The discovery of small noncoding microRNAs (miRNAs) increases the complexity of gene expression regulatory mechanisms. The basic mechanism of miRNA biogenesis and regulatory functions of target genes have been widely elucidated. Recently, it has been recognized that regulation of each step of miRNA biogenesis is critical for generating functional miRNAs. Interestingly, cell signaling pathways, including the transforming growth factor-β (TGF-β) signaling pathway, have been found to modulate miRNA biosynthesis. TGF-β signaling regulates expression of several miRNAs that are implicated in the development and homeostasis of vascular smooth muscle cells (VSMCs). This review describes recent understanding of the transcriptional and post-transcriptional regulation of miRNA expression by TGF-β signaling in VSMCs.

Key Words: Vascular smooth muscle cells, TGF-β signaling, MicroRNA
ive precise gene expression during development and homeostatic maintenance of VSMCs.

**VASCULAR SMOOTH MUSCLE CELLS**

VSMCs compose the medial layer of the vessel wall and control blood pressure. VSMCs, unlike other terminally differentiated muscle cells, can switch between differentiated and dedifferentiated states in response to physiological and pathological signals [12]. The differentiated state of VSMCs is termed the 'contractile phenotype' and is characterized by a very low rate of proliferation, appropriate contractility to contractile cues, and expression of smooth muscle cell (SMC)-specific contractile genes, such as smooth muscle α-actin (α-SMA), smooth muscle myosin heavy chain (SM-MHC), SM22α, and calponin. In contrast, the dedifferentiated state of VSMCs is termed the 'synthetic phenotype' and has demonstrated increased proliferation, enhanced migration, and a reduced expression of SMC-specific contractile genes [13]. Control of this phenotype switch is critical for vascular development, homeostasis, and injury repair. Abnormal control of the VSMC phenotype leads to progression of certain vascular diseases, such as atherosclerosis, pulmonary artery hypertension (PAH), or restenosis after angioplasty.

TGFβ signaling is implicated in normal vascular development and homeostasis. Malfunction of the TGFβ signaling pathway results in vascular disorders, such as PAH and hereditary hemorrhagic telangiectasia [14]. The TGFβ superfamily of growth factors, such as TGFβ and bone morphogenetic proteins (BMPs), has been demonstrated to increase the expression of SMC-specific contractile genes and inhibit cell proliferation and migration, leading to the contractile phenotype of VSMCs [15].

**TGFβ SIGNALING**

TGFβ family ligands bind to specific sets of heteromeric receptor complexes, the type I and type II TGFβ receptors, which are both serine/threonine kinases [16]. After formation of this heterotetrameric receptor complex, active type II receptor kinase phosphorylates the type I receptor. Subsequently, active type I receptor phosphorylates the downstream Smad signal transducers. Smads are classified into three classes, including receptor-specific Smads (R-Smads), common Smads (co-Smads), and inhibitory Smads (I-Smads). The active receptor complex phosphorylates R-Smads, leading to their association with the co-Smad, Smad4. This complex translocates to the nucleus and modulates gene expression via DNA binding in a sequence-specific manner. Several downstream transcription factors upon TGFβ signaling bind to the CArG box [CC(ATT)6GG] in the promoters of contractile genes. In association with SRF, Myocardin and Myocardin-related transcription factors (MRTFs) activate the transcription of SMC-specific contractile genes [15]. In contrast, the Kruppel-like zinc finger family 4 (KLF4) is involved in remodeling the chromatin of CArG box-containing promoters, thereby repressing transcription of SMC-specific contractile genes [18].

**MicroRNA**

MiRNAs have been demonstrated to act by fine-tuning gene expression during development and tissue homeostasis. MiRNA genes are transcribed by RNA polymerase II, and the pri-miRNAs transcripts range from a few hundred nucleotides (nt) to a few kilobases in length [19]. Pri-miRNAs fold into hairpin structures and are recognized by RNase III endonuclease Drosha together with the DiGeorge syndrome critical region gene 8 (DGCR8) [20,21]. The Drosha complex also contains other cofactors that modulate the catalytic activity of Drosha, such as RNA helicases, transcription factors, and RNA-binding proteins. Pri-miRNAs are cleaved into precursor miRNAs (pre-miRNAs), which are approximately 70 nt in length and form a long stem-loop structure. The pre-miRNAs are exported from the nucleus to the cytoplasm by Exportin-5 (Xpo5) in cooperation with the guanine triphosphatase (GTPase) Ran. In the cytoplasm, the pre-miRNAs are associated with the cytoplasmic RNase III enzyme Dicer and cleaved into an approximately 22 nt-long double-stranded miRNA containing a guide strand (mature miRNA) and a passenger strand (miRNA*) [22]. While the miRNA* is often degraded, the mature miRNAs associate
with the RNA-induced silencing complex (RISC) and guide RISC to partially complementary sequences within the target mRNAs. The mature miRNAs typically interact with the 3' untranslated regions (UTR) of mRNA targets through Watson-Crick base pairing between the second and seventh bases at the 5' end of the miRNA, termed the seed sequence [23]. Translation of target mRNAs is repressed by sequestering translational machinery from the miRNAs or mRNA destabilization through the recruitment of poly(A) nuclease, followed by deadenylation.

