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BMP Signaling Goes Posttranscriptional in a microRNA Sort of Way

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BMP Signaling Goes Posttranscriptional in a microRNA Sort of Way

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Abstract

Aberrant microRNA (miRNA) expression correlates with human diseases such as cardiac disorders and cancer. Treatment of such disorders using miRNA-targeted therapeutics requires a thorough understanding of miRNA regulation in vivo. A recent paper in *Nature* by Davis et al. expands our understanding of miRNA biogenesis and maturation, elucidating a mechanism by which extracellular signaling directs cell differentiation via posttranscriptional regulation of miRNA expression.

Current analyses predict approximately 700 human miRNAs, and several hundred more may exist. These miRNAs are likely to regulate thousands of human genes. Many miRNAs are expressed in tissue-specific patterns and function in differentiation and morphogenesis of those tissues. Misexpression of such miRNAs frequently results in aberrant development. Given the importance of precisely controlling miRNA function, there is significant interest in characterizing factors required to regulate miRNA gene expression.

MicroRNAs comprise a single class of small, noncoding regulatory RNAs, which are most easily distinguished by their mode of biogenesis (Figure 1; reviewed in Murchison and Hannon, 2004). They are encoded by genes in animals, plants, and viruses and are transcribed primarily by RNA polymerase II. A primary miRNA (pri-miRNA) transcript contains an inverted repeat that folds into a short hairpin. The hairpin is cleaved from the rest of the transcript by the nuclear Microprocessor complex, which includes the RNase III enzyme DROSHA, DGCR8, and the associated RNA helicases p68 and p72. The resulting pre-miRNA hairpin product is exported from the nucleus to the cytoplasm via interaction with the nuclear export protein Exportin-5. In the cytoplasm, Dicer processes the pre-miRNA hairpin into a 21–24 nucleotide duplex RNA and subsequently aids in the assembly of the miRNA effector complex, the miRNA-induced silencing complex (miRISC). Active miRISC contains only one of the two RNA strands, either the mature miRNA or the mature miRNA(*) species, which guides effector complex interaction with target messenger RNAs via complementary base pairing. While recent work has elucidated factors required for the transcriptional control of miRNAs and the downstream effect of mature miRNAs on their targets, the regulation of miRNA biogenesis by cellular events that produce miRISC is less well understood.

Recently, the miRNA miR-21 has come under scrutiny and provided us with some surprising revelations. It was shown previously that miR-21 expression is significantly upregulated in damaged cardiovascular tissue (Ji et al., 2007). In Davis et al., 2008, a new

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study published in *Nature*, the authors demonstrate that miR-21 upregulation is a consequence of TGF-β superfamily signaling during normal development and homeostasis of the vasculature. Moreover, they show that TGF-β superfamily signaling controls the miRNA processing machinery to achieve upregulation of miR-21.

It is well established that the TGF-β superfamily of growth factors directs vascular development and homeostasis, including induction of the contractile phenotype in human vascular smooth muscle cells (VSMCs), by increasing the expression of VSMC genes (Lagna et al., 2007). Davis et al. explored the role of miR-21 in VSMC differentiation by neutralizing miR-21 function using 2′-O-methyl antagomirs. They observed a reduction in VSMC gene expression. Conversely, overexpression of miR-21 with a viral vector increased expression of VSMC markers. They then examined the effect of signaling by BMP4 and TGF-β on miR-21 and discovered a several-fold increase in miR-21 expression. Previous reports have demonstrated a role for cell signaling in the regulation of miRNA expression. However, with a few exceptions, the signals control the rate of miRNA transcription (Lee et al., 2008; Obernosterer et al., 2006; reviewed in Smallheiser, 2008; Thomson et al., 2006) Significantly, when Davis et al. looked at which step of miR-21 biogenesis was affected by BMP4, they found the levels of pri-miR-21 unchanged, suggesting that signaling was affecting miR-21 expression posttranscriptionally.

BMPs transduce their signal through receptor-mediated phosphorylation of cytoplasmic SMAD proteins. Once phosphorylated, SMADs are able to enter the nucleus and regulate gene transcription by binding to specific DNA sequences in target genes. To elucidate the potential role of SMADs in miR-21 induction, Davis et al. used RNAi knockdown to deplete SMAD1 and SMAD5 and observed a reduction in BMP4-mediated induction of both pre- and mature miR-21, with no corresponding effect on the levels of pri-miR-21. These results suggested that SMADs regulated, directly or indirectly, a posttranscriptional step in miR-21 expression.

Fortuitously, Davis et al. had some hint as to how SMADs might regulate miR-21 maturation, since it was reported that the MH2 domain of SMAD1 interacts with a subunit of the Microprocessor complex, the RNA helicase p68 (Fukuda et al., 2007; Warner et al., 2004). The authors were able to confirm this interaction and demonstrate, using GST pull-down assays, that the physical interaction between SMAD1 or SMAD5 and p68 could be induced by BMP4. Furthermore, RNA-chromatin immunoprecipitation showed that BMP4 also stimulated association of pri-miR-21 with SMAD1, SMAD3, and SMAD5. Notably, the observed SMAD-p68 interaction was RNaseA-resistant and thus not directly dependent upon the presence of pri-miR-21 RNA. The authors also observed an interaction between SMADs and DROSHA that was triggered by BMP stimulation. However, this interaction was somewhat RNaseA sensitive. Thus, the authors postulate that miR-21 maturation is stimulated as a result of the association of activated R-SMADs and DROSHA, by means of interaction with p68 (Figure 1). This association could result in increased processing of pri-miR-21 to pre-miR-21. In vitro processing assays confirmed that pre-miRNA formation was increased upon BMP4 stimulation.

