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Molecular Mechanisms of Smooth Muscle Cell Differentiation from Adipose-Derived Stem Cell

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Abstract

Smooth muscle cell can be differentiated from adipose-derived stem cell via various approaches: mechanical force, growth factor or cytokine stimulation as well as cell coculture. The smooth muscle cell derived from stem cell has been employed as a promising cell source to replace the damaged tissue in cardiovascular, gastrointestinal and bladder diseases. The goal of the review is to summarize recent knowledge of methodologies of smooth muscle cell differentiation from adipose-derived stem cell and possible molecular mechanism.

Keywords: Mesenchymal stem cell, Adipose-derived stem cell, Smooth muscle cell differentiation, Molecular mechanism.

Abbreviations

AA	ascorbic acid
ASC	adipose-derived stem cell
A-SMA	α-smooth muscle actin
AT1	angiotensin II receptor type 1
BMP4	bone morphogenetic protein 4
BMPs	bone morphogenetic proteins
CaM	calmodulin

ECM	extracellular matrix
ERK	extracellular signal-regulated kinase
GPCR	G protein-coupled receptor
IL-1β	interleukin-1 beta
LPA	lysophosphatidic acid
MAPK	mitogen-activated protein kinase
MLCK	myosin light chain kinase
MRTF-A	myocardin-related transcription factor-A
MRTF-B	myocardin-related transcription factor-B
PDGF	platelet-derived growth factor
PKC	protein kinase C
PLCL	lactic acid and ε-caprolactone
S1P	sphingosine 1-phosphate
SMC	smooth muscle cell
SM-MHC	smooth muscle myosin heavy chain
SPC	sphingosylphosphorylcholine
SRF	serum response factor
SVF	stromal vascular fraction
TGF-β1	transforming growth factor-β1
VSMC	vascular smooth muscle cell.

9.1 Introduction

Smooth muscle cells (SMCs) constitute the wall of blood vessels, gastrointestinal tracts, respiratory tract, bladder and uterus, thus playing a critical role in homeostasis and a number of physiological processes. Degradation of functional SMC or switching of phenotype is associated with various diseases including atherosclerosis, hypertension, urinary and faecal incontinence [1]. Nowadays, cell-based treatment has been a novel solution involving SMC-related diseases; thus, obtaining fresh functional SMC from stem cells is a critical step in regeneration medicine and tissue engineering involving smooth muscle.

Although embryonic stem cells and born marrow-derived mesenchymal stem cells (BM-MSC) have been extensively investigated, there are still some limitations for their practical clinical application including ethical consideration, low stem cell numbers, painful procedure and donor site morbidity. In contrast, adipose-derived stem cells (ASCs) have been an attractive stem cell source due to its easy accessibility, abundant quantities and no ethical

issues. ASC possesses high proliferation capacity allowing rapid expansion *in vitro* and have been differentiated into adipose tissue, bone, cartilage, smooth muscle cell, cardiac muscle, endothelial cells and even neurons [2].

To date, a number of studies have described the differentiation of SMC from ASC via mechanical forces, cytokine and growth factors stimulation, cell–cell coculture. Replacement of damaged SMC with differentiated SMC in cell-based treatment or tissue engineering has been a novel solution, and the efficacy of differentiated SMC has been evaluated in some animal trials [1]. The aim of this review is to summarize recent knowledge in terms of SMC differentiation from ASC and possible molecular mechanism.

9.2 SMC and ASC Characterization

SMC, as a heterogeneous subpopulation, originates from at least eight progenitors including neural crest, secondary heart field, somites, mesoangioblasts, proepicardium, splanchnic mesoderm, mesothelium and various stem cells [3]. SMCs have two different phenotypes: synthetic phenotype and contractile phenotype. Both visceral and vascular SMCs are not terminally differentiated cells in adult organism and are capable of switching between phenotypes due to changes of environmental cues [4]. Contractile phenotypical SMCs are characterized by the expression of specific contractile proteins including α-smooth muscle actin (α-SMA), SM22, calponin, caldesmon, smooth muscle myosin heavy chain (SM-MHC) and smoothelin, which contributes to perform cell-specific contractile function. In contrast, synthetic SMC exhibits the ability to proliferate, migrate and secrete matrix proteins but are also associated with the loss of contractility [5].

