Deletion of the PDGFR-β Gene Affects Key Fibroblast Functions Important for Wound Healing

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This study provides new perspectives on the unique aspects of platelet-derived growth factor β-receptor (PDGFR-β) signaling and biological responses through the establishment of a mutant mouse strain in which two loxP sequences were inserted into the introns of PDGFR-β gene sequences. Isolation of skin fibroblasts from the mutant mice and Cre recombinase transfection in vitro induced PDGFR-β gene deletion (PDGFR-βΔΔ). The resultant deletion of the PDGFR-β protein significantly attenuated platelet-derived growth factor (PDGF)-BB-induced cell migration, proliferation, and protection from H2O2-induced apoptosis of the cultured PDGFR-βΔΔ dermal fibroblasts. PDGF-AA and fetal bovine serum were mitogenic and anti-apoptotic but were unable to induce the migration in PDGFR-βΔΔ fibroblasts. Concerning the PDGF signaling, PDGF-BB-induced phosphorylation of Akt, ERK1/2, and JNK, but not p38, decreased in PDGFR-βΔΔ fibroblasts, but PDGF-AA-induced signaling was not altered. Overexpression of the phospholipid phosphatases, SHIP2 and/or PTEN, inhibited PDGF-BB-induced phosphorylation of Akt and ERK1/2 in PDGFR-βΔΔ fibroblasts but did not affect that of JNK and p38. These results indicate that disruption of distinct PDGFR-β signaling pathways in PDGFR-βΔΔ dermal fibroblasts impaired their proliferation and survival, but completely inhibits migratory response, and that PDGF-BB-induced phosphorylation of Akt and ERK1/2 possibly mediated by PDGFR-α is regulated, at least in part, by the lipid phosphatases SHIP2 and/or PTEN. Thus, the PDGFR-β function on dermal fibroblasts appears to be critical in PDGF-BB action for skin wound healing and is clearly distinctive from that of PDGFR-α in the ligand-induced biological responses and the underlying properties of cellular signaling.

Platelet-derived growth factors (PDGFs)† are major mitogens for connective tissue cells that are involved in diverse biological processes including physiological development, tissue repair, tumorigenesis, and atherosclerosis (1). PDGF family members, PDGF-A, -B, -C, and -D, are assembled as disulfide-linked homo- or heterodimers and exert their activity by binding to and activating specific high affinity cell surface receptors. Two receptor subtypes with protein-tyrosine kinase activity have been identified that can form homo- and heterodimeric receptor complexes: the α-subunit, which can bind to the A-, B-, and C-chains of PDGF, and the β-subunit, specific for the B-, C- and D-chains (1–4). Dermal fibroblasts are one of the major target cells of PDGF in the initiation and propagation of wound healing in the skin (5). Levels of PDGF receptor (PDGFR)-α expression are high in fibroblasts during early embryogenesis, and disruption of PDGFR-α results in a reduction in fibroblasts throughout the embryo (6, 7). In contrast, targeted deletion of PDGFR-β and analysis of blastocyst chimeras demonstrated no effect of PDGFR-β on fibroblast development (8, 9). However, in mice prepared from the blastocyst chimeras that contain a combination of wild-type and PDGFR-β−/− cells, analysis of granulation tissue formation following the subcutaneous implantation of sponges demonstrated that PDGFR-β−/− cells were depleted in the granulation tissue (10). These reports suggest that the two subtypes of PDGFR play distinct roles in development and that PDGFR-β is important in wound healing by dermal fibroblasts. Analysis of mutant mice in which the cytoplasmic signaling domain of the PDGFR-β was used to replace the PDGFR-α cytoplasmic domain showed no obvious defects in any of the PDGFR-α-dependent cell types (11). On the other hand, when the PDGFR-β was dependent upon PDGFR-α cytoplasmic domain, multiple abnormalities occurred in vascular smooth muscle cell development (11). These data suggest that PDGFR-β has unique signaling capacities compared with PDGFR-α.

PDGF binding to the receptors activates a variety of intracellular signaling molecules (12). One of these is phosphatidylinositol 3-kinase (PI3K), which results in the local accumulation of P(3,4,5)P3 at the plasma membrane. Synthesized

BrdUrd, 5-bromo-2’-deoxyuridine; DMEM, Dulbecco’s modified Eagle’s medium; ERK, extracellular signal-related kinase; ES cells, embryonic stem cells; FBS, fetal bovine serum; JNK, c-Jun NH2-terminal kinase; MAPK(s), mitogen-activated protein kinase(s); m.o.i., multiplicity of infection; neo-TK, neomycin-thymidine kinase; PDGFR, platelet-derived growth factor receptor; PI(3,4,5)P3, phosphatidylinositol 3,4,5-trisphosphate; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; SHIP2, Src homology domain 2-containing inositol phosphatase 2; pfu, plaque-forming units.

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PI3(3,4,5)P3 recruits the pleckstrin homology domain-containing signaling molecule Akt/PKB to the membrane (12, 13). PDGFs are also known to activate the mitogen-activated protein kinase (MAPK) families, including the extracellular signal-regulated kinase (ERK1/2), c-Jun NH2-terminal kinase (JNK), and p38 MAPK. ERK1/2 is predominantly activated by receptor tyrosine kinases of growth factors, whereas JNK and p38 are preferentially activated by stress-inducing stimuli such as UV light, heat shock, pro-inflammatory cytokines, and by mitogenic stimuli as well (14). The PI3K/Akt and MAPKs pathways play important roles in the regulation of cell growth, migration, and survival during the wound healing process (13, 15, 16), although the relative importance of these kinases in various cellular phenomena differ depending on the cell type (14). SHIP2 and PTEN are also known to inhibit PDGF activation of Akt and Akt-mediated signaling are still poorly understood.

To elucidate the biological properties and signaling pathways of PDGFR-β, it is critical to analyze its function in nontransformed cells that physiologically express the receptor. Because homozygous disruption of PDGF-B or PDGFR-β results in perinatal death in mouse embryos (8, 19), it has been difficult to evaluate the role of the PDGF-B/PDGFR-β system in repair of adult tissues, such as wound healing. To address this problem, we established a new mouse strain in which the PDGFR-β exons are flanked by two loxP sequences. The Cre-mediated recombination by adenovirus-gene transfer markedly reduced the expression of PDGFR-β in the dermal fibroblasts derived from the mutant mice without affecting the expression of PDGFR-α. By using these dermal fibroblasts, we studied the PDGF-induced biological effects, including proliferation, migration, and apoptosis, which are central processes in wound healing. In addition, we examined the involvement of PDGFR-β in the activation of Akt and MAPKs to clarify the intracellular signals activated by the PDGFs. Finally, we examined the effect of the phospholipid phosphatases, SHIP2 and PTEN, in the regulation of PDGF-BB signaling in dermal fibroblasts lacking PDGFR-β.

