



Review

Smad-mediated regulation of microRNA biosynthesis

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ABSTRACT

microRNAs (miRNAs) are small non-coding RNAs conserved in metazoans. Depletion of miRNAs results in embryonic lethality, suggesting they are essential for embryogenesis. Similarly, pathways induced by growth factors of the transforming growth factor β (TGF- β) superfamily control cell growth, differentiation, and development. Recently Smad proteins, the signal transducers of the TGF- β pathway, were found to regulate miRNA expression, which, in turn, affects expression of numerous proteins. Smads modulate miRNA expression through both transcriptional and post-transcriptional mechanisms illustrating the complexity of gene regulation by TGF- β . In this chapter we summarize the current knowledge of mechanisms underlying Smad-mediated regulation of miRNA biogenesis.

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1. Introduction

miRNA profiling study suggests that miRNAs are globally reduced in tumor samples as compared to normal tissues [1]. Another study found that miRNA signatures are a better indicator of tumor source and prognosis than similarly developed mRNA signatures [2]. Deregulation of miRNAs is a critical component of both developmental defects and pathogenesis. Thus, miRNAs are integral to basic physiological functions in metazoans similar to the TGF- β super family of growth factors. It is not too surprising, then, that miRNAs are themselves critical components of the TGF- β signaling pathway, nor that the Smad proteins have developed several different mechanisms for regulating miRNA expression and activity. Below, we discuss the recent advances made in understanding the diverse mechanisms of miRNA regulation utilized by the TGF- β signaling pathway and the implications of this regulation to human diseases.

2. miRNA biogenesis

Since the original description of miRNAs in 1993 [3], they have emerged as critical regulators of nearly every aspect of mammalian biology. By one estimation more than 60% of protein-coding re-

gions in the human genome display areas of deep conservation in their 3' UTR indicative of targeting by miRNAs [4]. miRNAs are important regulatory factors that mediate the expression of large numbers of proteins. Profiling studies show that a single miRNA may tune the expression of over 100 different proteins [5]. Partial complementarity between miRNA sequences and their target mRNAs usually occurring in the 3'-UTR, mediates translational repression or, more commonly, destabilization of the mRNAs [6,7]. Interestingly, specific recognition of target mRNAs by a miRNA does not require 100% sequence complementarity [6]. Rather, the 6–8 nucleotides at the 5' end of the miRNA, known as the “seed sequence” or miRNA recognition element (MRE), confer the most critical determinants for targeting mRNAs; though other nucleotides may also be important under certain circumstances [8]. Because a relatively small number of bases is required to induce silencing, a large number of transcripts can be targeted by a single miRNA, however, the degree of change for any single transcript is relatively small at ~30–50% reduction [9].

miRNA biogenesis begins in the nucleus where they are transcribed by RNA polymerase II (RNA Pol II) as long transcripts known as primary miRNA (pri-miRNA) [10] (Fig. 1). Like mRNAs, pri-miRNAs are poly-adenylated at the 3' end and bear a 7-methyl-guanosine cap at the 5' end [11]. Pri-miRNA is cleaved by the RNase III enzyme Drosha into a shorter (<100 bp) transcript known as the precursor miRNA (pre-miRNA) composed of a stem-loop structure which encodes the mature miRNA sequence in the stem [12]. Drosha acts as part of a large processing complex that consists of multiple proteins, including the DiGeorge critical

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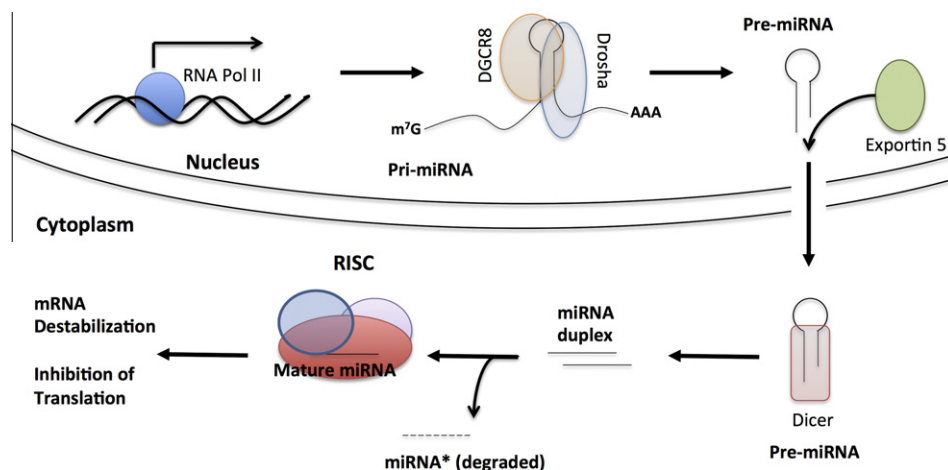