Stromal vessel fraction (SVF) containing ASC is produced when adipose tissue is digested with collagenase and centrifuged to remove mature adipocytes. Abundant number, ease of harvest, less invasiveness and immunocompatibility render it a promising stem cell source for repair of damaged tissue or diseased organs. ASCs are characterized by their negative expression of haematopoietic antigens (CD45, CD31) and positive expression of stromal-associated markers (CD29, CD44). Moreover, it is different from BM-MSC due to its positive expression of CD49d and negative expression of CD106. However, due to the lack of single definitive marker, the identification of ASC still needs to consider multifactorial markers including tissue origin, CD marker profile, self-renewal ability and pluripotency [1, 6].

9.3 Differentiation of SMC from ASC and Possible Molecular Mechanism

SMC forms a heterogeneous population with distinct origins, SMC in the body exhibits distinct states of differentiation, and SMC differentiation is controlled by different intracellular mechanism; therefore, SMC differentiation and phenotype switching *in vivo* are extremely complex process [3]. The differentiation is determined by numerous local environmental cues and extrinsic factors including oxygen tension, mechanical influences, cell–cell contact, cell–extracellular matrix (ECM) interactions, humoral factors and neurotransmitters [4, 5]. Among these affecting factors, biochemical factors associated with signalling pathways are undoubtedly a critical component controlling SMC differentiation. Several groups have used a number of different protocols to drive the differentiation of ASC towards a smooth muscle-like cell type, exhibiting both similar morphology and gene and protein expression profiles characteristic of smooth muscle as well as contractility induced by muscarinic agents. An example of changes in morphology is seen in Figure 9.1.

Rodríguez et al. [7] used 100 unit/ml heparin in medium MCDB131 for 6 weeks to successfully drive human ASC to differentiate into phenotypic and functional SMC [7]. This result was confirmed by our group showing when human ASC was cultured in smooth muscle inductive medium (medium MCDB 131 containing 100 unit/ml heparin) for 6 weeks, SMC-specific markers were enhanced after 4 weeks of induction, but decreased from

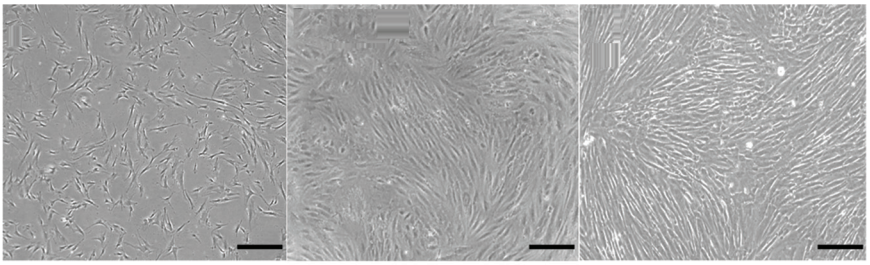

Figure 9.1 Representative images of adipose-derived stem cells before and after differentiation with treatment of TGF-β1 (5 ng/ml) and BMP4 (2.5 ng/ml) in combination for 2 weeks as well as human aortic smooth muscle cells showing the morphological changes. (a) ASC exhibits fibroblast-like shape (b) ASC acquires the spindle-like morphology and the typical "hill and valley" pattern after differentiation 2 weeks similar to (c) human aortic smooth muscle cells. Scale bar, 50 μm for all images.

week 4 to week 6 [8]. Another study indicated that heparin (6–3,200 μg/ml) induced changes in SMC contractile phenotype in dose-dependent fashion [9]. Inhibitory effect of heparin on the SMC proliferation has been extensively investigated: heparin can bind directly to the SMC surface or bind to growth factors of cell surface, thus inhibiting cellular proliferation [10]. Savage et al. [11] employed anti-heparin receptor antibodies to disclose the involvement of heparin receptor on vascular smooth muscle cell growth, which showed anti-heparin receptor antibodies to decrease mitogen-activated protein kinase (MAPK) activity levels after activation in a manner similar to heparin resulting in inhibition of SMC proliferation [11]. In general, vascular SMC proliferation and differentiation are two independent and opposite processes [12]. Then, it is reasonable to speculate that heparin has a great potential to promote ASC to express SMC-like specific contractile proteins by means of binding to growth factors of ASC cell surface, thereby inhibiting ASC growth, although the exact mechanism by which heparin can promote contractile gene expression of SMC has yet to be resolved.

