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Review

The pivotal role of PDGF and its receptor isoforms in adipose-derived stem cells

Won-Serk Kim[1], Hyoung-Sook Park[2] and Jong-Hyuk Sung[2]

[1]Department of Dermatology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul and [2]College of Pharmacy, Yonsei University, Incheon, Korea

Summary. Platelet-derived growth factor (PDGF) is one of the growth factors that reportedly regulates cell growth and division of mesenchymal cells. Although PDGF isoforms and their receptors reportedly play a pivotal role in mesenchymal stem cell regulation, there is a paucity of literature reviewing the role of PDGF in adipose-derived stem cells (ASCs). Therefore, we summarized previous reports on the expression and functional roles of PDGF and its receptor isoforms in this review. In addition, we examined findings pertaining to underlying molecular mechanisms and signaling pathways with special focus on PDGF-D/PDGFRβ.

ASCs only express PDGF-A, -C, -D, PDGFRα, and PDGFRβ. PDGFRα expression decreases with adipocyte lineage, while PDGFRβ inhibits white adipocyte differentiation. In addition, PDGFRβ induces proliferation, migration, and angiogenesis and up-regulates the expression of paracrine factors in ASCs. Although PDGF-B and -D mediate their functions mainly by PDGFRβ and ROS generation, there are many differences between them in terms of regulating ASCs. PDGF-D is endogenous, generates ROS via the mitochondrial electron transport system, and regulates the autocrine loop of ASCs *in vivo*. Furthermore, PDGF-D has stronger mitogenic effects than PDGF-B.

Key words: Adipose-derived stem cells, Platelet-derived growth factor, Receptor, Reactive oxygen species, Mitogenic effect

Offprint requests to: Jong-Hyuk Sung, Ph.D., College of Pharmacy, Yonsei University, #162-1, Songdo-dong, Yeonsu-gu, Incheon, 406-840, Korea. e-mail: brian99@empal.com or brian99@empas.com
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Introduction

Adipose-derived stem cells (ASCs) exist in the perivascular region of adipose tissue and can be isolated from subculture of the stromal vascular fraction (Zuk et al., 2002; Kaewsuwan et al., 2012; Mendel et al., 2013). ASCs are a heterogeneous cell population with primitive mesenchymal cells that are primarily found in the perivascular adventitia and pericytes (Zannettino et al., 2008; Lin et al., 2010; Cai et al., 2011). ASCs typically have ultrastructural characteristics similar to those of primitive mesenchymal cells such as relatively high nuclear/cytosol ratio, prominent nucleoli, and immature cytoplasmic organelles (Ryu et al., 2013). ASCs exhibit specific surface markers, and immunohistochemistry staining demonstrated that platelet-derived growth factor receptor (PDGFR) is constitutively expressed in most ASCs regardless of passage number (Ryu et al., 2013). For example, the CD140a/PDGFRα subpopulation was regulated by basic fibroblast growth factor (bFGF), a critical factor for ASC self-renewal, and the number of CD140a/PDGFRα-positive cells declined as the ASCs differentiated (Mohsen-Kanson et al., 2013). Of interest, PDGFRα-positive cells exist in white adipose tissue that produces brown-like adipocytes in response to β-adrenergic stimulation (Lee et al., 2012). Flow cytometry and RT-PCR also showed that PDGFRα and β are highly expressed in ASCs, and they mediated ASC migration (Baek et al., 2011). Although PDGF isoforms and their receptors play a pivotal role in ASC regulation, there is a dearth of review articles addressing PDGF in ASCs.

PDGF is one of the growth factors that reportedly regulates cell growth and division (Hoch and Soriano,