Fig. 1. miRNA biogenesis pathway. Biosynthesis of miRNAs begins in the nucleus with the transcription of miRNA genes by RNA Pol II as long transcripts bearing a 7 methyl-guanosine cap at the 5'-end and a poly (A) tail at the 3'-end. The Drosha microprocessor complex, the minimal components of which are DGCR8 and Drosha, cleaves the pri-miRNA into pre-miRNA. Exportin 5 then exports the pre-miRNA into the cytoplasm where it undergoes a secondary processing step into a short miRNA duplex by Dicer. The miRNA duplex contains both a mature miRNA strand and a miRNA* strand. The mature miRNA is loaded into Ago proteins and mediates the silencing function as a part of the RISC, while the miRNA* gets degraded.

region 8 (Dgcr8 or Pasha) [13,14]. Dgcr8 is a double-stranded-RNA-binding protein that stabilizes the association of Drosha with pri-miRNA and determines the precise location of the processing; it is therefore essential for miRNA maturation [13]. Interestingly, Dgcr8 and Drosha cross-regulate expression of one another presumably to maintain homeostatic control of miRNA biogenesis [15]. Drosha destabilizes Dgcr8 mRNA through the hairpin structure in the 5'-UTR of the mRNA while Dgcr8 stabilizes the Drosha protein [15]. Although Drosha and Dgcr8 comprise the minimum components of the microprocessor complex, many more proteins have been identified that interact with this large structure. Two of these proteins are the DEAD-box helicase proteins p68 (also known as DDX5) and p72 (DDX17) [16]. Deletion of either p68 or p72 leads to embryonic lethality, however neither protein is absolutely essential for miRNA development as deletion of either from mouse embryonic fibroblasts (MEFs) disrupts the expression a distinct, but overlapping, set of miRNAs [16].

Following the cleavage of pri-miRNA by the Drosha microprocessor complex, the pre-miRNA is exported from the nucleus through the cooperative activity of Ran-GTP, which provides energy and exportin-5 (EXP5), which interacts with the stem-loops structure of pre-miRNAs [17]. Once in the cytoplasm, the pre-miRNA hairpin associates with another RNase III enzyme called Dicer, which further cleaves it into a double-stranded, miRNA duplex of approximately 22 nt, containing the guide strand (or mature miRNA) and the passenger strand (or miRNA*) [17]. The miRNA duplex is then loaded into argonaute (Ago) proteins, which select the guide strand and present it to the RNA-induced silencing complex (RISC) for targeting and silencing. Mature miRNAs can be generated from either the 5' or the 3' arm of the miRNA duplex, but it is rare for both strands to remain at high levels in the cell as the passenger strand is quickly degraded [14].

3. Smad signaling

The TGF- β family is a pleiotropic group of growth factors that activate an evolutionarily conserved signal-transduction cascade. TGF- β family ligands include TGF- β itself, integrins and bone morphogenic proteins (BMP). This ligand variety (46 human open reading frames or ORFs) is mirrored by a large degree of receptor diversity as well (12 human TGF- β receptors) [18]. However, this receptor diversity can be separated into two different classes of

receptors termed type I and type II receptors. Both receptor classes contain serine-threonine kinase domain and ligand binding induces heterodimerization between the two types of receptor. TGF- β is originally bound by the constitutively active type II receptors which induces binding, phosphorylation and activation of the type I receptor which propagates signal transduction into the cell.

