Developmental Cell
Previews

Cell
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MicroRNAs in a Cardiac Loop: Progenitor or Myocyte?

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Like transcription factors, microRNAs are emerging as regulators of cell fate decisions. In this issue, Wang et al. (2010) identify a critical microRNA pathway under the control of Bmp signaling that promotes outflow tract myocardial differentiation from cardiac progenitors in vivo.

Because congenital heart defects involving outflow tract morphogenesis are among the most common of birth defects, development of the outflow tract has been intensively studied (Laugwitz et al., 2008). Outflow tract myocardium is derived from second heart field progenitors, which actively proliferate prior to their entry into the forming heart (Kelly and Evans, 2010; Evans et al., 2010). Differentiation of progenitors occurs as they enter the heart and is accompanied by downregulation of progenitor markers and a period of relative cell cycle quiescence.

Among the pathways that regulate this process, BMP signaling promotes differentiation (Figure 1; Evans et al., 2010). In a comprehensive set of experiments reported in this issue of *Developmental Cell*, Wang et al. (2010) have now discovered that differentiation and concomitant downregulation of progenitor factors Islet and Tbx1 downstream of BMP signaling is mediated by microRNAs (miRNAs). miRNA array analysis of mice with second heart field ablation of BMP2 and BMP4 demonstrated aberrant regulation of multiple miRNAs, including reduced expression of the miRNA 17-92 cluster. Although BMP signaling can regulate miRNAs at the processing level (Davis et al., 2008), Wang et al. demonstrate that regulation of miRNA 17-92 by BMP is likely to occur at a transcriptional level, by Smad binding to conserved elements within the miRNA 17-92 locus.

Previous loss-of-function studies for miRNA 17-92 demonstrated a critical role in heart development, with mutants exhibiting thinner ventricular myocardium and ventricular septal defects (Ventura et al., 2008). In this more recent study, Wang et al. show that, as for mice with second heart field ablation of BMP2/4, mice with loss of function of miRNA 17-92 also exhibit outflow tract defects, including reduced myocardial differentiation, aberrant upregulation of Islet1 and Tbx1, and double outlet right ventricle. Mice null for BMP4 in the second heart field (BMP4 CKO) and heterozygous for miRNA 17-92 exhibit more severe cardiac phenotypes than BMP4 CKO mutants, supporting a common genetic pathway during heart development. Islet1 and Tbx1 contain miRNA 17-92 seed sequences within their 3′ UTRs, and their cognate transcripts are up- or downregulated with miRNA 17-92 loss- or gain-of-function mutants, respectively. Thus, activation of miRNA 17-92 by BMP signaling results in decreased expression of Islet1 and Tbx1, presumably promoting differentiation by interfering with proliferative progenitor pathways.

In proliferating progenitors, Tbx1 negatively regulates BMP signaling by directly binding to Smad1, and overexpression of Tbx1 has been shown to interfere with myocardial differentiation (Fulcoli et al., 2009). Results of Wang et al. demonstrate that once the brake has been released from BMP signaling, BMP signaling in turn downregulates Tbx1 expression, promoting differentiation at the expense of progenitor status, revealing feedback loops between Tbx1 and BMP to maintain progenitor status or promote differentiation. Nkx2-5 is another transcriptional regulator that in the progenitor state negatively regulates BMP2. Importantly, BMP signaling is required for Nkx2-5 expression, demonstrating another feedback loop controlling progenitor expansion (Prall et al., 2007).

In keeping with the observation that ablation of BMP2/4 results in upregulation of Islet1, previous studies have shown that BMP signaling through ALK3 is required for Tbx20 expression in outflow tract myocardium, and Tbx20 is necessary to repress Islet1 expression (Evans et al., 2010). Mechanisms by which Tbx20 represses Islet1 are not known, and it will be important to examine whether miRNA 17-92 and/or other miRNAs are involved. Effects of Islet1 overexpression in second heart field are also not known, but it is interesting to note that overexpression of Islet1 inhibits differentiation of craniofacial muscle (Harel et al., 2009), whereas maintenance of Islet1 in cardiomyocytes in Nkx2-5 null mutants is compatible with differentiation (Prall et al., 2007).