2003; Andrae et al., 2008). The PDGF families and their receptors are derived from a primordial VEGF system, and both families retain close sequence homology and structural relationships (Shibuya, 2001). PDGF is mitogenic during early developmental stages, driving the proliferation and migration of undifferentiated mesenchyme and some progenitor populations (Hannink and Donoghue, 1989; Heldin, 1992). In addition, it plays a significant role in blood vessel formation (Keck et al., 1989; Battegay et al., 1994). The PDGF signaling network consists of four ligands: PDGF-A, -B, -C, and -D. All PDGFs function as disulfide-linked homodimers, and PDGF-A and -B can form functional heterodimers (Andrae et al., 2008). The PDGFR is classified as a receptor tyrosine kinase, and two receptor types (PDGFRα and PDGFRβ) have been identified to date (Levitzki, 2004; Demoulin and Essaghir, 2014). Depending on the PDGFR expression profile, ligand binding can induce the formation of PDGFR homodimers and/or a heterodimer (PDGFRαβ) (Andrae et al., 2008). PDGF-B preferentially interacts with PDGFRβ, but also has a lower binding affinity for PDGFRα. PDGF-A can only interact with PDGFRα, and with higher affinity than PDGF-B. PDGF-C preferentially interacts with PDGFRα and PDGF-D with PDGFRβ (Fig. 1). However, the expression and functional role of PDGF isoforms and their receptors has not been fully elucidated in ASCs. Therefore, this review

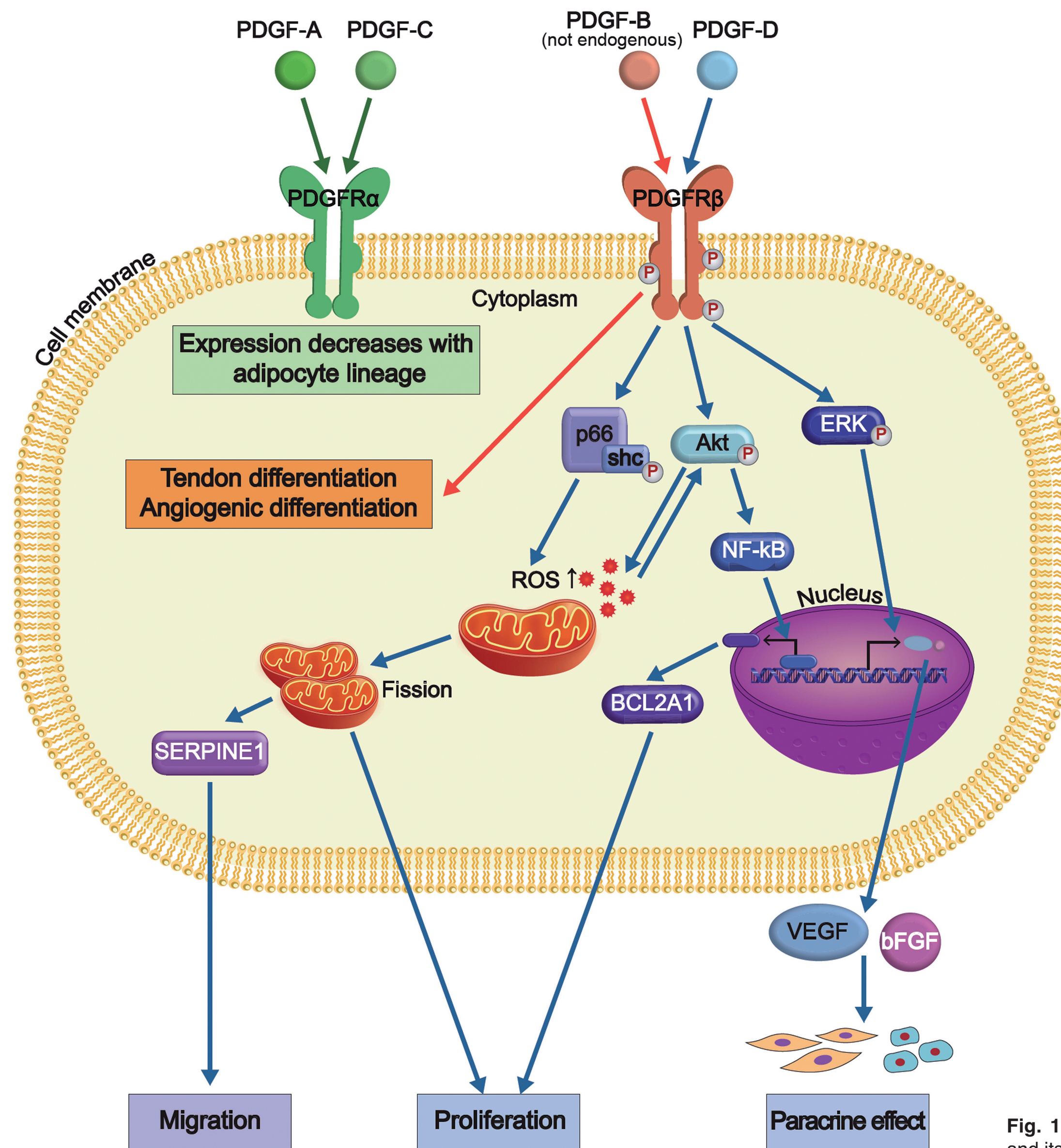

Fig. 1. The pivotal role of PDGF and its receptor in ASCs.

