⁶ cells per well (~5000 cells/cm²). Recording of cell motility was started 10 minutes after seeding to allow the setting of the microscope. Three or 4 fields of view (movies) were recorded for SMCs from each animal, and 2 animals of each genotype were used.

The live cell culture imaging system consists of a custom-made biochamber with humidified atmosphere at 37°C with 5% CO₂ incorporating a digitalized controlled x-y-z motorized stage driven by a stepper motor drive systems mounted on an inverted microscope (Nikon TE 2000-U, Nikon, Tokyo, Japan). Images were acquired with a high-resolution cooled charge-coupled device camera (Photometrics, Hopkinton Scientific, Tucson, AZ) at 5-minute intervals over 24 hours with a ×10 phase-contrast objective lens. Image sequences were processed with Nikon Elements Software (version 3.0; Nikon), converted to an 8-bit image, and enhanced for contrast and brightness with Image J software (version 1.38x; National Institutes of Health, Bethesda, MD). The percentage of spreading cells at a given time point after cell seeding was calculated from the movies. Five cells per movie were randomly chosen for velocity calculation, with the only criterion being that cells should be within an observation frame from recording start till the end. The center point of the selected cells, ie, the average of the x and y coordinates of all of the

**Morphometry and Histology**

Cross sections of the injured arteries of individual animals were serially obtained at 10 levels from the distal branch point of the femoral artery at 140-μm intervals (8 levels were sectioned for assessment of early cell infiltration/migration) and were stained with hematoxylin and eosin. Images were acquired with a charge-coupled device camera coupled to an inverted Nikon E600 microscope. Images were then digitized with Image Pro software (Media Cybernetics, Silver Spring, MD). Morphometric analysis was performed with a customized program (Phase 3 Imaging Systems, Glen Mills, PA) of Image Pro to measure the area of the lumen, the area inside the internal elastic lamina, and the area inside the external elastic lamina. This system was also used to count BrdU-labeled cells within the media and intima of vessels. Percentage stenosis was calculated as the ratio of the intimal area to the area inside the original internal elastic lamina, and the ratio of intima to media was determined. Sections with the maximal lesion area were selected to represent the animal from which femoral arteries had been harvested. Histology staining is detailed in the online-only Data Supplement.
pixels in the image or selection, was used to calculate the position of the cells at each time point. Distance traveled between each current time point and the immediate past time point was used to calculate the velocity at an individual time point.

Cell motility was also studied by both a scratch wound healing assay and a transwell assay. VSMCs were plated for 24 hours in complete media and were then serum starved overnight before the monolayer was scratched with a pipette tip for the scratch wound healing assay. Cell motility was recorded as described above every 30 minutes for 24 hours, and serial images were converted to TIFF and MPEG movie formats for analysis. The distance cells traveled between the time when the surface was scratched and 24 hours later

Figure 3. mPGES-1 modulates TN-C expression. A, Reduced TN-C expression in injured vessel of mPGES-1 KOs. TN-C was stained in WT (top) or KO (bottom) noninjured (left) and injured (right) vessels, as labeled. Scale bar=20 μm. B, TN-C expression in cultured VSMCs. TN-C was stained green and nuclei were stained blue by DAPI. TN-C expression was suppressed in mPGES-1 KO VSMCs compared with WT VSMCs (top). Whereas PGE₂ treatment only slightly increased the expression of TN-C in WT VSMCs (middle), iloprost reduced TN-C expression (bottom), with both drugs applied at 280 nmol/L. Bar=20 μm. C, Levels of PGE₂ and 6-keto-PGF₁α (the PGI₂ hydrolysis product) in cultured VSMCs. **P<0.01, n=6 per group. D, Expression of TN-C in SMCs treated with cicaprost (PGI₂ analog) at the indicated concentration as reflected by immunofluorescence (left) and quantitative reverse-transcriptase polymerase chain reaction (right). KO SMCs produced less TN-C than WT SMCs. *P<0.05; **P<0.01; ***P<0.001. n=4. All comparisons are relative to the WT group. Data presented are representative of 3 independent experiments.
mPGES-1 deletion also reduced thromboxane formation, which is a key modulator of platelet aggregation and vascular contraction. The endoperoxide, prostacyclin (PGI₂), and the prostaglandins (PGE₂ and PGD₂) were differentially regulated in the mPGES-1 KO mice. A representative graph showing the reduced production of thromboxane in KO mice compared to WT is shown in Figure 3A.