A number of studies have shown the family of transforming growth factor (TGF)-related proteins to be the most potent soluble growth factor-promoting SMC differentiation. Bone morphogenetic proteins (BMPs) represent the largest group in TGF cytokine superfamily [12, 13]. The combination of TGF-β1 (5 ng/ml) and BMP4 (2.5 ng/ml) stimulation for 1 week drove the ASC into mature contractile SMC [14]. Similarly, TGF-β1 and BMP4 were shown to reduce vascular smooth muscle cell (VSMC) proliferation and migration and promote expression of VSMC contractile genes. Additionally, 5 ng/mL TGF-β1 along with 50 ng/mL platelet-derived growth factor (PDGF)-BB or TGF-β1 alone (2 ng/ml for 3 weeks) enhanced SMC markers expression in ASC [15, 16]. It was shown that TGF-β1 proteins modulated SMC differentiation by directly binding to type-I receptor and thereafter activating downstream signals of Smad proteins. Activated Smad2 cooperated with serum response factor (SRF) and myocardin to induce expression of smooth muscle specific genes [14]. Because BMP4 belongs to the TGF-β superfamily, it is reasonable to speculate the activating effects of BMP4 on SMC differentiation might due to induction of TGF-β ligands or activate TGF-β type-I receptor. But the study from Lagna et al. provided evidence that SMC phenotype switch induced by BMP4 from synthetic to contractile was Smad and RhoA/Rho kinase-dependent and TGF-β receptor-independent signaling pathway. The BMP pathway activated transcription of SMC genes by inducing nuclear translocation of the transcription factors myocardin-related transcription factor-A (MRTF-A) and myocardin-related transcription factor-B (MRTF-B), binding

the CArG box of specific gene promoters. Therefore, either TGF-β1 or BMP4 has a capability to induce SMC contractile genes. In combination, they may exert a synergistic influence on differentiation through two independent, but crosstalk pathways [17].

Sphingosine 1-phosphate (S1P), sphingosylphosphorylcholine (SPC) and lysophosphatidic acid (LPA) are all natural bioactive lysophospholipids with similar chemical structure containing one long hydrocarbon chain on a three-carbon backbone containing a phosphate group. They can activate specific G protein-coupled receptor (GPCR) superfamily on the membrane [18]. S1P (from 100 nM to 5 μM) and SPC (from 1 to 10 μM) can stimulate differentiation of ASC towards SMC [19]. Another study indicated that ASC cultured in media containing 2 μM SPC for 4 days exhibited a SMC-like contractile phenotype [15]. Cancer-derived LPA induces expression of α-SMA in human ASC after 4 days of stimulation [20].

Since these three phospholipids have similar structure, it is likely to have similar mechanism instructing the ASC differentiation along SMC lineage. With respect to SPC, on one hand, it activated GPCR (Gi/o) extracellular signal-regulated kinase (ERK)-dependent pathway, which stimulated the secretion of TGF-β isoforms inducing late activation of Smad2 through TGF-β type-I receptor kinase. Activated Smad2 cooperated with SRF and myocardin to induce expression of smooth muscle-specific genes [21]. On the other hand, SPC induced RhoA/Rho kinase-dependent nuclear translocation of MRTF-A. Normally, MRTFA/B are inactivated in the cytoplasm due to interaction with G-actin, RhoA/Rho kinase-mediated actin polymerization can make MRTF free from G-actin and enter the nucleus to stimulate SRF-dependent transcription of SMC-specific genes [22]. As to S1P, GPCR receptors S1P2 and S1P3 are relatively important for myogenic differentiation; in addition, S1P has been shown to cross-activate TGF-β signaling pathway in renal mesangial cells and thereby mimicing TGF-β-induced cell responses [23]. Likewise, LPA stimulated differentiation of ASC to myofibroblast-like cells by activating autocrine TGF-β1-Smad signaling pathway as shown by Jeon et al. [20]. These studies suggested that differentiation process of SMC induced by S1P, SPC or LPA might be in part associated with the TGF-β signaling pathway.