Despite its receptor and ligand diversity, TGF- β family signal transduction is carried out by a relatively small number of conserved proteins known as Smads. Smads can be grouped into two distinct sets of proteins, the receptor-specific Smads (R-Smads) and the common Smad (co-Smad/Smad4). R-Smads are only phosphorylated upon receptor dimerization by their specific receptors; with the BMP receptors activating Smads 1, 5 and 8 and the TGF- β receptors activating Smads 2 and 3 [18]. Upon phosphorylation, R-Smads dimerize with Smad-4 and translocate into the nucleus as a complex. This interaction with Smad4 is essential for Smad-mediated transcriptional activity [19]. Once in the nucleus the R-Smad-Smad4 complex binds to the Smad-binding element (SBE) canonically identified as the heptamer 5'-CAGAC-3'. Though the SBE is sufficient to modulate Smad binding and activation, additional co-factors are necessary for strong activation of any ORF [20]. In addition to these primary effector molecules a number of other modulatory pathways exist including two inhibitory Smads, Smad6 and Smad7, and the Smad inhibiting protein 1 (SIP1).

Because of their deep conservation it is probably not surprising that Smad pathways are integral for a diverse range of biological processes. Furthermore, deregulation of Smad-controlled miRNAs has been implicated in numerous pathological conditions including cancer [21–24], cardiovascular disease [25], and fibrosis [26,27]. Understanding how Smad proteins regulate expression of miRNAs and how these unique molecules in turn feed back onto Smad expression are therefore critical elements in unraveling the diverse, context-dependent affect of TGF- β signaling.

That miRNAs comprise critical components of Smad signaling pathways is illustrated by the large number of miRNAs whose expression change in response to stimulation with TGF- β -family ligands. One recent study found ~20 miRNAs induced and another ~10 miRNAs inhibited in pulmonary arterial smooth muscle cells (PASMC) stimulated with either TGF- β 1 or BMP4 [28]. Similarly, in mouse granulosa cells, TGF- β treatment was found to significantly alter the expression of 16 miRNAs [29]. In contrast, interferon (IFN) stimulation of macrophages induced a significant

change in only one miRNA [30]. These data illustrate that miRNAs are likely to play an important role in the physiological activity of TGF- β signaling.

4. Transcriptional regulation of miRNAs by Smads

Chromatin immunoprecipitation (CHIP) analyses reveal that miRNA promoter elements closely resemble those of protein coding regions [31,32]. Therefore, it is not surprising that Smad proteins control the transcription of a variety of miRNA genes. Both *in vitro* and *in vivo* miRNA expression-profiling studies confirm that R-Smad proteins modulate a unique, but overlapping set of miRNAs [21,26,33,34]. For example, TGF- β induces both miR-216a and miR-217 in glomerular mesangial cells via Smad binding elements (SBEs) in the miR-216 promoter [35]. Conversely, TGF- β -induced Smad3/4 complex binding to the miR-24 promoter inhibits the expression of miR-24 in myoblasts [36]. Transcriptional activation of miRNAs has distinct physiological significance *in vivo*. For instance, transcriptional repression of miR-29 by Smad-binding to the promoter promotes renal fibrosis [33].

Transcriptional activation of miRNA genes by Smad is sensitive to canonical TGF- β signaling cascades. For instance, in mouse kidney epithelial cells, induction of miR-192 by the TGF- β -specific R-Smad, Smad3, can be reduced not only by siRNA against Smad3 but also by overexpression of the antagonistic Smad protein Smad7 [26]. Furthermore, many miRNAs transcriptionally regulated by Smads are known to be dependent on Smad4. One recent study of TGF- β stimulated murine mammary gland epithelial cells indicates that Smad4 knockdown results in deregulation of 28 miRNAs [21]. In these cells, a Smad4 binding site within the miR-155 promoter is critical for induction of this miRNA and subsequent down-regulation of its target gene RhoA [21].

In addition to direct association of the Smad complex with the miRNA promoters, Smads can indirectly modulate miRNA levels through activation of transcription factors that associate with their promoters. For instance, the miR-143~145 gene locus, which encodes both miR-143 and miR-145, is transcriptionally regulated by a complex composed of serum response factor (SRF) and myocardin or myocardin related transcription factors (MRTFs) [37,38]. TGF- β and BMP signaling increase miR-143 and miR-145 through activation of myocardin and MRTF, respectively [38]. Similarly, induction of let-7d and let-7a by TGF- β is mediated by the binding of Smad3 to a SBE in their promoters [27]. Interestingly, higher let-7d expression is found in the lungs of patients with idiopathic pulmonary fibrosis (IPF) as compared with control individuals [27] and increased expression of let-7d and decreased expression of its target HMGA2 correlate with higher TGF- β activity [26]. Therefore, deregulation of Smad-controlled miRNA may have serious health consequences despite the relatively small effect of a single miRNA:mRNA interaction.