**CONTROL OF MicroRNA BIOGENESIS BY TGFβ SIGNALING**

Recently, it has been reported that TGFβ signaling pathway can regulate the biogenesis of small noncoding miRNAs [24]. Expression of miRNAs can be transcriptionally controlled by TGFβ signaling, similarly to mRNAs, or post-transcriptionally in either of the processing steps (Fig. 1).

1. **Transcriptional regulation**

Nucleosome positioning and chromatin immunoprecipitation analyses of the promoter regions of miRNA genes demonstrate that miRNA promoter regions are similar to those of protein coding genes [25,26]. Smad signal transducers of TGFβ bind to promoter regions and modulate the transcription of miRNA genes. For example, miR-216a/217 and miR-192 are transcriptionally induced by Smads in glomerular mesangial cells and in mouse kidney epithelial cells, respectively [27,28]. Similarly, let-7a/d in human alveolar basal epithelial cells and miR-155 in murine mammary gland epithelial cells are induced by TGFβ-mediated Smad binding [29]. Conversely, transcription of miR-24 in myoblasts and miR-29 in fibroblasts and tubular epithelial cells are repressed by TGFβ-mediated Smad binding to their promoters [30,31].

Recently, we demonstrated that TGFβ/BMP signaling down-regulates transcription of the miRNA-302-367 gene cluster in various cells, including VSMCs [10]. This transcriptional repression of miR-302 by BMP signaling is mediated by Smads. Smad4 associates with the miR-302 promoter and recruits HDAC to repress miRNA-302 transcription. MiRNA-302c targets a partially complementary sequence localized in the 3' UTR of type II TGFβ receptor and type II BMP receptor genes (BMPRII) and leads to destabilization of the transcripts and downregulation of TGFβ/BMP signaling. The functional consequence of miR-302c-dependent downregulation of BMPRII on the BMP signaling pathway is inhibition of the contractile phenotype of VSMCs.

In addition to direct association of the Smad proteins with the miRNA promoters, Smads indirectly regulate miRNA expression through activation of transcription factors that associate with miRNA promoters. For example, TGFβ/BMP-mediated activation of transcription factors, such as Myocardin and Myocardin-related transcription factor (MRTF), activate transcription of miR-143/145 [18]. MiR-143 and miR-145 function to regulate the phenotype switching of VSMCs.

2. **Post-transcriptional regulation**

Recently, a novel role for Smads in the post-transcriptional regulation of miRNA processing has been elucidated [8]. Upon TGFβ and BMP signaling, the mature miRNA-21 level is increased in VSMCs without a corresponding increase in pri-miRNA-21, unlike transcriptionally regulated miRNAs. Smads interact with p68 in the Drosha Microprocessor complex and promote cleavage of pri-miR-21 into pre-miR-21.
For the Drosha-mediated miRNA-21 processing, Smads are required as Drosha binding to pri-miRNA-21 and miRNA processing are decreased when Smads are down-regulated by small interfering RNA (siRNA). Interestingly, unlike in the canonical Smad signaling pathway, a co-Smad is not essential for induction of miRNA processing. Therefore, TGFβ and BMP signals regulate gene expression via miRNAs independently of Smad4. The increased level of miR-21 by TGFβ and BMP signals represses the expression of proteins such as programmed cell death protein-4 (PDCD4) and multiple members of the dedicator of cytokinesis (DOCK) family, leading to the contractile phenotype of VSMCs [8,9].

In addition to miRNA-21, TGFβ and BMP signals modulate expression of a cohort of miRNAs through Smad-mediated post-transcriptional regulation. Interestingly, those miRNAs contain a conserved sequence (5'-CAGAC-3') toward the center of the mature miRNA region, which is identical to the consensus sequence for DNA binding by Smads [32]. Mutation of the conserved Smad binding sequence in pri-miRNAs abolishes the induction of miRNAs by TGFβ and BMP signals. Moreover, the mutation results in a reduction of Smad association with the pri-miRNA, as well as binding of Drosha and DGCR8 to the pri-miRNA. Therefore, Smad binding to both DNA and RNA is essential for the regulation of miRNA processing, as well as miRNA transcription upon TGFβ and BMP signals.

The role of Smads in the regulation of Drosha-mediated pri-miRNA processing has been further supported by Smad nuclear interacting protein 1 (SNIP1) [33]. SNIP1, a nuclear protein partner of Smads, was demonstrated to associate with Drosha and modulate miRNA biogenesis. Downregulation of SNIP1 reduces the expression of a subset of miRNAs, including miR-21. Therefore, Smads might alternatively regulate miRNA biogenesis by modulating the association of SNIP1 with Drosha.

CONCLUSION

Many miRNAs have been shown to function in the maintenance of a contractile or synthetic VSMC phenotype [24]. Control of the VSMC phenotype is critical for normal vascular development and homeostasis, and several signaling pathways are involved in this process. Therefore, understanding the complex interactions between miRNAs and signaling pathways involved in the vascular system may offer potential miRNA-based therapeutic applications. In this review, we summarized the regulation of miRNA expression at the transcriptional and post-transcriptional levels, focusing on TGFβ signaling in VSMCs. This study provides insights into how growth factor signaling pathways are integrated into miRNA biogenesis. We anticipate that future studies will identify additional important signal transduction networks and elucidate the mechanisms underlying the specificity of regulated miRNAs by different signaling pathways.

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