**EXPERIMENTAL PROCEDURES**

**Materials**—Human recombinant PDGF-AA was provided by Strathmann Biotech AG (Hamburg, Germany), and human recombinant PDGF-BB was purchased from Invitrogen. Polyclonal anti-PDGFR-β antibody was from Upstate Biotechnology, Inc. (Lake Placid, NY). Polyclonal anti-PDGFR-α antibody, polyclonal anti-Tyr685 phoshospho-specific PDGFR-β antibody, monoclonal anti-phosphotyrosine antibody (PY99), monoclonal anti-Akt1 antibody, and monoclonal anti-Pten antibody were from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal anti-Thr187 phosphospecific Akt antibody, polyclonal ERK1/2 antibody, polyclonal anti-PTEN antibody, monoclonal anti-PI3K p85α 185/186 phosphorylated JNK antibody, polyclonal anti-p38 antibody, and polyclonal anti-Thr180/Tyr182 phosphospecific p38 antibody were from Cell Signaling Technology, Inc. (Beverly, MA). Polyclonal anti-SHIP2 antibody was generated as described previously (20). Fetal bovine serum (FBS) was obtained from BioWhittaker A Cambrex Inc. (Walkersville, MD). Dulbecco’s modified Eagle’s medium (DMEM) was from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). All other reagents were of analytical grade and were purchased from Sigma or Wako Pure Chemical Industries Ltd. (Osaka, Japan).

**Generation of PDGFR-βfl/fl Knockout Mouse**—For the construction of the targeting vector, a 13.5-kb BamHI/SpeI genomic fragment of the PDGFR-β gene from the 129X1/SvEv mouse was cloned into pBluescript SK– (Stratagene, La Jolla, CA). We inserted a neomycin-thymidine kinase (neo-TK) selection cassette and one-loxP sequence at the 5′- and 3′-ends, respectively, of the 3.3-kb FspI/HpaI fragmentic genomic that includes exons 4–7 encoding the extracellular domain of the PDGFR-β (Fig. IA). The neo-TK selection cassette was flanked by two loxP sequences (PDGFR-βfllox/fllox) were then injected into blastocysts to obtain chimeric mice. The chimeras were bred with C57BL/6 mice (Sankyo Laboratory, Tokyo, Japan) to obtain the F1 progenies containing the recombinant allele (PDGFR-βfllox/fllox). These F1 progenies were cross-bred to obtain PDGFR-βfllox/fllox homozygous mice. Genotyping was performed by both Southern blotting and PCR-based analysis. The probes for Southern blotting are shown in Fig. 1A. The primers for genomic PCR were as follows: primer 1, 5′-TAGCCATGGACTCTCTCTCAGCTCTAATTCC-3′; primer 2, 5′-CTGCTCAATAGTACCTCACAATCCTGTGTA-3′; primer 3, 5′-TTCTTGTTCTGAGACGTCCTTGTGATGAGA-3′; primer 4, 5′-TGTCGACAGATTCTCCTGCTGGGAAATC-3′; and primer 5, 5′-AGCAAGGCTCGGCAAGGATAACAGC-3′.

**Cell Culture and Infection with Adenovirus**—Mouse dermal fibroblasts were prepared from 8- to 12-week-old male control C57BL/6 mice and PDGFR-βfllox/fllox mice (21). Briefly, mice were anesthetized with pentobarbital (50 mg/kg body weight), and a full thickness of the back skin was cut out by scissors. The skin tissues were cut into small pieces and were implanted into plastic tissue culture dishes containing DMEM containing 10% FBS. The fibroblast cultures were used after seven passages. Cre recombinase, PTEN, and SHIP2 were transiently expressed in cultured cells by adenovirus-mediated gene transfer. Cells were infected in DMEM containing 5% FBS at a multiplicity of infection (m.o.i.) of 10 pfu/cell. After 16 h, the virus was removed by replacing the medium. Experiments were conducted 24–48 h after the initial addition of the virus.

**DNA Synthesis Assay**—The cultured fibroblasts obtained from wild-type or PDGFR-βfllox/fllox mice were infected with Cre-adenovirus as described above. The cells were cultured in DMEM containing 10% FBS for an additional 24 h. The cells were then plated into 96-well plates at 1.5 × 104 cells/well in DMEM containing 10% FBS. After 24 h, the cells were serum-starved for 24 h and then were incubated for 16 h with 1 nM thymidine (MTS) or 1 nM PDGF-BB, or 10% FBS in DMEM containing 1 nM 5-bromo-2′-deoxyuridine (BrdUrd). BrdUrd incorporation into DNA was determined with a 5-bromo-2′-deoxyuridine labeling and detection kit (Roche Applied Science) according to the manufacturer’s instructions and detected with an enzyme-linked immunosorbent assay plate reader (Nippon InterMed. Ltd., Tokyo, Japan).

**Wound Scratch Assay**—Confluent cultured fibroblasts obtained from wild-type or PDGFR-βfllox/fllox mice were grown in 6-well plates and were infected with Cre-adenovirus as described above. The cells were cultured in DMEM containing 10% FBS for an additional 24 h. After the cells were serum-starved for 24 h in DMEM, they were scratched with a 1-ml plastic pipette tip and washed twice with PBS to remove the floating cells. The cells were then cultured in DMEM supplemented with 1 nM PDGF-AA, 1 nM PDGF-BB, or 10% FBS. After 48 h, the cells were viewed with an inverted phase contrast microscope (Olympus CK30, Tokyo, Japan) and photographed.