Statistical Analysis
Data are expressed as mean±SEM unless otherwise indicated. Comparisons of multiple groups were performed by ANOVA, and when only 2 mean values were compared, the 2-tailed t test was used. Bonferroni correction or the Dunnett test was applied when appropriate as indicated in the text. In all cases, statistical significance was defined as P<0.05.

Results
Deletion of mPGES-1 Reduces Neointimal Hyperplasia in the Wire-Injured Femoral Artery
To examine the role of mPGES-1 in vascular remodeling, mice were subject to endothelial denudation by wire injury to the femoral artery and examined 28 days later. Injured vessels from mPGES-1–deficient mice showed a reduced neointimal thickness compared to WT mice (Figure 1A). Further, the percentage of proliferating cells detected after BrdU incorporation was reduced in KO mice compared to WT (Figure I in the online-only Data Supplement). Cellular infiltration/migration to the subendothelium was also limited by mPGES-1 deletion 7 days after the injury (Figure II in the online-only Data Supplement).

Vascular injury resulted in a procedure-related increase in PG biosynthesis. Deletion of mPGES-1 significantly reduced synthesis of PGE₂ while augmenting PGI₂ and PGD₂. This contrast in product formation was even more pronounced when WT and KO mice were subject to injury. In that setting, formation of thromboxane was also augmented in the KOs (Figure 2 and Figure III in the online-only Data Supplement).

mPGES-1 Deletion Modulates TN-C Expression and VSMC Migration
Expression of TN-C was upregulated in medial and neointimal VSMCs in response to injury, and this response was attenuated by mPGES-1 deletion (Figure 3A). Consistent with these in vivo studies, mPGES-1 deletion also reduced TN-C expression in cultured VSMCs (Figure 3B). Formation of the 2 most abundant prostanoids formed by VSMCs, PGE₂ and PGI₂, was differentially regulated by mPGES-1 deletion in vivo, and a similar divergent pattern of formation was observed in VSMCs cultured ex vivo (Figure 3C). Here, we failed to detect the production of PGD₂ by VSMCs, and the trivial amounts of thromboxane formation were unaltered by gene deletion (data not shown). Treating WT VSMCs with PGE₂ at 28 or 280 nmol/L slightly increased TN-C expression. In contrast, the IP agonist iloprost strikingly decreased TN-C expression at the same concentrations (Figure 3B and data not shown). Cicaprost, another IP agonist, also dose-dependently suppressed TN-C expression.

Statistical Analysis
Data are expressed as mean±SEM unless otherwise indicated. Comparisons of multiple groups were performed by ANOVA, and when only 2 mean values were compared, the 2-tailed t test was used. Bonferroni correction or the Dunnett test was applied when appropriate as indicated in the text. In all cases, statistical significance was defined as P<0.05.
expression (Figure 3D). Thus, the increased levels of PGI2 may substantially contribute to the suppression of TN-C in mPGES-1 KO SMCs.

Increased expression of TN-C after injury provides a scaffold on which VSMCs may migrate in the process of neointima formation.41 Consistent with the restraint in expression of TN-C, migration of mPGES-1 KO VSMCs on a collagen-coated surface was impaired (Figure 4A and representative movies in the online-only Data Supplement). During the initial portion of the motility assays, attachment and spreading of VSMCs from KO mice were delayed (Figure IV in the online-only Data Supplement). mPGES-1 deletion also attenuated VSMC migration in a scratch-induced wound healing assay (Figure 4B). Knockdown of TN-C (Figure V in the online-only Data Supplement) similarly impaired VSMC migration in this assay (Figure 4C). Treatment with exogenous TN-C partially rescued the impaired migration of mPGES-1 KO VSMCs in the transwell migration assay (Figure 4D). Consistent with their divergent effects on TN-C expression, the IP agonists iloprost (28 or 280 nmol/L) and cicaprost42 (10 nmol/L) inhibited VSMC migration (Figure 5A and 5C and data not shown), whereas PGE2 (28 nmol/L) rescued the impaired migration of mPGES-1 KO VSMCs (Figure 5B and 5D).

