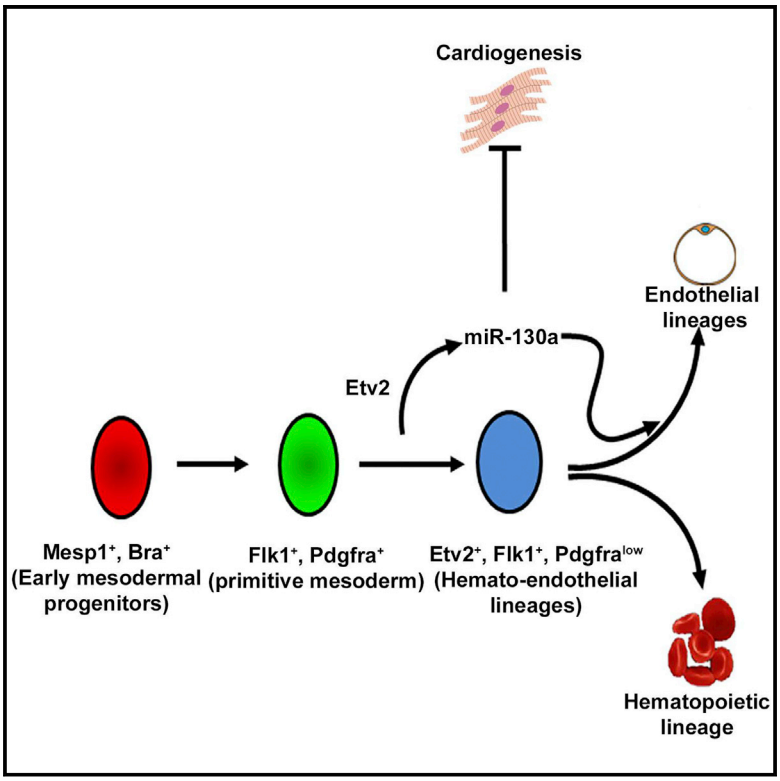
Report

Cell Reports

The *Etv2*-miR-130a Network Regulates Mesodermal Specification

Graphical Abstract



Highlights

- Etv2 transactivates miR-130a in the endothelial progenitors
- miR-130a specifically promotes the endothelial fate without affecting hematopoiesis
- miR-130a regulates Pdgfra expression

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In Brief

Endothelial and hematopoietic lineages emerge from common progenitors. Here, Singh et al. identify miR-130a as a direct target of Etv2 and demonstrate its role in segregating bipotent hemato-endothelial progenitors toward the endothelial lineage. Mechanistically, miR-130a directly suppresses *Pdgfra* expression and promotes the endothelial program by blocking Pdgfra signaling.

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The *Etv2*-miR-130a Network Regulates Mesodermal Specification

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SUMMARY

MicroRNAs (miRNAs) are known to regulate critical developmental stages during embryogenesis. Here, we defined an Etv2-miR-130a cascade that regulates mesodermal specification and determination. Ablation of *Dicer* in the *Etv2*-expressing precursors resulted in altered mesodermal lineages and embryonic lethality. We identified miR-130a as a direct target of Etv2 and demonstrated its role in the segregation of bipotent hemato-endothelial progenitors toward the endothelial lineage. Gain-of-function experiments demonstrated that miR-130a promoted the endothelial program at the expense of the cardiac program without impacting the hematopoietic lineages. In contrast, CRISPR/Cas9-mediated knockout of miR-130a demonstrated a reduction of the endothelial program without affecting hematopoiesis. Mechanistically, miR-130a directly suppressed *Pdgfra* expression and promoted the endothelial program by blocking Pdgfra signaling. Inhibition or activation of Pdgfra signaling phenocopied the miR-130a overexpression and knockout phenotypes, respectively. In summary, we report the function of a miRNA that specifically promotes the divergence of the hemato-endothelial progenitor to the endothelial lineage.

INTRODUCTION

During embryogenesis, mesodermal progenitors give rise to multiple lineages, including hemato-endothelial and cardiac lineages. For example, Flk1^{-}/Pdgfra^{+} (paraxial mesoderm) and Flk1^{+}/Pdgfra^{-} (lateral plate mesoderm) cells arise from the Flk1^{+}/Pdgfra^{+} unpatterned mesoderm (Kataoka et al., 2011; Sakurai et al., 2006). These lineages respond to distinct transcriptional networks and signaling cues. Precise control of the specification of these lineages is necessary for proper development and embryogenesis. The transcriptional regulators and signaling pathways that govern these mesodermal progenitors are incompletely defined (Kattman et al., 2006; Loebel et al., 2003).

Studies have established a hierarchy of transcriptional regulators including Mesp1, Vegf/Flk1, and Etv2 as modulators of hemato-endothelial development (Shalaby et al., 1995; Saga et al., 1999; Ferdous et al., 2009). Ablation of Etv2 results in embryonic lethality by embryonic day 9.5 (E9.5) with complete absence of the hemato-endothelial lineages (Ferdous et al., 2009; Koyano-Nakagawa et al., 2012). Etv2 serves as a key regulator of hemato-endothelial lineages through its interaction with multiple factors including *Tie2, Lmo2,* Gata2, and FoxC2 (De Val et al., 2008; Rasmussen et al., 2011; Koyano-Nakagawa et al., 2012; Shi et al., 2014). While the transcriptional hierarchy in hemato-endothelial development has been well described, the role of miRNAs in these progenitors are unknown.

MicroRNAs (miRNAs) govern the molecular switch by suppressing gene expression, thereby modulating and fine-tuning cell fate decisions (Ivey and Srivastava, 2010). Although global deletion as well as hypomorphic mutants of *Dicer* (an miRNA-processing enzyme) results in embryonic lethality (Bernstein et al., 2003; Yang et al., 2005), it is unclear whether Dicer and miRNAs play any role in the hemato-endothelial segregation and vascular develoment.

In the present study, we deciphered the requirement of miRNA biogenesis in early mesodermal precursors. We discovered an Etv2-miR-130a-Pdgfra network that directs the hemato-endothelial progenitors toward the endothelial fate without affecting the hematopoietic lineage. These findings define a factor that directs endothelial development without affecting the hematopoietic lineage.

RESULTS

Etv2-Cre-Mediated *Dicer* Deletion Results in Altered Mesodermal Lineages and Embryonic Lethality

Analysis of mesodermal transcripts during embryonic stem cell/embryoid body (ESC/EB) differentiation indicated that *Mesp1* was transiently but robustly expressed at day 3 of differentiation with subsequent expression of both *Flk1* and *Etv2* at d4, marking the appearance of the mesodermal lineages (Figure S1A). To evaluate the functional role of Dicer (miRNA dependent and independent) in the mesodermal progenitors, we conditionally deleted floxed-*Dicer* using Cre recombinase under the control of *Mesp1*, *Flk1*, or *Etv2* promoter elements. qPCR analysis revealed efficient deletion of the *Dicer* allele from sorted cells using fluorescence-activated cell sorting (FACS)