**Apoptosis Assay**—Apoptosis was detected using an APOPercentage™ kit (Biocolor Ltd., Belfast, Northern Ireland). Briefly, confluent wild-type or PDGFR-βfllox/fllox fibroblasts grown in 6-well plates were infected with Cre-adenovirus as described above. After infection, the cells were cultured for an additional 24 h in DMEM containing 10% FBS. Apoptosis was induced by 1 μM H2O2 in 1 ml of DMEM containing 1 nM PDGF-AA, 1 nM PDGF-BB, or 10% FBS, and 50 μl of APOPercentage Dye. After 60 min, the medium was drained from the wells, and the cells were washed twice with PBS. The cells were then observed with an inverted phase contrast microscope.

**Immunoprecipitation and Western Blotting**—Confluent wild-type and PDGFR-βfllox/fllox fibroblasts grown in 6-well plates were infected for...
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16 h with the Cre-adenovirus at an m.o.i. of 10 pfu/cell. The cells were then incubated for 24 h in DMEM containing 10% FBS. Next, the cells were serum-starved for 48 h and then treated with various concentrations of PDGF-AA or PDGF-BB at the indicated times. The cells were lysed for 15 min at 4 °C in a buffer consisting of 50 mM Tris, 150 mM NaCl, 10 mM EDTA, 0.5% sodium deoxycholate, 1% Triton X-100, 2 mM Na3VO4, 150 mM sodium fluoride, 2 mM phenylmethysulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin (pH 7.4). Lysates obtained from the same number of cells were centrifuged to remove insoluble materials. The supernatants were incubated with the indicated antibodies for 4 h at 4 °C and then immune complexes were mixed with glutathione-Sepharose for 2 h at 4 °C. The immune complexes were precipitated by centrifugation. Immune complexes or whole cell lysates were separated by SDS-PAGE and then electrophoretically transferred to polyvinylidene difluoride membranes. The membranes were incubated for 1 h at 20 °C in a buffer containing 50 mM Tris, 150 mM NaCl, 0.1% Tween 20, and 5% nonfat dry milk. The membranes were then probed with specific antibodies for 16 h at 4 °C. After the membranes had been washed in a buffer containing 50 mM Tris, 150 mM NaCl, and 0.1% Tween 20, the blots were incubated with an appropriate horseradish peroxidase-conjugated secondary antibody. Immunoreactive bands were detected using enhanced chemiluminescence reagents (Amersham Biosciences) according to the manufacturer’s instructions.

Immunofluorescence—Cultured wild-type or PDGFR-βflox/flox fibroblasts were infected with the Cre-adenovirus as described above and were replated in chamber slides. All staining procedures were performed at room temperature. After growth for 24 h, the cells were fixed with 1% formalin in PBS for 10 min. Nonspecific immunoreactions were blocked by incubation for 20 min with 10% goat serum in PBS. Cells were then incubated for 2 h with 1:100 anti-PDGFR-β, washed three times with PBS, and then incubated for 1 h with 1:1,000 Alexa Fluor 488-conjugated goat anti-rabbit IgG (Molecular Probes, Eugene, OR). The cells were mounted with Vectashield mounting medium containing 4,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA) and observed with an Olympus AX80 microscope.

Statistical Analysis—All data were presented as means ± S.D. Student’s t test was used to determine the p values, and p < 0.05 was considered statistically significant.

RESULTS

PDGFR-βflox/flox Mutant Mice Are Healthy and Show No Gross Abnormalities—After transfection of the targeting vector (Fig. 1A) and selection with neomycin, we obtained four ES cell clones with homologous recombination out of the 319 clones examined. In addition to a 12.8-kb fragment, clones that had undergone recombination generated a 7.3-kb fragment in Southern blotting using a 5’-probe after digestion of the genomic DNA with XbaI (Fig. 1B). Generation of a 5.7-kb fragment after SacI digestion and an 8.8-kb fragment after XbaI digestion was detected by Southern blotting using a 3’-probe and neo probe, respectively (Fig. 1B).

ES cell clones that had undergone homologous recombination were transfected with Cre-expressing plasmid (pCre-Pac; gift from Dr. Takeshi Yagi, Osaka University, Japan) to delete the neo-TK selection cassette. Deletion of the neo-TK selection gene was confirmed by Southern blotting with an internal probe (data not shown). The resulting ES clones (PDGFR-βflox/−) were used for the generation of chimera mice.

The PDGFR-βflox/− ES clones were also transfected with the pCre-Pac plasmid to confirm that the Cre recombinase can induce recombination and eliminate the floxed PDGFR-β allele. After transfection with the pCre-Pac plasmid, genomic PCR with primers 1 and 2, and 5 generated bands of 271, 329, and 410 bp, which corresponded to the PDGFR-β genes for wild-type, floxed, and deleted alleles (PDGFR-βΔ), respectively (Fig. 1C).

These results confirmed that the Cre recombinase caused PDGFR-β gene deletion in the PDGFR-βflox/− ES cells.

The chimeras generated from PDGFR-βflox/− ES cells were bred with C57BL/6 mice, generating F1 progenies containing the recombinant allele (PDGFR-βflox/fox). These mice were crossed with each other to obtain homozygous PDGFR-βflox/fox mice. The PDGFR-βflox/fox mice were healthy, and no gross abnormalities were detected. The genotype of the mice was confirmed by PCR with primers 1 and 2 (Fig. 1D) and primers 3 and 4 (data not shown). PCR with primers 1 and 2 generated a 271-bp band from the wild-type PDGFR-β gene and a 329-bp band from the floxed allele.

Adenovirus-mediated Cre Expression Eliminates the Expression of PDGFR-β in PDGFR-βflox/fox Dermal Fibroblasts—We first investigated whether Cre adenovirus infection could abrogate the expression of PDGFR-β in PDGFR-βflox/fox dermal fibroblasts. Similar levels of PDGFR-β were expressed in both wild-type and PDGFR-βflox/fox fibroblasts in the absence of Cre-adenovirus infection (Fig. 2A). Treatment with 1 nM PDGF-BB induced similar levels of tyrosine phosphorylation of PDGFR-β in both types of fibroblasts (Fig. 2A). Infection with the Cre-adenovirus caused a marked reduction in the expression and tyrosine phosphorylation of PDGFR-β in PDGFR-βflox/fox but not wild-type fibroblasts. However, Cre transfection had no effect on the expression of PDGFR-α in wild-type or PDGFR-βflox/fox fibroblasts (Fig. 2A). Immunofluorescent studies revealed that the cytoplasmic immunoreactivity for the PDGFR-β was eliminated in greater than 90% of PDGFR-βflox/fox fibroblasts after Cre transfection (PDGFR-βΔ/Δ) (Fig. 2B).