5. Epigenetic regulation of miRNAs

Like most transcription factors, Smad activity on the promoters of target genes may be epigenetically modulated. For example, mouse embryonic fibroblasts (MEFs) transfected with the oncogene RasV12 become senescent as part of the response to aberrant cell proliferation. Profiling of methylated loci following the expression of RasV12 identified multiple targets of the BMP-specific R-Smad Smad1, that are induced or repressed by this process [39]. The promoter/enhancer structure of miRNAs largely reflects that of RNA Pol II transcripts; similarly, the chromatin structure of miRNA promoters reflects that of RNA Pol II transcripts [31]. Thus, it is likely that miRNA expression is susceptible to epigenetic changes similar to protein coding genes. Epigenetic silencing is documented

for several miRNAs, including miR-203 [40], miR-9 [41], and miR-124 [42]. Therefore, we postulate it is only a matter of time before epigenetic control of miRNAs by the TGF- β signaling pathway is identified. Additionally, two TGF- β regulated miRNAs, miR-206 and miR-29, post-transcriptionally repress histone deacetylase 4 (HDAC4) induction and thereby inhibit myogenic differentiation [43]. Thus TGF- β regulated miRNAs can themselves alter epigenetic patterns as a way to mitigate more long-term gene regulation.

6. Post-transcriptional regulation of miRNAs by Smads

As described above, regulation of miRNA transcription by TGF- β signaling largely conforms to the determinants of normal Smad transcription. Close molecular dissection of TGF- β signaling recently identified a novel role of Smads in post-transcriptionally regulating miRNA biogenesis. In vascular smooth muscle cells (VSMCs), TGF- β and BMP4 signaling promotes the maintenance of a synthetic “contractile” phenotype, while lack of TGF- β or BMP4 signaling results in a more proliferative phenotype [44]. This phenotype is maintained, at least partially, due to the induction of miR-21, which leads to downregulation of the miR-21 target programmed cell death protein 4 (PDCD4) [45]. Interestingly, the induction of miR-21 by TGF- β or BMP4 occurs without a corresponding increase in pri-miR-21 and is not repressed by the RNA Pol II inhibitor α -amanitin [45]. Furthermore, unlike transcriptionally regulated miRNAs, BMP and TGF- β -mediated induction of miR-21 occurs independent of Smad4, though it is completely dependent on the R-Smads [45]. RNA CHIP experiments confirm that R-Smads bind directly to the pri-miR-21 hairpins in a ligand-dependent manner [45]. Thus, mature miR-21 expression is induced by R-Smad activity through a post-transcriptional mechanism (Fig. 2).

The mechanism for this process was actually hinted at by a study performed to identify DNA regulatory proteins that promote Smad3 activity. Through a yeast two-hybrid system using the carboxyl-terminal Mad homology 2 (MH2) domain of Smad3 as bait, the DEAD-box RNA helicase protein p68 was identified as a Smad3 interacting protein [46]. As discussed above, p68 is an emerging factor in pri- to pre-miRNA processing [16]. Consistent with this observation the induction of miR-21 by BMP is dependent on expression of p68 [45]. These data suggest a new mechanism of miRNA regulation by Smads in which ligand stimulation induces translocation of Smads to the nucleus where an association with the Drosha microprocessor complex occurs. Further, it provides a potential mechanism for Smad4-independent gene regulation [47]. Interestingly, while many Smad4 deficient cell types develop normally, they are more prone to tumorigenic transformation [47]. This observation is consistent with the oncogenic function of many TGF- β regulated miRNAs, including miR-21, which is known to promote cell growth and migration as well as to inhibit apoptosis in various types of tumors [48]. These results further suggest that Smad-mediated regulation of miRNA processing may be important during development.

To assess the structural characteristics of pri-miRNA regulated by Smads, miRNA microarray profiling studies were performed [28]. These studies found that, approximately 5% (20 of 377) of miRNAs examined are induced by stimulation with both TGF- β and BMP [28]. Interestingly, a subset of these miRNAs contain an SBE-like RNA sequence (R-SBE) at the 3' end of their mature miRNA sequence [28]. The R-SBE motif (5'-CAGAC-3') is necessary for direct binding of Smad proteins to pri-miRNA as illustrated by the fact that mutation of this region abrogates Smad binding [45]. When introduced to miRNAs that do not contain R-SBE, this sequence motif is sufficient to confer not only the association with Smad but also TGF- β -dependent recruitment of Drosha and processing [45].

