Pioneer transcription factor two-pronged way to promote endothelial cell differentiation

Single-cell analyses defined the mechanisms by which the pioneer transcription factor ETV2 drives endothelial cell specification from mesodermal progenitors in vitro. ETV2 interaction with a transcription factor (GABPA) promoted endothelial cells differentiation, and ETV2 interaction with a repressor (REST) inhibited differentiation to alternative cell types.

This is a summary of:
Chen, D. et al. Pioneer factor ETV2 safeguards endothelial cell specification by recruiting the repressor REST to restrict alternative lineage commitment. *Nat. Cardiovasc. Res.* https://doi.org/10.1038/s44161-025-00660-y (2025).

Published online: 16 June 2025

The question

Pioneer transcription factors are a type of transcription factor that can bind DNA on nucleosomes (bead-like structures of densely packed DNA and proteins) and create accessible DNA-binding sites for other transcription factors. Understanding the mechanisms by which pioneer transcription factors drive the specification of specific cell types is a major question in developmental and regenerative biology[1]. ETS translocation variant 2 (ETV2), a pioneer transcription factor essential for vascular development[2,3], efficiently and rapidly drives endothelial cell (EC) specification from progenitor cells in the mesoderm (an embryonic tissue layer)[4]. We used this model system to dissect the mechanisms by which ETV2 directs mesodermal cell differentiation towards ECs. Insights from these studies might enhance efforts to repair or regenerate the vasculature, and more broadly inform our understanding of lineage specification.

The discovery

We optimized a system in which doxycycline (Dox)-induced overexpression of *ETV2* in human mesodermal progenitors derived from inducible pluripotent stem cells (iPSCs) rapidly and efficiently generated ECs. We studied this model system using single-cell RNA sequencing to analyze transcriptomes and single-cell assay for transposase-accessible chromatin using sequencing to determine the DNA regions available for transcription factors binding. Analyses of these data nominated additional transcriptional regulators of EC specification, which we prioritized through a focused CRISPR loss-of-function screen, and we performed detailed analyses of the top two candidates.

Transcriptomic experiments demonstrated that normally only a subset of mesodermal progenitors express ETV2, and these cells differentiate to ECs with venous features. Most mesodermal progenitors differentiate along alternative trajectories to form other cell types, including cells in the cardiac lineage. ETV2 overexpression in mesodermal progenitors drove nearly all mesodermal progenitors to differentiate along a separate trajectory, ultimately yielding ECs with arterial features (Fig. 1a). Analysis of ETV2 binding and changes in accessible chromatin identified candidate transcription factors that cooperate with ETV2 to promote EC differentiation. A CRISPR screen of these candidates led us to focus on two factors, GAPBA and REST, that had not been previously implicated in EC specification. GABPA, a transcription factor, interacted with ETV2 and co-occupied genomic regions to promote EC differentiation. REST, a repressive transcription factor, interacted with ETV2 to co-occupy different genomic regions and repress cardiac and other alternative fates. Indeed, EC specification had reduced efficiency in REST-knockout cells, with increased activation of cardiac lineage genes (Fig. 1b).

The implications

Our study highlights the activating and repressive mechanisms by which the pioneer transcription factor ETV2 promotes lineage specification. In addition to the well-established roles of pioneer factors in activating expression of genes that promote lineage specification, our study indicates that they might have an important role to repress alternative lineage genes[5]. In keeping with previous studies showing that ETV2 inactivation in mouse embryos resulted in loss of ECs and expansion of cardiac and other mesodermal lineages[3], our study demonstrates how ETV2 balances mesodermal commitment by recruiting REST to suppress non-EC fates.

An important limitation of this study is that it was performed in vitro in iPSC-derived mesodermal progenitors. It will be important to evaluate whether the mechanisms identified in this study are relevant for vascular development in vivo.

In future studies, we will investigate the participation of GABPA and REST in vascular development in vivo. ETV2 overexpression in more translationally relevant cell types, such as fibroblasts, promotes EC transdifferentiation, albeit with scarce efficiency. By extending our approach to these other contests, we will endeavor to identify and overcome bottlenecks in ETV2-mediated EC reprogramming.

Danyang Chen & William T. Pu
Boston Children's Hospital, Boston, MA, USA.

EXPERT OPINION

"This report contains extensive carefully conducted genome-wide analyses of the consequences of overexpression of the master regulator *ETV2*, which are then followed up by a series of elegant functional studies. In particular, the observation that increased ETV2 levels result in arterial endothelial cells rather than venous endothelial cells is very interesting."
An anonymous reviewer.