The effects of Cre transfection were further assessed by examining PDGFR-AA- and PDGFR-BB-induced tyrosine phosphorylation of PDGFR-α and PDGFR-β. Stimulation by either PDGFR-AA or PDGFR-BB induced equivalent and dose-dependent tyrosine phosphorylation of PDGFR-α in both Cre-transfected wild-type and PDGFR-βflox/fox fibroblasts (Fig. 2C, a and b). In contrast, PDGFR-BB-induced tyrosine phosphorylation of PDGFR-β was not detected in PDGFR-βΔ/Δ fibroblasts, although it was clearly observed in wild-type fibroblasts following Cre transfection (Fig. 2C, c). Thus, Cre transfection of PDGFR-βflox/fox fibroblasts selectively abrogated the expression and function of PDGFR-β but not PDGFR-α.

PDGFR-βflox/fox fibroblasts selectively abrogated the expression of PDGFR-β, although it was clearly observed in wild-type fibroblasts following Cre transfection (Fig. 2C, c). Thus, Cre transfection of PDGFR-βflox/fox fibroblasts selectively abrogated the expression and function of PDGFR-β but not PDGFR-α.

PDGFR-βflox/fox fibroblasts were isolated from the PDGFR-βflox/fox mutant mice generated from the Cre-expressing ES clones. The effects of PDGFR-βflox/fox fibroblasts were then assessed in the wound healing assay. As shown in Fig. 3, 1 nM PDGF-AA induced similar levels of BrdUrd incorporation in wild-type and PDGFR-βflox/fox fibroblasts. As shown in Fig. 3, 1 nM PDGF-AA induced similar levels of BrdUrd incorporation in wild-type and PDGFR-βΔ/Δ cells. In contrast, 1 nM PDGF-BB and 10% FBS-induced BrdUrd incorporation in wild-type and PDGFR-βΔ/Δ cells were significantly decreased in PDGFR-βflox/fox fibroblasts compared with wild-type fibroblasts, 48.4 ± 1.2 and 34.2 ± 1.9%, respectively. These results demonstrate a significant contribution of PDGFR-β to cell proliferation in response to PDGF-BB and FBS, but not PDGFR-α.

In Vitro Repair of a Scratch Wound Is Inhibited after PDGFR-β Gene Deletion—Because PDGFR-β is also known to be implicated in cell migration (12), we examined the effect of the deletion of the PDGFR-β gene on the combined migratory and proliferative response to scratch wounding that mimics aspects of wound healing. A wound was formed in confluent monolayers by scratching with a plastic pipette tip. In wild-type fibroblasts cultured for 48 h with 1 nM PDGF-BB, the wound was completely closed (Fig. 4, A and B). Similarly, substantial numbers of wild-type fibroblasts had filled in the scratched area following treatment with 10% FBS for 48 h (Fig. 4, E and F). In contrast, the scratched wound remained unclosed at 48 h after culture with 1 nM PDGF-BB in PDGFR-βΔ/Δ fibroblasts (Fig. 4, C and D). Wound closure was mostly suppressed in PDGFR-βflox/fox cells after 48 h of culture with 10% FBS (Fig. 4, G and H). These results indicate that the PDGFR-β/PDGFR-β system is essential for the combined migration and proliferation required...
FIG. 1. Construction of PDGFR-β gene targeting vector and genotyping. A, diagram of the targeting vector. Three *loxP* sequences and neo-TK and diphtheria toxin selection cassettes were inserted into the PDGFR-β locus, and it was subcloned into the pBluescript SK– plasmid. XbaI and SacI sites were artificially introduced in the indicated positions. WT, wild type. B, Southern blotting of ES cells after homologous recombination (RE) using three different probes. Genomic DNA prepared from isolated ES cells was digested with XbaI or SacI. C, Cre-mediated gene depletion in ES cells. PDGFR-β*flx/flx* ES cells were treated with Cre recombinase, and genomic PCR was performed. The 410-bp band corresponds to the PDGFR-β-deleted allele (PDGFR-βΔ) after Cre treatment. D, PCR-based genotyping of mice. Genomic PCR products obtained from mouse tail DNA show distinct band patterns in PDGFR-β*flx/flx*, PDGFR-β*+/−*, and PDGFR-β*flx/+* mice.
FIG. 2. Depletion of PDGFR-β protein expression after Cre-adenovirus infection. A, wild-type (WT) and PDGFR-β<sup>flx/flx</sup> fibroblasts were infected with the Cre-adenovirus at an m.o.i. of 10 pfu/cell and were stimulated with 1 nM PDGF-BB for 5 min. Whole cell lysates were immunoblotted with anti-PDGFR-α, anti-PDGFR-β, or anti-Tyr<sup>857</sup> phosphospecific PDGFR-β antibody. B, the Cre-adenovirus-infected wild-type or PDGFR-β<sup>flx/flx</sup> fibroblasts were immunostained with anti-PDGFR-β antibody (green). Representative immunofluorescent staining for PDGF-β is shown. Fibroblasts were unambiguously identified by 4,6-diamidino-2-phenylindole staining of nuclei (blue). C, PDGF-induced phosphorylation of PDGFR after deletion of the PDGFR-β gene. Wild-type and PDGFR-β<sup>flx/flx</sup> fibroblasts were infected with the Cre-adenovirus at an m.o.i. of 10 pfu/cell and serum-starved for 24 h. a, the cells were stimulated with various concentrations of PDGF-AA for 5 min. The cell lysates were immunoprecipitated with anti-PDGFR-α antibody and immunoblotted with anti-phosphotyrosine antibody (PY99) or anti-PDGFR-α antibody. b, cells were stimulated with various concentrations of PDGF-BB for 5 min. Proteins were immunoprecipitated from cell lysates using anti-PDGFR-α antibody, and then immunoblotting was performed with PY99 or anti-PDGFR-α antibody. c, cells were stimulated with various concentrations of PDGF-BB for 5 min. Proteins were immunoprecipitated from cell lysates using anti-PDGFR-β antibody, and immunoblotting was performed with PY99 or anti-PDGFR-β antibody.
to fill in a scratch wound with PDGF-BB- and 10% FBS as stimulants and suggest that the role of PDGFR-α is minimal. This concept was further supported by the fact that PDGFR-AA did not induce apparent wound closure in either wild-type or PDGFR-βΔα cells (Fig. 4, I–L).