examines research on this topic. Of these proteins, special focus is placed on PDGF-B, -D and PDGFRβ signaling, which play a pivotal role in proliferation, migration, and differentiation of ASCs.

PDGF and it receptor expression in ASCs

Although it is well-reported that PDGF-B has multiple effects (i.e., increased proliferation, migration, and angiogenic differentiation) on ASCs, PDGF-B is not expressed in ASCs (Devarajan et al., 2012; Kim et al., 2015). Using RT-PCR amplification and Western blotting, Devarajan first reported that PDGF-B is not, but PDGF-A, -C, and -D are expressed in ASCs (Devarajan et al., 2012). Instead of PDGF-B, PDGF-D released by tissue-resident ASCs mediated the epithelial-mesenchymal transition of breast cancer lines (Devarajan et al., 2012). Our group also demonstrated that ASCs only express PDGF-A, -C, and -D by RT-PCR and quantitative PCR analysis (Kim et al., 2015). The cycle threshold (Ct) values for PDGF-A, -C, and -D are 32.90 (±0.08), 25.90 (±0.02), and 27.90 (±0.04), respectively. In addition, PDGFRα and PDGFRβ are highly expressed in ASCs (Ct values =23.84 (±0.08) and 28.16 (±0.03), respectively). PDGFRα-positive cells are precursors of adipocytes and PDGFRα expression diminishes with adipocyte lineage (Berry and Rodeheffer, 2013). Baek et al. also reported that PDGFRα and β are highly expressed at both the protein and mRNA levels in ASCs, and PDGF-AB showed the strongest migratory effects on human ASCs (Baek et al., 2011).

Table 1. Function of PDGF and its receptors in ASCs.

PDGF or receptor type	Functions in ASCs	Reference
PDGF-D, PDGFRβ	Increased mtROS generation Increased proliferation and migration Up-regulated growth factor secretion	Kim et al., 2015
PDGF	Secretion of extracellular vesicles Enhanced angiogenic potential	Lopatina et al., 2014
PDGF-B	Enhanced proliferation and osteogenic differentiation via ERK pathway Suppressed adipocyte differentiation	Jin et al., 2014
PDGF-B	High PDGF-B and low BMP-2 enhanced tenogenesis	Min et al., 2014
PDGF-B	Enhanced tenogenesis	Goncalves et al., 2013
PDGFRα	PDGFRα–positive cells recruited macrophages and remodeled adipose tissue	Lee et al., 2013
PDGFRα	PDGFRα is a marker of preadipocytes, and decreased with adipocyte lineage	Berry and Rodeheffer, 2013
PDGF	PDGF in collagen-nanoparticle fibers promoted proliferation and tenogenic differentiation	Cheng et al., 2014
PDGF-AB, PDGF-B	Enhanced proliferation	Josh et al., 2013
PDGF-B	Enhanced both vascular network stability and osteogenic differentiation	Hutton et al., 2013
PDGF-B, PDGFRβ	Increased ROS generation Increased proliferation and migration	Kim et al., 2013
PDGF-B	Improved tendon repair	Manning et al., 2013
PDGF-B	Increased proliferation Improved tendon repair	Raghavan et al., 2012
PDGF-D	Secreted PDGF-D from ASCsinduced epithelial-mesenchymal transition in breast cancer	Devarajan et al., 2012
PDGFRα	PDGFRα-positive cells proliferated and differentiated into multilocular UCP1-positive brown adipocytes in response to ADRB3 agonist treatment	Lee et al., 2012
PDGFRβ	Hypoxia-generated ROS phosphorylated PDGFRβ Increased proliferation and migration	Kim et al., 2011
PDGF-A	Secreted PDGF-A from ASCs and induced anagen hair in hair follicles	Festa et al., 2011
PDGF-D	Enhanced angiogenesis	Gehmert et al., 2011
PDGF-AB, PDGFRα, PDGFRβ	Increased migration	Baek et al., 2011
PDBG-B, PDGFRβ	Tumor cell-derived PDGF-BB increased migration via PDGFRβ	Gehmert et al., 2010
PDGF	Increased tube forming activity	Keerl et al., 2010
PDGF-D	Induced proliferation and migration	Kang et al., 2005
PDGF	Inhibited adipocyte differentiation by PKC pathway	Artemenko et al., 2005
PDGF	Increased proliferation Decreased adipocyte differentiation	Hauner et al., 1995
PDGF	Increased DNA synthesis	Butterwith and Goddard, 1991