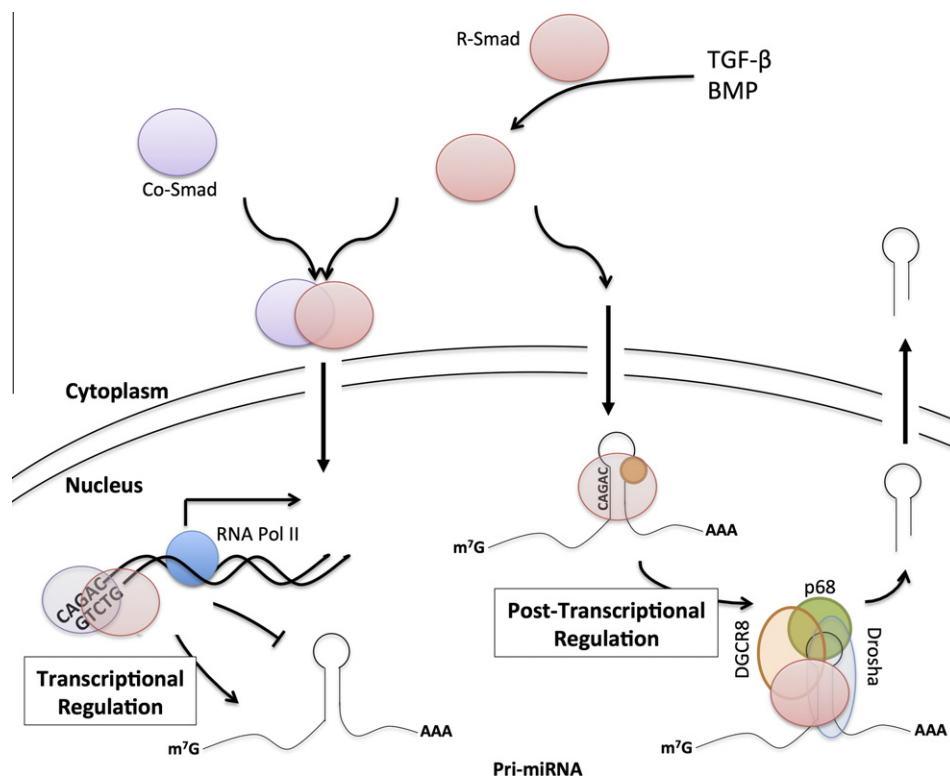


Fig. 2. Regulation of miRNA expression by Smads. Smad-mediated regulation of miRNAs can be classified into two mechanisms: (i) transcriptional regulation of miRNAs by Smads largely resembles the canonical TGF- β signaling pathway. Signal transduction leads to phosphorylation of an R-Smad, which induces the formation of an R-Smad/co-Smad heterodimer. This complex is imported into the nucleus, binds the SBE and regulates, either positively or negatively, the transcription of miRNA genes. The pri-miRNAs then undergoes normal miRNA processing. (ii) Post-transcriptional regulation of miRNA biogenesis acts on pri-miRNA in the nucleus. Phosphorylation of an R-Smad induces nuclear localization. In the nucleus, R-Smad recognizes and binds an SBE-like sequence localized in the stem region of the pri-miRNA, and recruits the Drosha/DGCR8 microprocessor complex to the pri-miRNA. The microprocessor then promotes efficient processing of the pri-miRNA into pre-miRNA.

Drosha is a largely non-specific RNase enzyme that requires interactions with other proteins, such as Dgcr8, to recruit and indicate the appropriate site for pri-miRNA cleavage [15]. Recent evidence suggests that proper RNA binding by microprocessor components is a vital determinant of Drosha cleavage and affects pre-miRNA and subsequently mature miRNA size and stability [15]. Thus the location of the R-SBE within the hairpin structures of the pri-miRNAs may be critical for modulating the final mature miRNA sequence.