**Apoptosis Induced by H₂O₂ Is Enhanced by Elimination of the PDGFR-β Gene**—We next examined the effect of the deletion of the PDGFR-β gene on H₂O₂-induced apoptosis in dermal fibroblasts. To detect apoptosis, we utilized the APOPercentage™ kit, which stains apoptotic cells but not necrotic cells with a purple-red color (24, 25). Few apoptotic cells were observed in wild-type or PDGFR-βΔα fibroblasts when the cells were cultured with 10% FBS (data not shown). In wild-type cells cultured with DMEM supplemented only with 0.2% bovine serum albumin, the addition of H₂O₂ increased the number of apoptotic cells by 78.3 ± 5.2%. The addition of 1 nM PDGF-BB protected wild-type cells from apoptosis. However, PDGFR-βΔα fibroblasts showed an increase in the number of apoptotic cells from 23.3 ± 3.7% in wild-type to 56.9 ± 6.5% in PDGFR-βΔα fibroblasts treated with 1 nM PDGF-BB (Fig. 5, A, B, and G). These data indicate that PDGF-BB mediates protection from H₂O₂-induced apoptosis through PDGFR-β. In contrast, H₂O₂ induced only a low level of apoptosis in wild-type and PDGFR-βΔα fibroblasts cultured in the presence of 10% FBS or 1 nM PDGF-AA, and there was no significant difference in the degree of apoptosis among the four treatments (apoptosis for wild-type cells in 10% FBS was 23.8 ± 3.8%; for wild-type cells in PDGF-AA, 21.3 ± 2.9%; for PDGFR-βΔα cells in 10% FBS, 24.3 ± 1.9%; and for PDGFR-βΔα cells in PDGF-AA, 23.2 ± 1.6%; Fig. 5, C–G). These data provide further data that PDGFR-α signaling by PDGFR-AA is not perturbed by conditional deletion of PDGFR-β and that it is sufficient to inhibit H₂O₂-induced apoptosis in fibroblasts.

**PDGFR-BB-induced Phosphorylation of Akt Is Decreased after PDGFR-β Gene Deletion**—Because various biological actions were impaired in PDGFR-βΔα fibroblasts, we next investigated the effect of PDGFR-β deletion on PDGF-induced intracellular signaling. Akt is one of the important signaling molecules involved in the biological effects of PDGF (12). As shown in Fig. 6A, stimulation with PDGF-BB for 5 min dose-dependently increased the phosphorylation of Akt in wild-type fibroblasts, and the levels of phosphorylation reached a maximum at 1 nM PDGF-BB. Compared with the wild-type cells, the levels of phosphorylation following treatment with PDGF-BB were significantly lower in PDGFR-βΔα fibroblasts. At 0.2 nM PDGF-BB, Akt phosphorylation was decreased by 50.8 ± 2.1% in PDGFR-βΔα cells compared with wild-type cells.

We further conducted time course studies of PDGF-BB-induced phosphorylation of Akt. In wild-type cells, PDGF-BB induced the phosphorylation of Akt in a time-dependent manner for up to 60 min, and the phosphorylation was sustained at high levels until 120 min after stimulation (Fig. 6C). Akt phosphorylation was significantly reduced at all time points in PDGFR-βΔα fibroblasts, whereas the dose (Fig. 6B) and time dependence (Fig. 6D) of PDGF-AA-induced phosphorylation of Akt were comparable in wild-type and PDGFR-βΔα fibroblasts.

**PDGFR-BB-induced Phosphorylation of ERK1/2 and JNK, but Not p38, Are Decreased after Deletion of the PDGFR-β Gene**—In addition to Akt, MAPKs, including ERK1/2, JNK, and p38, are known to be key molecules in the biological actions of PDGF (26). Therefore, we investigated the effect of PDGFR-β gene deletion on PDGF-induced phosphorylation of ERK1/2, JNK, and p38. In wild-type fibroblasts, PDGF-BB dose-dependently decreased the phosphorylation of ERK1/2 at up to 1 nM (Fig. 7A). In the presence of 1 nM PDGF-BB, the levels of phosphorylation reached a maximum after 5 min with a gradual decrease thereafter (Fig. 7C). PDGF-BB-induced ERK1/2 phosphorylation was significantly decreased at all doses and time points in PDGFR-βΔα cells (Fig. 7, A and C). Phosphorylation of JNK was induced by PDGF-BB in a similar dose- and time-dependent manner as that observed in ERK1/2. JNK phosphorylation was also decreased in PDGFR-βΔα cells compared with wild-type cells (Fig. 8, A and C). At 0.4 nM PDGF-BB, the levels of ERK1/2 and JNK phosphorylation were reduced by 42.7 ± 3.8% and 53.2 ± 2.7%, respectively, in PDGFR-βΔα cells compared with wild-type cells. The phosphorylation of ERK1/2 and JNK induced by PDGF-AA was essentially identical between wild-type and PDGFR-βΔα cells at all doses and time points examined (Figs. 7, B and D, and Fig. 8, B and D).

PDGF-BB and PDGF-AA dose-dependently induced the phosphorylation of p38 up to a concentration of 2 nM (Fig. 9, A and B). In addition, both 1 nM PDGF-BB and 1 nM PDGF-AA caused a transient phosphorylation that lasted for 15 min, decreasing thereafter and returning to a basal level at 120 min (Fig. 9, C and D). Most interestingly, both PDGFR-BB- and PDGF-AA-induced phosphorylation of p38 was nearly identical at all dose and time points in wild-type and PDGFR-βΔα cells.

**Effect of PTEN and SHIP2 Expression on the Phosphorylation of Akt and MAPKs in Wild-type and PDGFR-βΔα Fibroblasts**—PTEN and SHIP2 are phospholipid phosphatases that may act as negative regulators of PDGF signaling (17, 18). To understand their roles in PDGF-BB-induced phosphorylation of Akt and MAPKs, we overexpressed PTEN and/or SHIP2 in wild-type and PDGFR-βΔα fibroblasts. As shown in Fig. 10A, we were able to enhance the expression of PTEN and SHIP2 by 10-fold of endogenous expression. PDGF-BB-induced phosphorylation of Akt was only modestly decreased by the expression of either PTEN or SHIP2 but was significantly inhibited when both phosphatases were overexpressed in wild-type cells. In agreement with the results in Fig. 6, the levels of the Akt phosphorylation were decreased by the deletion of PDGFR-β, and expression of either PTEN or SHIP2 effectively inhibited PDGF-BB-induced phosphorylation of Akt in PDGFR-βΔα cells (64.2 ± 4.1% and 65.5 ± 3.1% reduction, respectively; Fig. 10B).