Functional role of PDGFand its receptors

As is summarized in Table 1, PDGF and its receptor isoforms mediate diverse functions in ASCs. Although the underlying mechanisms and receptor phosphorylation differ, PDGF-B and -D reportedly increase proliferation, migration, and paracrine effects of ASCs (Kim et al., 2011, 2013, 2015). PDGF-B also induces angiogenic and tendon differentiation, while it suppresses the adipogenic differentiation of ASCs (Hauner et al., 1995; Raghavan et al., 2012) (Fig. 1).

Proliferation

In general, supplementation of growth factors has proliferative effects on ASCs. Compared with PDGF-A and PDGF-C, PDGF-B and PDGF-D have strong proliferative effects on ASCs. Butterwith and Goddard first reported that PDGF increased DNA synthesis and proliferation of ASCs (Butterwith and Goddard, 1991). Hauner et al. demonstrated that PDGF (0.1-50 ng/ml) treatment decreased adipocyte differentiation, while increasing ASC proliferation (Hauner et al., 1995). PDGF phosphorylates SH2 domain-containing inositol 5-phosphatase, and activates JNK and ERK pathways in ASCs (Artemenko et al., 2005; Kang et al., 2005). Recently, our group demonstrated that PDGF-B and -D significantly increased proliferation of ASCs via ROS generation, followed by Akt and ERK phosphorylation (Kim et al., 2013, 2015). However, long-term treatment or high concentrations of PDGF-B and -D induced morphological changes and senescence of ASCs (Kim et al., 2015).

Migration

Like proliferation, PDGF-B and -D have strong migratory effects on ASCs. Baek et al. showed that PDGFRα and PDGFRβ are expressed at both the protein and mRNA levels, and PDGF-AB exhibited the strongest mitogenic effects of the tested growth factors or cytokines (Baek et al., 2011). It was also reported that breast cancer cells induced the migration of ASCs via the PDGF-B/PDGFRβ signaling pathway, and PDGF-B acts as an important factor governing the microenvironment interaction between tumor cells and local tissue-resident ASCs (Gehmert et al., 2010). Our group also found that PDGF-B and -D treatment significantly increased ASC migration (Kim et al., 2013, 2015). Serpine1expression is significantly up-regulated by PDGF-D treatment, which contributed to the PDGF-D-induced migration of ASCs (Kim et al., 2015).

Angiogenic differentiation

ASCs are of perivascular origin, and play a pivotal role in microvessel formation in adipose tissue (Lin et al., 2010; Cai et al., 2011). Although it is controversial, ASCs can be differentiated into endothelial cells *in vitro*, and Keerl first reported that PDGF and bFGF induced tube forming activity of ASCs (Keerl et al., 2010). Gehmert also found that capillary-like tube formation was enhanced by PDGF-B and significantly reduced by antibodies against PDGFRβ (Gehmert et al., 2011). PDGF-B (20 ng/mL) enhanced both vascular network stability and osteogenic differentiation, therefore inducing the development of vascularized bone tissue by ASCs (Hutton et al., 2013). In addition, it is of interest that PDGF regulated the secretion of extracellular vesicles, which enhanced the angiogenic potential of ASCs (Lopatina et al., 2014).