Direct association of Smads with the pri-miRNAs is carried out through the MH1 domain of Smad proteins [28], the same region responsible for binding the SME to DNA during canonical Smad signaling [20]. Although the pri-miRNA binding activity of R-Smads is mediated by a molecular mechanism resembling that of DNA-binding, Smad4 is essential for the association with DNA [49] but not with pri-miRNAs [45]. It is also important to note that the phosphorylation of R-Smads at the carboxyl-terminus serine residues by the type I TGF- β receptors is not essential for the pri-miRNA processing function of R-Smads. It was initially reported that the phosphorylation of R-Smads promotes association of R-Smads with Smad4 and translocation to the nucleus [49]. It is currently unclear whether there is another protein which substitutes for the role of Smad4 in nuclear import during processing of pri-miRNAs. A subset of Smad proteins can enter the nucleus in a basal non-phosphorylated setting by a mechanism that does not require import in 7 (Imp7) or Imp8, the canonical Smad nuclear-import factors [50]. Thus R-Smads present in the nucleus in a form not bound to Smad4 might preferentially interact with the Drosha/Dgcr8 microprocessor complex to promote miRNA maturation. However,

this hypothesis does not address the TGF- β inducibility of Smad-mediated miRNA maturation. Thus, inducible, Smad4-independent transport of R-Smads into the nucleus remains an interesting open avenue of research. Furthermore, kinases other than the TGF- β receptors, such as mitogen activated protein kinase (MAPK), glycogen synthase kinase 3 (GSK3) and extracellular signal-regulated kinase 2 (ERK2), can also affect the subcellular localization of R-Smads and thereby alter pri-miRNA processing [51,52].

7. p68/p72-interacting transcription factors in addition to Smads

The Drosha microprocessor complex is emerging as a critical site for post-transcriptional regulation of miRNA biogenesis. Two additional transcription factors, estrogen receptor- α (ER α) and p53, are known to interact with the Drosha complex similar to R-Smads [53,54]. In the case of p53, DNA damage induces p53 to interact with the Drosha complex through p68 which promotes pri- to pre-miRNA processing of multiple miRNAs, including miR-143, miR-145, and miR-16-1 [53]. ER α is also recruited to the Drosha microprocessor complex through p68 in response to estradiol (E2) stimulation, however, the recruitment of ER α with the Drosha complex inhibits the processing of pri-miR-143, pri-miR-145, pri-miR-16-1, pri-miR-195, and pri-miR-125a [54]. Unlike the Smads, the mechanisms of recognition for specific pri-miRNAs by p53 or ER α remain unknown, however, it is plausible that further analyses will unveil motifs within the pri-miRNA sequence recognized by these transcription factors. Interestingly, miRNA profiling experiments show that BMP4 and TGF- β stimulation in VSMCs also

results in the downregulation of a subset of miRNAs [28]. Furthermore, miR-206 is post-transcriptionally downregulated in the C2C12 myoblast cell line following BMP2 treatment [55]. These data suggest that in addition to positive regulation of pri- to pre-miRNA processing, the Smad/Drosha/Dgcr8 complex may also inhibit the maturation of pri-miRNAs.

The involvement of both p68 and p72 in Drosha processing induced by diverse regulatory pathways and involving divergent transcription factors suggests that these helicase proteins represent a general hub for the regulation of miRNA biogenesis. However, it remains unclear whether this mechanism is generally applicable to other transcription factors. To date several additional transcription factors, including MyoD [56], Runx2 [56], androgen receptor [57] and β -catenin [54], have been found to interact with p68 and p72. Thus it is likely that at least some of these proteins might contribute to miRNA biogenesis. Interestingly, a *meta*-analysis of miRNA profiling found that there is no correlation between the level of pre-miRNA and mature miRNA in cancer cells [58]. This observation suggests that post-transcriptional regulatory mechanisms play a pivotal role under pathological conditions as well as in normal cells [58]. Finally, it is noteworthy that Smads may not be the only members of the TGF- β signaling pathway that work by promoting miRNA biogenesis. The protein small nuclear interacting protein 1 (SNIP1), which was originally identified based on its nuclear interaction with Smads, also associates with Drosha [59]. Whether this interaction affects miRNA biogenesis, has not yet been determined.