Differential Regulation of VSMC Cell Cycle by PGs in mPGES-1 KOs
VSMC proliferation was also influenced by mPGES-1 deletion. S-phase entry was suppressed in VSMCs cultured from KOs (Figure 6A and 6B). PGE2 dose-dependently promoted proliferation and rescued this phenotype (Figure 6A). PGE2 similarly affected proliferation in both WT and IP KO SMCs (Figure 6B) without changing PGI2 release into the media (data not shown), indicating that the proliferative effect of PGE2 does not depend on signaling via the IP. On the other hand, cicaprost inhibits S-phase entry in WT VSMCs, whereas CAY10441,43 an IP antagonist, promotes this phenomenon (Figure 6A). Thus, both suppression of PGE2 and augmentation of PGI2 may contribute to the restraint on...
integrated injury-induced VSMC proliferation observed in mPGES-1 KOs.

**Discussion**

The role of COX-2 inhibition and mPGES-1 deletion in cardiovascular remodeling is complex. Although COX-2 inhibition restrains neointima formation in response to wire or balloon vascular injury, it exacerbates the remodeling response to restriction of blood flow in ligated arteries. Although global mPGES-1 deletion, unlike COX-2 inhibition, does not increase mortality after coronary artery ligation in mice, it does adversely influence myocardial remodeling. Here, we report that, in contrast, the vascular remodeling response to injury is restrained in mPGES-1–deficient mice. Although the mechanistic distinctions that account for this phenotypic divergence in mPGES-1 KOs are currently unclear, they likely reflect 2 fundamental properties: the contrasting biological responses evoked by prostanooids and the contrasting predominant products of substrate rediversion products of mPGES-1 deletion in distinct cell types. Furthermore, despite mPGES-1 deletion, PGE2 formation is actually sustained in the peri-infarct myocardium as a result of infiltration of myeloid cells expressing alternate PGES enzymes, whereas in the setting of vascular injury, biosynthesis of PGE2 and its formation by VSMCs are suppressed.

Intimal hyperplasia is a feature of vascular remodeling caused by either injury or changes in blood flow. It is characterized by abnormal migration and proliferation of SMCs coincident with de novo deposition of the surrounding ECM. TN-C, an ECM glycoprotein, is upregulated during neointimal hyperplasia and associated with the synthetic proliferative phenotype of VSMCs after balloon injury, in pulmonary hypertension, and in vascular grafting. It may provide a scaffold along which proliferating VSMCs can migrate to form the neointima. Interestingly, TN-C is subject to regulation by PGs in this study and others. Thus, TN-C constitutes a focus for mechanistic elucidation of mPGES-1–modulated vascular remodeling. We have demonstrated that the deletion of mPGES-1 attenuates upregulation of TN-C in response to injury and that knockdown of TN-C impairs VSMC migration in vitro. In VSMCs, the dominant effect of mPGES-1 deletion on VSMC PG formation is to depress PGE2 and to augment PGI2. Cicaprost, a highly selective PGI receptor agonist, dose-dependently downregulates TN-C expression in VSMCs, whereas PGE2 slightly upregulates TN-C. In this study, we show that these prostanooids have contrasting effects on TN-C expression and thus may have contributed to the impact of mPGES-1 on VSMC migration and proliferation. It is possible that mPGES-1 deletion may regulate expression of other ECM molecules besides TN-C, which may mediate VSMC behavior. Global production of ECM was examined, as reflected by trichrome stain (Figure VI in the online-only Data Supplement). ECM was increased by injury in both WT arteries and mPGES-1 KOs; however, the composition of ECM cannot be identified by this approach and awaits elucidation.

Deletion of mPGES-1 also suppresses VSMC proliferation in response to vascular injury. This response is mimicked by knockdown of TN-C (Figure VII in the online-only Data Supplement). As with VSMC migration, the effects of mPGES-1 deletion on both PGE2 and PGI2 may have been relevant. Thus, PGE2 dose-dependently restores impaired proliferation of mPGES-1 in mPGES-1 KOs. In contrast, activation of the IP by cicaprost suppresses proliferation in both WT and KO VSMCs. Thus, both depression of PGE2 and augmented formation of PGI2 may have contributed to the suppression of VSMC proliferation in mPGES-1 KO mice.