Tendon differentiation

ASCs can be differentiated into and generate tendon, and PDGF reportedly enhanced tendon tissue repair by ASCs (Raghavan et al., 2012; Manning et al., 2013; Cheng et al., 2014). For example, aligned PDGF-releasing collagen-nanoparticle fibers promoted the proliferation and tenogenic differentiation of ASCs (Cheng et al., 2014). In addition, experiments using a porous membrane with reverse gradients of PDGF-B and BMP-2 showed that higher PDGF-B and lower BMP-2 concentrations provided a better environment for ASC tenogenesis (Min et al., 2014).

Adipocyte differentiation

The anti-adipogenic effect of PDGF was first demonstrated by Artemenko, and PDGF treatment significantly attenuated the protein expression of adipogenic transcription factors, such as PPAR-γ and C/EBP-α, as well as the levels of later differentiation markers, including adiponectin, aP2, and fatty acid synthase (Artemenko et al., 2005). Jin et al. also found that oil-red O staining and adipogenesis marker gene expression was suppressed by recombinant PDGF-B treatment, and the ERK pathway mediated the anti-adipogenic effect of PDGF-B (Jin et al., 2014).

Brown adipocyte differentiation

PDGFRα expression is related to brown adipocyte differentiation. PDGFRα-positive ASCs are responsive to β-adrenergic stimulation, which induces the expression of uncoupling proteins (i.e., UCP1) and produces brown-like adipocytes (Lee et al., 2012). In addition, cold stress did not increase proliferation of inguinal white adipose tissue, but triggered the proliferation and differentiation of PDGFRα-positive preadipocytes into brown adipocytes (Lee et al., 2015).

Paracrine effect

ASCs exhibit paracrine effects, and secretion of PDGF from ASCs is related to local tissue maintenance and regeneration (Festa et al., 2011; Devarajan et al., 2012). For example, Festa et al. showed that ASCs secret

PDGF-A, which enhanced hair follicle stem cell activity (Festa et al., 2011). They also found that ASCs act as skin niche cells, and are necessary and sufficient to drive follicular stem cell activation. Devarajan et al. identified an important role of PDGF-D in cancer progression, and found that ASC-generated PDGF-D promotes the growth of breast cancer cells and mediates the epithelial-mesenchymal transition of cancer cells (Devarajan et al., 2012).

Transplanted ASCs secrete diverse growth factors to regenerate damaged tissue, and transplanted ASCs reportedly contribute to wound-healing and hair regeneration (Kim et al., 2009; Won et al., 2010; Jeong et al., 2013). We recently found that PDGF-D can be used as an ASC-preconditioning agent, and up-regulates the expression of a diversity of growth factors such as VEGFA, FGF1, FGF5, LIF, INHBA, IL11 and HBEGF in ASCs (Kim et al., 2015). Therefore, PDGF-D preconditioning (i.e., pretreatment at 10 ng/ml concentration for one day) enhanced the hair-regenerating potential of ASCs.

PDGFRβ-related signaling pathway

PDGF-B, -D, and PDGFRβ primarily regulate the proliferation and migration of ASCs, and up-regulate the expression of paracrine factors in ASCs (Fig. 1). Although PDGF-B and -D mediate their functions mainly by PDGFRβ and ROS generation, there are many differences between them in terms of regulating ASCs.

Inhibition of PDGFRβ

As described above, both PDGFRα and β are expressed by ASCs. PDGFRα is highly expressed in ASCs compared with PDGFRβ (Kim et al., 2015). However, PDGF-A and -C had marginal effects on ASCs, while PDGF-B and -D significantly increased the proliferation and migration of ASCs in a concentration-dependent manner (Kim et al., 2015). Pharmacological inhibition of PDGFRβ (i.e.,CP673451) and transfection of siRNA specific for PDGFRβ significantly down-regulated ROS generation and decreased the phosphorylation of Akt and ERK1/2 molecules in ASCs (Kim et al., 2013, 2015). Therefore, pharmacological or molecular inhibition attenuated the proliferation and migration of ASCs. Gehmert also reported that neutralizing antibodies to PDGFRβ decreased the migration of ASCs (Gehmert et al., 2010). Collectively, these findings indicate that the PDGFRβ signaling pathway plays a key role in ASC proliferation and migration.