In wild-type cells, PDGF-BB-induced phosphorylation of the ERK1/2 did not appear to be affected by the overexpression of SHIP2 or PTEN. However, in PDGFR-βΔα cells, overexpres-
FIG. 4. Effect of PDGFR-β deletion on wound scratch assay. Confluent wild-type (WT) and PDGFR-β<sup>+/-</sup>/flox/flox fibroblasts were infected with the Cre-adeno-virus. The cells were subjected to injury by scratching with a plastic pipette tip. The cells were then treated for 48 h with 1 nM PDGF-BB (A–D), 10% FBS (E–H), or 1 nM PDGF-AA (I–L). Large black dots marked the bottom surface of the culture dish to specify the site of observation. Representative images are shown from three separate experiments.
FIG. 5. Effect of PDGFR-β deletion on H2O2-induced apoptosis. Wild-type (WT) and PDGFR-βlox/lox fibroblasts were infected with the Cre adenovirus at an m.o.i. of 10 pfu/cell and then were cultured in 12-well plates. After 24 h, 1 μM H2O2 and dye from the APOPercentage™ kit were added along with 1 nM PDGF-BB (A and B), 10% FBS (C and D), or 1 nM PDGF-AA (E and F). After 1 h, the cells were washed twice with PBS and then photographed. G, results represent the means ± S.D. of three separate experiments. *p < 0.05 versus PDGF-BB incubation in wild-type fibroblasts.

In the current studies, we developed a new mouse line in which a portion of the PDGFR-β exons were flanked by two loxP sequences that did not affect development of the mutant mouse. The expression of PDGFR-α and -β and the level of ligand-induced phosphorylation were comparable between dermal fibroblasts isolated from wild-type and mutant mice without Cre transfection. The adenovirus-mediated Cre transfection in PDGFR-βlox/lox cells efficiently abrogated PDGFR-β expression without affecting PDGFR-α expression and its signaling pathways. Therefore, this is a powerful approach for unambiguously determining the role of the two PDGFR subtypes in the biological effects of and signaling pathways stimulated by PDGF.

In wild-type fibroblasts, PDGF-AA and PDGF-BB induced the incorporation of BrdUrd, but PDGF-BB- and 10% FBS-induced BrdUrd incorporation were significantly lower in PDGFR-βlox/lox fibroblasts than in wild-type fibroblasts. These results indicate that PDGFR-β plays an important role in mediating PDGF-BB- and 10% FBS-induced DNA synthesis. However, because the depletion of PDGFR-β caused only a partial decrease of BrdUrd incorporation, PDGFR-α appears to partly mediate the effects of PDGF-BB in dermal fibroblasts. In addition, PDGF-AA-induced DNA synthesis was unaffected by depletion of PDGFR-β, consistent with all of the effects of PDGF-AA being mediated by PDGFR-α.

In the wound scratch assay, PDGF-BB and 10% FBS, but not PDGF-AA, induced wound closure in wild-type fibroblasts. The wound closure induced by PDGF-BB and 10% FBS was significantly inhibited by depletion of the PDGFR-β. Wound closure is known to be dependent on both cell motility and proliferation. Because PDGF-AA clearly induced cell proliferation in both wild-type and PDGFR-βlox/lox cells, the lack of wound closure by PDGF-AA suggests that PDGFR-α signaling is insufficient to stimulate cell motility. Similarly, studies with aortic endothelial cells transfected with equal numbers of PDGFR-α or -β showed that chemotactic migration was mediated only by PDGFR-β (27). Also, in dermal fibroblasts, PDGF-BB is known to be the major inducer of chemotaxis on type I collagen, although the subtype of PDGFR that was involved was not determined (28). Furthermore, the contribution of PDGFR-β−/− fibroblasts to granulation formation was reduced by 85% after subcutaneous implantation of sponges in the blastocyst chimeric mice expressing mixtures of wild-type and PDGFR-β−/− cells (10). Taken together, our results clearly indicate that in dermal fibroblasts PDGFR-β, but not PDGFR-α, plays a crucial role particularly in the mediation of PDGF-induced cellular motility.

PDGF-AA, PDGF-BB, and 10% FBS protected wild-type fibroblasts from H2O2-induced apoptosis to similar extents. In PDGFR-βlox/lox fibroblasts, the effect of PDGF-BB was mostly lost, whereas the protective effects of PDGF-AA and 10% FBS were essentially unchanged. These results indicate that the PDGFR-β plays an important role in the ability of PDGF-BB to protect dermal fibroblasts from H2O2-induced apoptosis and that the remaining PDGFR-α appears to be sufficient for the protection by PDGF-AA and 10% FBS.