These studies raise the possibility of mPGES-1 inhibition as a strategy to limit restenosis after percutaneous coronary intervention. However, a limitation may prove to be the contrasting effect on myocardial remodeling after coronary ligation, & preliminary evidence suggests that in patients receiving platelet inhibitors to limit this risk, restenosis might be reduced by this purposely designed NSAID selective for inhibition of COX-2. Whereas mPGES-1 inhibitors might be expected to confer a diminished risk of myocardial infarction compared with COX-2 inhibitors, the comparative safety and efficacy of these compounds in the setting of percutaneous coronary intervention merits further consideration.

**Conclusions**

Deletion of mPGES-1 decreases vascular injury–induced VSMC proliferation, in part by regulating TN-C expression and subsequent neointimal hyperplasia. Both suppression of PGE2 and augmented formation of PGI2 may contribute to these effects. The therapeutic potential of locally delivered mPGES-1 inhibitors as an adjuvant for percutaneous coronary intervention merits further consideration.

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**Disclosures**

Dr FitzGerald is the McNeil Professor of Translational Medicine and Therapeutics. He has consulted in the past year for Astra Zeneca, Daiichi Sankyo, Logical Therapeutics, Lilly, and Nicox on NSAIDs and related compounds. The other authors report no conflicts.

**References**


**CLINICAL PERSPECTIVE**

Nonsteroidal antiinflammatory drugs specific for the inhibition of cyclooxygenase-2 (COX-2) confer a risk of myocardial infarction and stroke. Microsomal (m) prostaglandin (PG) E2 synthase (S)-1 represents an alternative antiinflammatory target downstream of COX-2. Inhibition of mPGES-1 may be less likely to predispose patients to thrombotic events than inhibition of COX-2. Despite the risk of myocardial infarction conferred by celecoxib in placebo-controlled trials, preliminary evidence suggests that in patients receiving platelet inhibitors to limit this risk, restenosis might be reduced by this purposefully designed nonsteroidal antiinflammatory drug selective for inhibition of COX-2. Here, we demonstrate that deletion of mPGES-1 in mice attenuates neointimal hyperplasia after vascular injury. Both suppression of PGE2 and redversion of the accumulated PGH2 substrate to PGI2 may contribute to dysregulated expression of tenascin-C, an extracellular matrix glycoprotein, resulting in impaired vascular smooth muscle cell migration and proliferation in the knockouts. These studies raise the possibility of mPGES-1 inhibition as a strategy to limit restenosis after percutaneous coronary intervention. However, a limitation may prove to be the contrasting effect on myocardial remodeling after coronary ligation. Although mPGES-1 inhibitors might be expected to confer a diminished risk of myocardial infarction and mortality after myocardial infarction compared with COX-2 inhibitors, the safety and efficacy of these compounds in the setting of percutaneous coronary intervention remain to be determined. The therapeutic potential of locally delivered mPGES-1 inhibitors as an adjuvant for percutaneous coronary intervention merits further consideration.
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Histology

Paraffin sections were deparaffinized, rehydrated, and then incubated with 3% H₂O₂ to block endogenous peroxidase activity for immunohistochemistry. After antigen-retrieval and blocking non-specific IgG binding, anti-TN-C rabbit polyclonal antibody (Chemicon International Inc., Temecula, CA) was applied followed by incubation with biotinylated species-specific secondary antibodies in conjunction with a Vectastain Elite ABC kit (Vector Laboratory Inc, Burlingame, CA). Sections were exposed to diaminobenzidine (DAB) (Vector Laboratory Inc, Burlingame, CA), prior to counter-staining with hematoxylin to visualize antigens. Fluorescence conjugated anti-rabbit IgG (Invitrogen, Carlsbad, CA) was applied to the fixed cells following primary antibody incubation for detection of TN-C in SMC. Nuclei were stained with DAPI (KPL Inc., Gaithersburg, Maryland) and mounted with fluorescent mounting media (KPL Inc). The same exposure time for fluorescence microscope imaging was used in all groups for comparison. Staining of mPGES-1 was carried out as described above with the corresponding primary antibody (Cayman Chemicals, Ann Arbor, MI, Cat. 160140). Rabbit Antibody Enhancer and Polymer-HRP for rabbit (Golden Bridge International, Mukilteo, WA, Cat. 40-6) was used as secondary antibody. Non-immunized rabbit IgG was used for negative control staining.
Supplemental Figure 1. mPGES-1 expression in injured artery. A cross section from an artery, harvested four weeks after the injury, was stained for mPGES-1 (demarked by brown). N denotes neointima; M denotes media; ▶ indicates internal elastin; ▼ indicates external elastin. Scale bar denotes 10 µm.