PDGF-B and -D signaling pathways differ

Although PDGF-B and -D mediate their functions largely by PDGFRβ, there are many differences between them in regulating ASCs. PDGF-B is not expressed in ASCs, and the mitogenic potential of exogenous/recombinant PDGF-B is not as potent as PDGF-D (Gehmert et al., 2010; Kim et al., 2015). Therefore, it is reasonable to assume that PDGF-D activates PDGFRβ and influences the autocrine loop of ASCs *in vivo* without PDGF-B, a classical PDGFRβ ligand. In addition, the regulatory mechanism of ASC is quite different between PDGF-B and -D. PDGF-B generates ROS via NADPH oxidase in ASCs, while PDGF-D does so via the mitochondrial electron transport system (Kim et al., 2013, 2015).

We first demonstrated that hypoxia induces PDGFRβ phosphorylation by ROS generation, which increased the proliferation and migration of ASCs (Kim et al., 2011). We also found that hypoxia increased ROS generation by NADPH oxidase 4 (Kim et al., 2012). Furthermore, hypoxia up-regulates miR-210, and increases the proliferation and migration of ASCs through PTPN2 down-regulation (Kim et al., 2013). ROS donors also significantly up-regulate miR-210 via NF-κB and Elk1 phosphorylation. During that study, we found that PDGF-B generated ROS, thereby up-regulating miR-210 expression, and increased the proliferation and migration of ASCs (Kim et al., 2013). Transfection of siRNA specific for PDGFRβ significantly down-regulated miR-210 expression, which indicates that the PDGF-B/PDGFRβ signaling pathway plays a key role in miR-210 up-regulation.

Recently, we found that PDGF-D further increases the proliferation and migration of ASCs via ROS generation. However, PDGF-D treatment does not generate ROS via NADPH oxidase 4, nor does it up-regulate miR-210 expression. Instead, PDGF-D increases the proliferation and migration of ASCs through mitochondrial (mt) ROS generation and mitochondrial fission (Kim et al., 2015). mtROS generation and mitochondrial fission are mediated by p66Shc phosphorylation, and transfection of p66Shc siRNA attenuated PDGF-D-induced proliferation and migration of ASCs (Fig. 1). mtROS generation upregulated Serpine1 to increase ASC migration while upregulating BCL2A1 to enhance proliferation. However, PDGF-D treatment did not alter Oct4, Nanog, or Sox2 mRNA levels, but it did change cell morphology at high concentrations (Kim et al., 2015).

PDGF-D treatment increased the phosphorylation of Akt and ERK1/2 molecules in ASCs. The PI3K/Akt pathway is primarily involved in the PDGF-D-induced mitogenic effects of ASCs, while the MAPK pathway mediates paracrine effects in ASCs (Fig. 1). For example, pharmacological inhibition of Akt by LY294002 significantly attenuated the proliferation and migration of ASCs, but inhibition of ERK1/2 by U0126 significantly reduced PDGF-D-induced growth factor expression (Kim et al., 2015).

Conclusion

In this review, we summarized the expression and

functional roles of PDGF and its receptor isoforms in ASCs. In addition, we discussed their underlying molecular mechanisms and signaling pathways with special focus on PDGF-B, -D and PDGFRβ signaling pathways. As described above, ASCs only express PDGF-A, -C, -D, PDGFRα, and PDGFRβ. PDGF isoforms regulate the proliferation, migration, and differentiation of ASCs. PDGFRα expression decreases with adipocyte lineage, while PDGFRβ inhibits white adipocyte differentiation. In addition, PDGFRβ enhances the proliferation, migration, and angiogenesis of ASCs, and up-regulates the expression of paracrine factors in ASCs. Although PDGF-B and -D mediate their functions mainly by PDGFRβ and ROS generation, there are many differences between them in terms of regulating ASCs. PDGF-B generates ROS via NADPH oxidase, while PDGF-D does so via the mitochondrial electron transport system. PDGF-D has stronger mitogenic effects than PDGF-B through PDGFRβ, and regulates the autocrine loop of ASCs *in vivo* without the involvement of PDGF-B. In addition, PDGF-D treatment increased the phosphorylation of Akt and ERK1/2 molecules in ASCs. The PI3K/Akt pathway is primarily involved in the PDGF-D-induced mitogenic effects of ASCs, while the MAPK pathway mediates paracrine effects in ASCs. Therefore, PDGF and its receptor signaling pathways play important roles in the functional regulation of ASCs.

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