Based on these results, the functional importance of PDGFR-β and PDGFR-α appears to differ, depending on the specific biological effects triggered by PDGF-AA, PDGF-BB, and 10% FBS. Our studies have further clarified the functional significance of PDGFR-β signaling in mediating its biological effects by examining the activation of two of its downstream pathways, Akt and MAPKs. Compared with wild-type fibroblasts, there was a decrease in PDGF-BB-induced phosphorylation of Akt, ERK1/2, and JNK in PDGFR-βlox/lox fibroblasts.
FIG. 6. Effect of PDGFR-β depletion on PDGF-induced phosphorylation of Akt. Wild-type (WT) and PDGFR-β<sup>flx/flx</sup> fibroblasts were infected with Cre-adenovirus at an m.o.i. of 10 pfu/cell, and the cells were serum-starved for 48 h. The cells were then treated at 37 °C for 5 min with the indicated concentrations of PDGF-BB (A) or PDGF-AA (B), or they were treated at 37 °C for the indicated times with 1 nM PDGF-BB (C) or 1 nM PDGF-AA (D). Cell lysates were separated by 7.5% SDS-PAGE and immunoblotted with anti-Thr<sup>308</sup> phosphospecific Akt antibody or anti-Akt1 antibody. The relative amount of phosphorylated Akt was determined by densitometry. Results represent means ± S.D. of three separate experiments. *, p < 0.05 versus Akt phosphorylation in wild-type fibroblasts.
FIG. 7. Effect of PDGFR-β depletion on PDGF-induced phosphorylation of ERK1/2. Wild-type (WT) and PDGFR-β<sup>flox/flox</sup> fibroblasts were infected with the Cre-adenovirus at an m.o.i. of 10 pfu/cell, and the cells were serum-starved for 48 h. The cells were treated at 37 °C for 5 min with the indicated concentrations of PDGF-BB (A) or PDGF-AA (B), or they were treated at 37 °C for the indicated times with 1 nM PDGF-BB (C) or 1 nM PDGF-AA (D). Cell lysates were separated by 7.5% SDS-PAGE and immunoblotted with anti-Thr<sup>202</sup>/Tyr<sup>204</sup> phosphospecific ERK1/2 antibody or anti-ERK1/2 antibody. The relative amount of phosphorylated ERK1/2 was determined by densitometry. Results represent the means ± S.D. of three separate experiments. *, p < 0.05 versus ERK1/2 phosphorylation in wild-type fibroblasts.
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Fig. 8. Effect of PDGFR-β depletion on PDGF-induced phosphorylation of JNK. Wild-type (WT) and PDGFR-β<sup>flx/flx</sup> fibroblasts were infected with the Cre adenovirus at an m.o.i. of 10 pfu/cell, and the cells were serum-starved for 48 h. The cells were treated at 37 °C for 5 min with the indicated concentrations of PDGF-BB (A) or PDGF-AA (B), or they were treated at 37 °C for the indicated times with 1 nM PDGF-BB (C) or 1 nM PDGF-AA (D). Cell lysates were separated by 7.5% SDS-PAGE and immunoblotted with anti-Thr<sup>183</sup>/Tyr<sup>185</sup> phosphospecific JNK antibody or anti-JNK antibody. The relative amount of phosphorylated JNK was determined by densitometry. Results represent means ± S.D. of three separate experiments. *, p < 0.05 versus JNK phosphorylation in wild-type fibroblasts.
FIG. 9. Effect of PDGFR-β depletion on PDGF-induced phosphorylation of p38. Wild-type (WT) and PDGFR-β<sup><br>lox/lox</sup> fibroblasts were infected with the Cre-adenovirus at an m.o.i. of 10 pfu/cell, and the cells were serum-starved for 48 h. The cells were treated at 37 °C for 5 min with the indicated concentrations of PDGF-BB (A) or PDGF-AA (B), or they were treated at 37 °C for the indicated times with 1 nM PDGF-BB (C) or 1 nM PDGF-AA (D). The cell lysates were separated by 7.5% SDS-PAGE and immunoblotted with anti-Thr<sup>180</sup>/Tyr<sup>182</sup> phosphospecific p38 antibody or anti-p38 antibody. The relative amount of phosphorylated p38 was determined by densitometry. Results represent means ± S.D. of three separate experiments.
FIG. 10. Effect of co-infection with PTEN and SHIP2 on PDGF-induced phosphorylation of Akt, ERK1/2, JNK, and p38 in wild-type and PDGFR-β<sup>−/−</sup> fibroblasts. Wild-type (WT) and PDGFR-β<sup>−/−</sup> fibroblasts were co-infected with the Cre-adenovirus, and LacZ-, PTEN-, or SHIP2-adenovirus at an m.o.i. of 10 pfu/cell. The cells were serum-starved for 48 h and then treated at 37 °C for 5 min with 1 nM PDGF-BB. The cell lysates were separated by 7.5% SDS-PAGE and immunoblotted with PTEN or SHIP2 antibody (A), anti-Thr<sup>308</sup> phosphospecific Akt and anti-Akt1 antibodies (B), anti-Thr<sup>202</sup>/Tyr<sup>204</sup> phosphospecific ERK1/2 and anti-ERK1/2 antibodies (C), anti-Thr<sup>183</sup>/Tyr<sup>185</sup> phosphospecific JNK and anti-JNK antibodies (D), or anti-Thr<sup>180</sup>/Tyr<sup>182</sup> phosphospecific p38 and anti-p38 antibodies (E). The relative amounts of phosphorylated Akt, ERK1/2, JNK, and p38 were determined by densitometry. Results represent means ± S.D. of three separate experiments. *, p < 0.05 versus Akt phosphorylation in Cre- and LacZ-infected wild-type fibroblasts in B and versus ERK1/2 phosphorylation of Cre- and LacZ-infected PDGFR-β<sup>−/−</sup> fibroblasts in C. †, p < 0.05 versus Akt phosphorylation in Cre- and LacZ-infected PDGFR-β<sup>−/−</sup> fibroblasts in B.
Most interestingly, PDGF-BB-induced phosphorylation of p38 MAPK was unaffected by depletion of PDGFR-β. On the other hand, PDGF-AA-induced phosphorylation of Akt, ERK1/2, JNK, and p38 was comparable between wild-type and PDGFR-β<sup>+/−</sup> fibroblasts. These results indicate that PDGFR-β is involved in PDGF-BB-induced phosphorylation of Akt, ERK1/2, and JNK. Because the inhibition of these pathways was partial, we presume that PDGFR-α mediates part of the signal triggered by PDGF-BB. In addition, our data suggest that PDGFR-α mediates PDGF-AA-induced phosphorylation of Akt, ERK1/2, JNK, and p38, and, unexpectedly, PDGF-BB-induced phosphorylation of p38 as shown in our dose- and time-dependent course studies.

In NIH3T3 fibroblasts, PDGFR-α, but not PDGFR-β, mediates PDGF signals leading to the activation of JNK, although both PDGFR-α and PDGFR-β activate ERK1/2 (29). This is different from our current finding that ERK1/2 and JNK are possible downstream signaling molecules for both PDGFR-α and PDGFR-β. The apparent discrepancy may be due to the specific cell types examined and/or the experimental procedures employed. A recent study (30) reported that mouse embryonic fibroblasts lacking both JNK1 and JNK2 exhibit much slower proliferation than wild-type fibroblasts. In addition, studies with pharmacological inhibitors, antisense oligonucleotides, and c-Jun<sup>−/−</sup> fibroblasts suggest that JNK plays a key role in cell cycle progression (31). Furthermore, by using dominant-negative mutants, Kawano et al. (26) showed that ERK and JNK, but not p38, are involved in PDGF-BB-induced mesangial cell proliferation. In the current studies, we found that PDGFR-β<sup>+/−</sup> cells have reduced PDGF-BB-induced cell proliferation and decreased activation of ERK1/2 and JNK, but there was no effect on the activation of p38. Taken together, these findings support the idea that PDGFR-β mediates PDGF-BB-induced proliferation through ERK1/2 and JNK, but not through p38, in dermal fibroblasts.