These studies thus reveal an exciting connection between BMP signaling and nuclear miRNA maturation. Yet many questions remain unanswered. First, what factors are required for the pri-miR-21-SMAD interaction? If SMADs can associate with p68, why is the recruitment of SMADs to the p68-DROSHA complex pri-miRNA specific? Does the SMAD protein directly bind to the RNA in a structure-dependent fashion and facilitate Microprocessor association? Finally, it would be exciting to explore whether additional Microprocessor-interacting proteins regulate the maturation of other specific miRNAs,

which would demonstrate that cells contain a host of subtly distinct, yet functionally specific, miRNA processing complexes.

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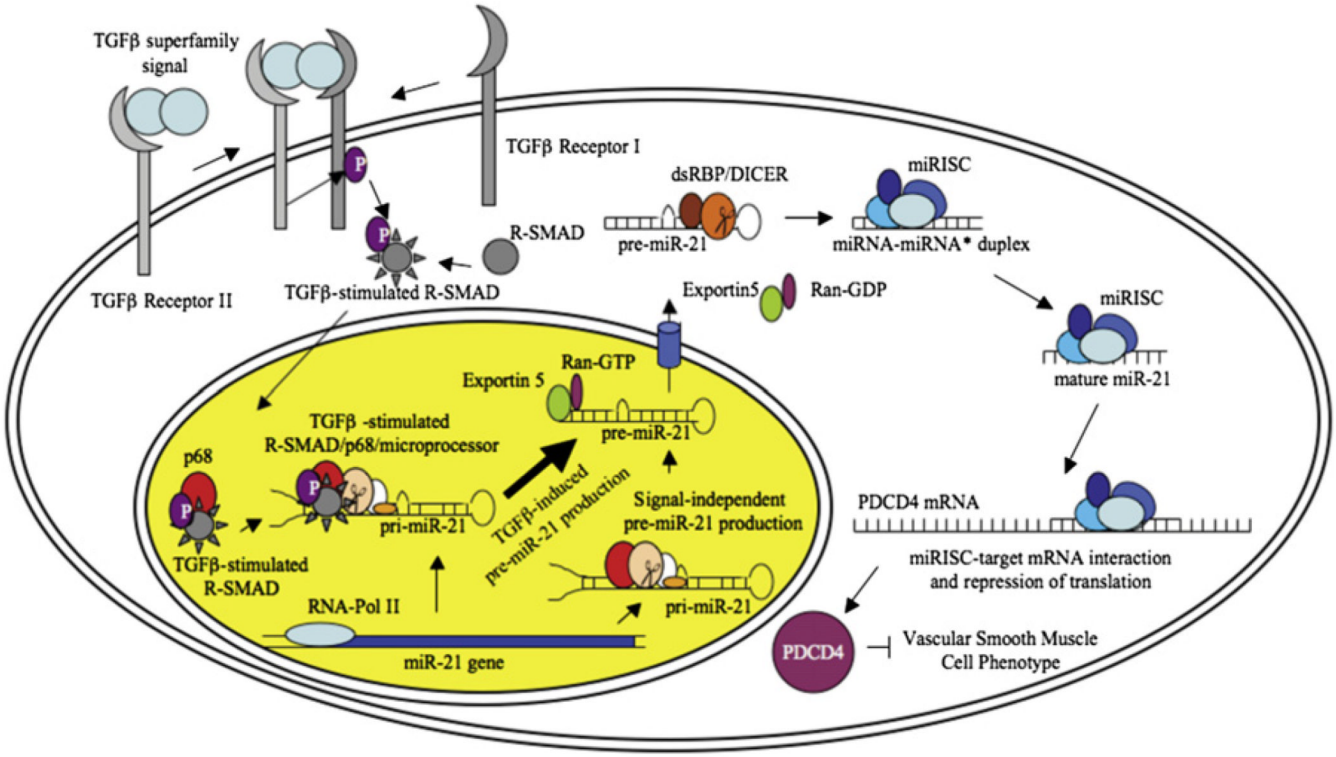

Figure 1. A Model for the Regulation of miRNA Maturation in Response to TGF-β Superfamily Signaling

BMP and TGF-β signaling stimulates the production of pre-miR-21, affecting downstream miR-21 targets such as PDCD4. Activated R-SMAD interaction with the pri-miRNA processing machinery is postulated to mediate posttranscriptional regulation of miRNAs. BMPs are proposed to affect the interaction between R-SMAD and pri-miRNAs primarily by controlling SMAD nuclear localization, as a nonphosphorylatable R-SMAD mutant retains the ability to interact with miR-21 in vitro.