Islet1 is downstream of β-catenin and upstream of smoothened signaling, both of which are required for proliferation of second heart field progenitors (Evans et al., 2010). Premature cardiomyocyte differentiation is observed when β-catenin or smoothened are ablated. In light of results by Wang et al., is it possible that miRNAs of the miRNA 17-92 cluster are upregulated in these situations, causing decreased Islet expression and promoting premature differentiation? Do signals that promote proliferation normally repress expression of miRNA 17-92? Although Wang et al. found that 1.7- to 2.0-fold overexpression of miRNA 17-92 in second heart field was insufficient to promote premature differentiation, perhaps higher levels of overexpression are required. Sustained signaling
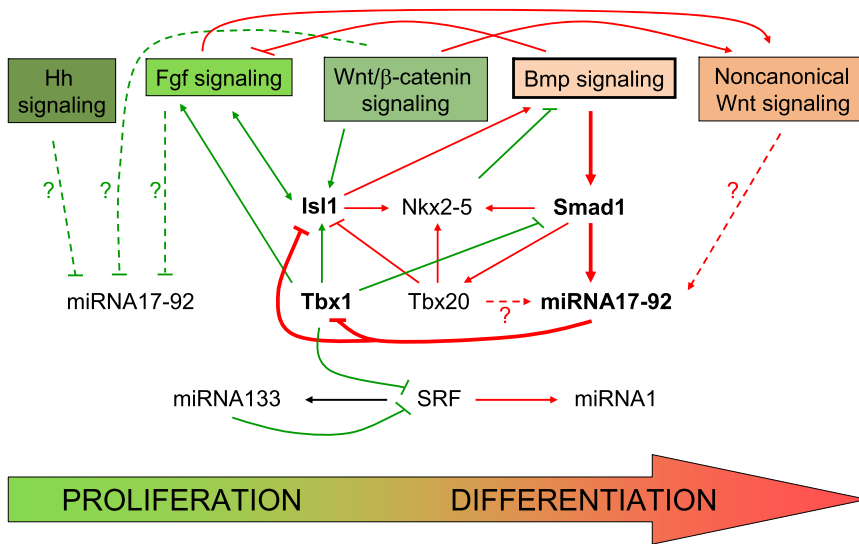
Figure 1. Major Intracellular Signaling Pathways and Regulatory Transcriptional Network during the Transition from Proliferative Second Heart Field Progenitor to Differentiated Outflow Tract Myocyte
Green and red lines indicate regulations promoting proliferation and differentiation, respectively. Thicker lines highlight the results from Wang et al. and dashed lines unknown regulations.

by β-catenin interferes with differentiation. What does expression of miRNA 17-92 look like in this situation?

If progenitor pathways function to put the brake on differentiation pathways, what is it that allows differentiation to progress, and what are additional potential roles of miRNAs in this process? Like BMP signaling, noncanonical Wnt signaling by Wnt11 promotes cardiomyocyte differentiation. Canonical β-catenin signaling, which is required for proliferation of second heart field progenitors, activates expression of Islet1, but also activates transcription of Wnt11 to initiate a differentiation pathway. In this way, while promoting proliferation of undifferentiated progenitors, the same pathway can also initiate the next phase of development, supporting differentiation. Timing and levels of expression are probably critical and different signaling thres-

holds may be required for an effect on proliferation or myocardial differentiation. Results of Wang et al. suggest that micro-RNAs may be instrumental in setting these thresholds. Are miRNAs regulated by BMP also modulated by Wnt11 to synergistically regulate differentiation? Does noncanonical Wnt signaling also downregulate Islet1 through miRNA 17-92?

The studies by Wang et al. have focused on differentiation of outflow tract myocardium, leaving open the question as to a broader role for miRNA 17-92 or related miRNAs in myocardial differentiation within other cardiac compartments. Mechanisms underlying thin walled ventricle and ventricular septal defects in miRNA 17-92 null mutants remain to be explored. Although miRNA 17-92 is the focus of the studies of Wang et al., other miRNAs were observed to be misregu-

lated in BMP mutants, both up- or down-regulated. Previous studies have demonstrated a role for miR-1 and miR-133 in cell cycle withdrawal and myocardial differentiation (Liu and Olson, 2010). The transcription factor SRF is required for differentiation, regulating expression of a number of myofibrillar genes, as well as a number of miRNAs, including miR-1/133. Future investigation into the role of these miRNAs, and others, in the complex regulation involved in cardiac progenitor expansion and differentiation, will be of great interest.

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