8. Reciprocal regulation: Control of Smad proteins by miRNAs

In addition to controlling miRNA expression, Smad proteins can themselves be targeted by miRNAs. To date, most Smad proteins are validated targets of at least one miRNA (see Table 1). Smad expression can be modulated by miRNAs under normal physiological conditions. For example, BMP2 signaling promotes osteoblast differentiation at least in part by inhibiting the expression of miR-135 and miR-199* which target Smad5 and Smad1, respectively [21,60]. Smads are also targeted by miRNAs under pathological states; for instance in diffuse large B cell lymphoma miR-155 promotes proliferation by downregulating Smad5 expression [23]. Even exogenous signals can alter Smad expression through miRNAs. For example, miR-21 targets the antagonistic Smad; Smad7, thus increasing basal TGF- β signaling in hepatocytes following infection with the hepatitis C virus

(HCV) [61]. Recently, Smad3 was identified as a target of miR-140 which is regulated only at the protein level [62]. To identify this protein, the authors first identified mRNAs that were indirect targets of miR-140 using mRNA arrays to find genes modulated by miR-140, but lacking a seed-sequence [62]. Next, they characterized promoter elements that were underrepresented in the off-target transcripts to identify putative binding sites of the altered transcription factors [62]. Such studies illustrate both the difficulty and the importance of considering the translational effects of miRNA activity. It is likely that many more miRNAs that target Smads remain to be discovered by studies that consider changes occurring only at the protein levels.

Additional proteins related to the TGF- β -Smad signaling pathways, such as Smad inhibiting protein 1 (SIP1) [63,64] and TGF- β super family receptors, such as ALK4 [65], the type II TGF- β receptor [66] and the type II BMP receptor (BMPRII) [67], as well as TGF- β ligand itself [68] are targeted by miRNAs. The case of BMPRII is especially interesting as it is targeted by miR-21 whose expression is elevated by Smad activity [67]. It is currently unclear whether miR-21-dependent regulation of BMPRII plays a role in Smad signaling as BMPRII expression remains high throughout the course of BMP stimulation, at least in VSMCs (A.H., unpublished data). It is possible that miRNA regulation of BMPRII acts as a negative feedback loop to limit the duration of Smad activity.

9. Conclusion

In this chapter, we summarized a critical role of Smad proteins in the regulation of miRNA biogenesis. Two primary modes of regulation by Smads are: (i) transcriptional regulation of miRNA genes by nuclear translocation of R-Smad/co-Smad heterodimers, association with the DNA sequence motif known as SBE, and modulation of transcription, and (ii) post-transcriptional regulation of pri- to pre-miRNA processing by R-Smad through the recruitment and activation of the Drosha/Dgcr8/p68 microprocessor complex. Additional mechanisms of miRNA regulation by the TGF- β super family of growth factors are likely to be uncovered in the future. Such mechanisms are an important component of understanding how cells integrate the complex repertoire of miRNAs whose expression levels are fine-tuned by TGF- β signaling pathways and transmit a precise signal in order to control normal development and maintain homeostasis.

Table 1
miRNAs targeting Smads. miRNAs have been shown to target numerous Smad proteins under various cellular conditions. Below is a list of miRNAs that have been experimentally validated to target individual Smad proteins and the biological significance of this activity.

Target	miRNA	Activity	Ref.
miR-18a	Smad2	Regulates TGF- β signaling in neuroblastoma cells	[69]
miR-18a	Smad4	Regulates TGF- β signaling in neuroblastoma cells	[69]
miR-21	Smad7	Enhances TGF- β signaling in HCV infected livers	[61]
miR-135	Smad5	Inhibits osteoblast differentiation	[60]
miR-140	Smad3	In 3T3 cells, regulates expression only at the protein level	[62]
miR-145	Smad2	Alters macrophage sensitivity to TGF- β	[70]
miR-146-5p	Smad4	Suppresses TGF- β signaling in thyroid cancer	[71]
miR-155	Smad1	Induced by Epstein-Bar Virus (EBV) in B cells to inhibit BMP signaling and virus reactivation	[72]
miR-155	Smad2	Alters macrophage sensitivity to TGF- β	[70]
miR-155	Smad5	Induced by Epstein-Bar Virus (EBV) in B cells to inhibit BMP signaling and virus reactivation	[72]
miR-155	Smad5	Promotes tumorigenesis in diffuse large B cell lymphoma	[23]
miR-192	Smad3	Contributes to collagen production by mouse renal cells and fibrosis in the kidney in vivo	[26]
miR-199a*	Smad1	Inhibits differentiation into chondrocytes	[73]
miR-200	Smad3	Promotes epithelial-mesenchymal transition in gastric cancer	[74]
miR-224	Smad4	Enhances proliferation in mouse granulosa cells	[29]
miR-483-3p	Smad4	Promotes proliferation of pancreatic cancer cells	[24]

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