Angiotensin II-induced contraction of smooth muscles are involved in multiple pathways including the activation of angiotensin II receptor type 1 (AT1), Ca^{2+} influx, protein kinase C (PKC), MAPK and Rho kinase [24]. Kim et al. also demonstrated that both angiotensin II and bradykinin drove ASC differentiation into contractile SMC phenotype through ERK-dependent

activation of the autocrine of TGF-β1-Smad2 crosstalk pathway [25]. Mimetic thromboxane A2 induced differentiation of human ASC to contractile smooth muscle-like cells through calmodulin (CaM)/myosin light chain kinase (MLCK) and RhoA-Rho kinase-dependent actin polymerization [26]. Additionally, it was reported that interleukin-1 beta (IL-1β)-activated macrophages were capable of differentiating ASC into SMC by means of a prostaglandin F2α-mediated paracrine mechanism, involving extracellular signal-regulated kinase, Smad2 and myocardin pathways [27]. All these cytokines belong to vasoactive factors; although upstream signal molecular is varied depending on cytokine varieties, their downstream signal pathways are involved in either of TGF-β1-Smad2 crosstalk pathway or RhoA-Rho kinase-dependent actin polymerization.

Obtaining myocytes from ASC through mechanical stimulation or coculture methods has been tried in some studies but most publications concluded that myogenic markers rather than SMC-specific markers were enhanced via a variety of mechanical stimulation [28]. Other studies employed a combination of methods consisting of cyclic strain and cytokines to increase the SMC-specific marker expression: for instance, when ASC was stimulated with uniaxial cyclic strain (10% strain at 1Hz) with or without 1ng/ml TGF-β1 for 7 days, strain alone decreased the expression of early SMC markers α-SMA and *h1*-calponin in ASC; however, cyclic strain combination with cytokines increased the SMC marker expression [29]. Similarly, ASC seeded on electrospun lactic acid and ε-caprolactone (PLCL) scaffold was subjected to combined strain and varying biochemical effects for 2 weeks (5% uniaxial strain,1HZ; 1 ng/ml TGF-β1; 50 μM β-mercaptoethanol and 0.3 mM ascorbic acid (AA); 1 μM all-*trans* retinoic acid; 10 ng/ml PDGF-BB; 1 μM angiotensin II), and α-SMA and MHC expressions were significantly increased by combined effects with retinoic acid, AA or TGF-β1 [30]. With respect to coculture method, only one publication showed that primary rat bladder smooth muscle cells cocultured with ASC for 2 weeks induced ASC differentiation into SMC, and study indicated that microenvironment cues rather than nuclear fusion induced differentiation [31]. Interesting, distinct pattern of seeding substrate also could change the commitment of stem cell which was verified by one study: uniaxial alignment increased the expression of SM22a and a-SMA, but the other two patterns altering cell direction to different extent promote the expression of chondrogenic and osteogenic markers [28]. These studies suggest that mechanical strain, additional chemical factors, coculture condition and substrate pattern might result in different cellular responses via respective signalling pathways, although it still remains unclear.

9.4 Conclusion and Perspectives

ASC can be driven into functional SMC via various approaches and has been a preferred stem cell source in the repair of damaged tissue and reconstruction of diseased organs. Potential differentiation mechanism is considerably complicated and is an integration of multiple signaling pathways based on varying differentiation approaches, different biochemical factors and extrinsic environmental cues.

Since the implantation of exogenous SMC has been considered as a promising therapy for injured SMC tissue repair and replacement, an optimal differentiation approach is critical important. Oxygen tension influences not only *in vitro* expansion and differentiation but also *in vivo* efficacy after implantation. In addition, oxygen also modulates the paracrine activities of stem cells leading to changes of various secretory factors, which might indirectly affect the differentiation process [32]. Therefore, controlling the differentiation process via modulating oxygen concentration is important in future applications.

Most studies in this field concentrate on *in vitro* differentiation employing biochemical factors using monolayers of culture cells. But an increasing data show how ECM substrate markedly affects SMC phenotypic shift. It has been reported that laminin and collagen type-IV substrates inhibit cell proliferation and increased SMA content and, conversely, substrates of fibronectin and collagen type I enhances SMC proliferation and reduces contractile protein expression [33, 34]. When *in vitro* differentiated SMCs are implanted into the distinct tissues or organs, SMCs have to adapt to a varied 3D physiological structure and a possibly unfavourable environment; therefore, integration of more relative factors to mimic *in vivo* environment is still a major challenge in smooth muscle tissue engineering.

9.5 Conflict of Interest

The authors declare that they have no conflict of interest.

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