FIGURE

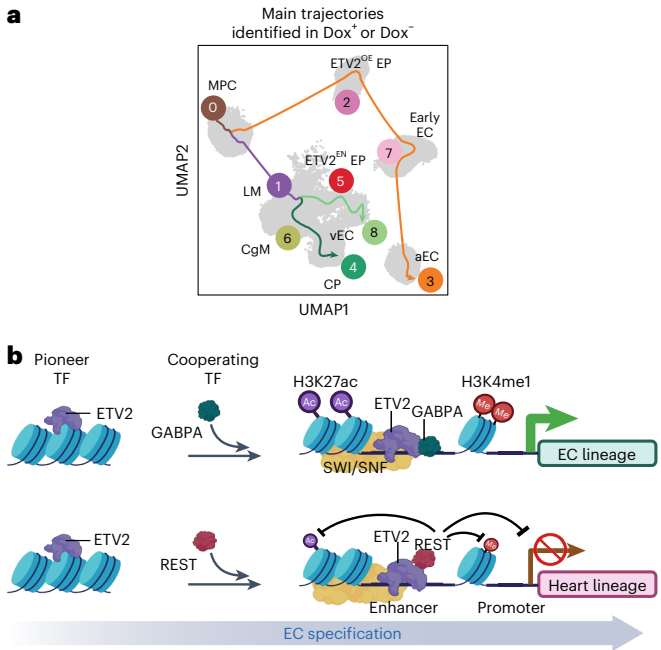

Fig. 1 | ETV2 drives EC specification by promoting the EC lineage and repressing alternative lineages. **a**, Uniform manifold approximation and projection (UMAP) of single-cell accessible chromatin during EC differentiation from mesodermal progenitor cells (MPCs; 0). Orange trajectory indicates ETV2 overexpression ($ETV2^{OE}$), which leads to $ETV2^{OE}$ endothelial progenitor (EP) cells (2), then early ECs (7) and eventually arterial ECs (aECs; 3). Purple and green indicate trajectories taken during differentiation with endogenous ETV2 expression ($ETV2^{EN}$), which result in $ETV2^{EN}$ EPs (5), lateral mesoderm (LM; 1), cardiogenic mesoderm (CgM; 6), cardiac progenitors (CPs; 4) and venous ECs (vECs; 8). **b**, Model of pioneer transcription factor (TF) ETV2-driven EC specification. ETV2 recruits GABPA to promote EC lineage commitment, and the repressor REST to restrict alternative lineage commitment. H3K4me1, mono-methylation of Lys4 on histone 3; H3K27ac, acetylation of Lys27 on histone 3. Panel **b** created with BioRender.com. © 2025, Chen, D. et al.

BEHIND THE PAPER

This paper was built on foundational studies on ETV2 and EC specification by numerous investigators, and particularly the work of our colleagues Kai Wang and Juan Melero-Martin[4]. By combining our expertise in single-cell techniques and gene regulation with the inducible ETV2 system developed by Wang, Melero-Martin and their colleagues, we were able to determine the mechanisms by which this pioneer transcription factor drives EC specification. Following a similar strategy to study ETV2 overexpression in translationally relevant cell types might lead to enhanced approaches for vascular repair and regeneration. **D.C. & W.T.P.**

REFERENCES

1. Balsalobre, A. & Drouin, J. Pioneer factors as master regulators of the epigenome and cell fate. *Nat. Rev. Mol. Cell Biol.* **23**, 449–464 (2022).
   A review article on pioneer transcription factors and their role in cell fate specification.
2. Lee, D. et al. ER71 acts downstream of BMP, Notch, and Wnt signaling in blood and vessel progenitor specification. *Cell Stem Cell* **2**, 497–507 (2008).
   This paper describes that ETV2 (also known as ER71) is required for EC specification in vivo.
3. Rasmussen, T. L. et al. ER71 directs mesodermal fate decisions during embryogenesis. *Development* **138**, 4801–4812 (2011).
   This paper describes the role of ETV2 in balancing the fates of mesodermal cells, with expansion of the cardiac lineage in its absence.
4. Wang, K. et al. Robust differentiation of human pluripotent stem cells into endothelial cells via temporal modulation of ETV2 with modified mRNA. *Sci. Adv.* **6**, eaba7606 (2020). **This paper established the efficient specification of ECs by doxycycline-induced overexpression of *ETV2* in mesodermal progenitors derived from iPSCs.**
5. Matsui, S. et al. Pioneer and PRDM transcription factors coordinate bivalent epigenetic states to safeguard cell fate. *Mol. Cell* **84**, 476–489.e10 (2024).
   This paper describes how pioneer transcription factors recruit repressors of alternative fates in endoderm differentiation.

FROM THE EDITOR

"This work stood out because it uncovers the mechanism by which ETV2 directs human iPSC-derived progenitors into endothelial cells, which might affect cellular reprogramming and therapeutic vascular regeneration in the future."
Gerburg Schwaerzer, Associate Editor, *Nature Cardiovascular Research*.