The involvement of p38 and JNK in cell migration is controversial, possibly because of different findings in various cell types and different methods to evaluate cell migration. Ras-mediated activation of p38, but not JNK, has been reported to be crucial for the migration of PDGFR-transfected porcine aortic endothelial cells (32). In contrast, Javeluda et al. (33) showed that transforming growth factor-β stimulates cell motility via the activation of JNK and c-Jun in JNK<sup>−/−</sup> mouse embryonic fibroblasts and human dermal fibroblasts. Consistent with these findings, our data showed that, in PDGFR-β<sup>−/−</sup> cells, the phosphorylation of JNK was decreased, whereas that of p38 was unaffected. Collectively, our results suggest that the decrease in PDGF-BB-induced phosphorylation of JNK in PDGFR-β<sup>−/−</sup> fibroblasts may contribute to the lack of cell movement and that normal activation of p38 is not sufficient to induce cell motility.

Ligand binding- or stress-induced activation of PDGFR-β is known to mediate Akt activation resulting in the protection of cells from apoptosis (34, 35). After PDGF-BB stimulation, association of the activated Akt with 14-3-3 appears to be a mechanism for protection of human skin fibroblasts from apoptosis (36). In PDGFR-BB-stimulated wild-type cells, the phosphorylation of Akt sustained at high levels until 2 h after stimulation, whereas the phosphorylation of MAPKs showed transient peaks and tended to decrease within 15–30 min after stimulation. The decrease of phosphorylation of Akt was marked until 2 h in PDGF-BB-stimulated PDGFR-β<sup>−/−</sup> fibroblasts. These features of Akt activation may imply that the Akt, rather than MAPKs, contributed to the protection of PDGFR-BB-stimulated wild-type cells from H₂O₂-induced apoptosis.

The phospholipid phosphatases SHIP2 and PTEN have been reported to be negative regulators of PDGF signaling. These phosphatases lower the level of PI(3,4,5)P<sub>3</sub>, which results in a reduction in Akt activation (17, 18, 37). We found that the overexpression of either SHIP2 or PTEN effectively inhibited PDGF-BB-induced phosphorylation of Akt in PDGFR-β<sup>−/−</sup> fibroblasts, whereas Akt phosphorylation was only partially inhibited by expression of both SHIP2 and PTEN in wild-type fibroblasts. In addition, SHIP2 is known to competitively inhibit Shc/Grb2 binding, which is required for the activation of p21 Ras and ERK1/2 (20). Consistent with this, PDGF-BB-induced phosphorylation of ERK1/2 was inhibited by the expression of SHIP2 but not PTEN in PDGFR-β<sup>−/−</sup> fibroblasts. These results indicate that the PDGF-induced phosphorylation of Akt and ERK1/2 mediated by PDGFR-α, and not PDGFR-β, is preferentially regulated by phospholipid phosphatases. However, we cannot rule out the possibility that SHIP2 or PTEN alone are insensitive for the negative regulation of PDGF-BB-induced Akt phosphorylation when it is mediated by both PDGFR-α and PDGFR-β. In contrast to the involvement of SHIP2 and/or PTEN in Akt and ERK1/2 activation, expression of SHIP2 and PTEN did not affect PDGF-BB-induced phosphorylation of JNK or p38 MAPK in either wild-type or PDGFR-β<sup>−/−</sup> fibroblasts. These results indicate that the phospholipid phosphatases are a negative regulator of certain, but not all, PDGF-BB-induced intracellular signaling events. Further studies are needed to determine whether phospholipid phosphatases play a direct role in the negative regulation of PDGFR-α- or PDGFR-β-mediated biological effects.

In summary, we examined the biological effects and signaling stimulated by PDGF in dermal fibroblasts in which PDGFR-β was depleted by the adenovirus-mediated Cre/loxP system. This system allowed us to assess the functional importance of PDGFR-β and to examine the role of its specific intracellular signaling pathways. Our results indicate the following. 1) PDGFR-β plays a crucial role in PDGF-BB-induced cell proliferation, migration, and protection from apoptosis. 2) PDGFR-α activation cannot mediate fibroblast migratory response to fill in a scratch wound, although it supports a proliferative response. 3) PDGFR-BB-induced phosphorylation of Akt, ERK1/2, and JNK, but not p38, is involved in the biological effects of PDGFR-BB. 4) Although PDGFR-α alone can mediate PDGF-BB-induced activation of p38, it is not enough to mediate the cellular responses in fibroblasts, including cell proliferation, migration, and protection from apoptosis. 5) SHIP2, and not PTEN, preferentially plays a role as a negative regulator of PDGFR-α-mediated phosphorylation of Akt and ERK1/2, but not of JNK or p38.

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REFERENCES
1. Heldin, C. H., and Westmark, B. (1999) Physiol. Rev. 79, 1283–1316
2. Li, X., Pontén, A., Aase, K., Karlsson, L., Abramsson, A., and Uutela, M. (2000) Nat. Cell Biol. 2, 302–309
3. Bergsten, E., Uutela, M., Li, X., Pietras, K., Östman, A., and Heldin, C. H. (2001) Nat. Cell Biol. 3, 512–516
4. LaRoche, W. J., Jeffers, M., McDonald, W. F., Chillakuru, R. A., Giese, N. A., and Lokker, N. A. (2001) Nat. Cell Biol. 3, 517–521
5. Singer, A. J., and Clark, R. A. (1999) Neogl. J. Med. 341, 738–746
6. Orr-Urtreger, A., and Lonai, P. (1992) Development (Camb.) 115, 1045–1058
7. Schattenman, G. C., Morrison-Graham, K., van Koppen, A., Weston, J. A., and Bowen-Pope, D. F. (1992) Development (Camb.) 115, 123–131
Deletion of the PDGFR-β Gene Affects Key Fibroblast Functions Important for Wound Healing

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