Supplemental Figure 2. Cellular infiltration/migration in subendothelium. Wire injury induced cell infiltration/migration to the subendothelial space (as indicated by arrow head) one week after the injury. Subendothelial cell number (right panel) was quantified on H&E stained sections across the injured arteries (average of 8 levels). (*: p<0.05, n=5).

Supplemental Figure 3. Consequences of mPGES-1 deletion on urinary metabolites of PGD2 and TxA2 in vascular remodeling. Urinary metabolites of PGD2 (A) and TxB2 (B), 11,15-dioxo-9alpha-hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid (tetranor PGDM) and 2,3 dinor - TxB2 (Tx-M), respectively, were compared between genotypes at baseline, day 1 and day 14 post injury. (t-test with Bonferroni correction. *: p<0.05; **: p < 0.01. n=14-16 per group)

Supplemental Figure 4. Impaired adhesion and spreading in mPGES-1−/− VSMCs. VSMC motility was examined for 24 hours after plating cells on collagen thin films, as detailed in methods. The percentage of spreading cells are plotted for WT (left panel) and KO (right panel) VSMCs. Each curve corresponds to a single observation field under the microscope over the 24-hour observation period. Seven and eight movies were recorded from two animals of each genotype. Representative movies are shown in online supplemental data.

Supplemental Figure 5. TN-C knockdown in VSMCs.
A: Staining of TN-C expression (green) in cultured VSMCs 60 hours after transfection. Nuclei shown in blue by DAPI staining. B: TN-C mRNA expression was analyzed 48 hours after transfection by quantitative PCR. Expression of TN-C mRNA was normalized to 18S rRNA and presented as percentage change relative to control siRNA. Data are representative of three independent experiments.

**Supplemental Figure 6. Masson's trichrome staining of wire-injured arteries.**
Remodeled arteries four weeks after wire-injury (right panels) and non-injured control arteries (left panels) from WT (upper panels) and mPGES-1 KO (lower panels) were stained by Trichrome. Blue: collagen, mucin; Black: nuclei; Red: cytoplasm, keratin, muscle fibers. Bar=20 µm.

**Supplemental Figure 7. Knock-down of TN-C reduces S phase entry in VSMCs.**
Mouse SMCs transfected with control or TN-C siRNA (100 nM) for 60 hours were serum starved for 48 h and stimulated with 2% FBS for 48 h in the presence of BrdU. The data are presented as mean ± SD of three independent experiments. (*: p<0.05, n=3).
Supplemental Figure 1
Supplemental Figure 2

WT  KO

![Image of WT and KO samples with annotations and bar graph showing internal cell number comparison]

*
Supplemental Figure 3

![Graph showing changes in PGD-M and Tx-M over time post-injury.](image-url)
Supplemental Figure 4

The figure shows the percentage of spreading cells over time for wild-type (WT) and knockout (KO) conditions. The x-axis represents relative units of time, and the y-axis represents the percentage of spreading cells. The graph indicates that the percentage of spreading cells increases over time for both WT and KO conditions, with some variations in the rate of increase.
Supplemental Figure 5

Control siRNA

TN-C siRNA

Tenascin C mRNA (Fold)

Control siRNA
TN-C siRNA 100 nM
TN-C siRNA 300 nM
Supplemental Figure 6

Non-injured

Injured

WT

KO
Supplemental Figure 7

The bar chart shows the % BrdU Incorporation for two groups: Control siRNA and TN-C siRNA. The chart indicates a significant difference between the two groups, with the TN-C siRNA group showing a lower % BrdU Incorporation compared to the Control siRNA group.
Supplemental video data

VSMC motility was examined for 24 hours after plating VSMCs on collagen thin films, as detailed in methods. Representative movies for WT and in mPGES-1 KO VSMCs are shown in online Supplementary video file WT and KO respectively. VSMC velocity was decreased in mPGES-1 KOs.