Endothelin signalling drives dermal sheath smooth muscle contraction for hair follicle regression

Contraction of the hair follicle-lining dermal sheath smooth muscle generates the forces necessary for the tissue remodelling that takes place during the regression phase of the hair growth cycle. This study reveals that endothelin signalling — from epithelial progenitors at the follicle bottleneck region to its neighbouring dermal sheath — is the main contraction-activating pathway.

This is a summary of:
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The question

In the natural hair growth cycle, follicles — after active hair shaft production — enter a regression phase marked by programmed cell death, tissue shrinkage and the relocation of the dermal papilla niche to hair follicle stem cells, all of which sets the stage for follicle regeneration of the next hair growth cycle[1]. Understanding the cellular and molecular mechanisms that regulate hair follicle regression not only advances basic science but might also help in developing hair loss therapies. Recently, the dermal sheath that lines hair follicles has been identified as a smooth muscle that contracts during hair cycle regression to push the hair shaft upwards and move the dermal papilla to the stem-cell reservoir of the resting-phase follicle[2,3]. However, the contraction-activating mechanism has remained unexplored. We set out to discover which contraction-regulating pathway controls sheath contraction, as well as probe its spatiotemporal and molecular signalling mechanisms.

The discovery

Hypothesizing that any of the major signalling pathways that regulate smooth muscle contraction might regulate sheath contraction, we first performed transcriptome-wide gene expression analysis of freshly isolated dermal sheath cells, enriched by cell sorting. This approach allowed us to systematically assess which of the many contraction-pathway receptors is produced by the dermal sheath smooth muscle. Subsequently, we tested the identified pathway with functional assays, including cell contraction and pharmacological signalling inhibition experiments, as well as targeted ablation of receptor genes in the dermal sheath and a ligand gene in follicle progenitors. Additionally, we complemented our genetic and biochemical assays with spatiotemporal ligand–receptor imaging and downstream calcium signalling experiments.

With our approach we discovered that endothelin signalling — one of the strongest smooth muscle contraction-activating pathways[4] — regulates dermal sheath contraction to propel follicle reorganization during the regression phase of the hair growth cycle (Fig. 1a). Of the 20-plus contraction-related receptors surveyed in the dermal sheath, we identified strikingly high expression of only the two endothelin receptor genes. Exposure to the endothelin ligand ET-1 functionally contracted cultured dermal sheath cells in a dose-dependent manner down to physiological ligand levels. Pharmacological blocking of endothelin signalling in skin with small-molecule antagonists of endothelin receptors impaired follicle regression. Simultaneous knockout of both endothelin receptor genes (ET_A and ET_B), specifically in the dermal sheath, also led to stalled follicle regression. To find the source of the ligand, we used transcriptome and spatiotemporal imaging analysis and identified high ET-1 expression by follicle progenitors at the bottleneck region (Fig. 1a), the site of maximal sheath contraction. Ablation of the gene encoding ET-1 (*Edn1*) in the follicle epithelium confirmed that ET-1 is required for activating dermal sheath contraction and regression. Finally, calcium imaging and influx-manipulation experiments established that the molecular mechanism of ET-1 signalling is via the calcium–calmodulin–myosin light-chain kinase pathway (Fig. 1b).

Future directions

Our study highlights that progenitors orchestrate the remodelling of their microenvironment during tissue regression, in addition to their well-known role as the main architects of the stem-cell niche during homeostasis and regeneration[5]. Whether endothelin-induced contraction is involved in regulating other hair cycle phases will be interesting to determine. From a translational point of view, contraction-blocking strategies might be an interesting avenue to explore to ameliorate hair loss conditions.

The pharmacological blocking and genetic ablation experiments indicate that endothelin signalling is the major pathway controlling dermal sheath contraction; however, we cannot rule out the possible contribution of other unexplored contraction-regulating mechanisms. Although our study clearly established epithelial progenitors as the major source of the ET-1 ligand required for dermal sheath contraction, we cannot exclude the possibility that other sources, such as endothelial cells of the surrounding vasculature, contribute to ET-1 signals.

Further studies are needed to determine the upstream transcriptional regulation of ET-1 expression in epithelial progenitors located in the bottleneck region of the follicle, which drives the production of ET-1 in a seemingly precise spatiotemporal fashion. Finally, given that in the absence of sheath contraction the stalled follicles lacked any signs of cell death, it will be interesting to dissect potential molecular links between contractile forces and apoptosis regulation.

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EXPERT OPINION

"This study investigates the regulation of dermal sheath smooth muscle contraction during hair follicle regression, which has not previously been determined. The results are novel and will ignite a new area for research in endothelin signalling and a wider understanding of epithelial–mesenchymal interactions. The research is intriguing, providing, for example, a mechanistic explanation for missense variants in the gene encoding the ETA receptor affecting hair growth." **Antony P. Davenport, University of Cambridge, Cambridge, UK.**

FIGURE

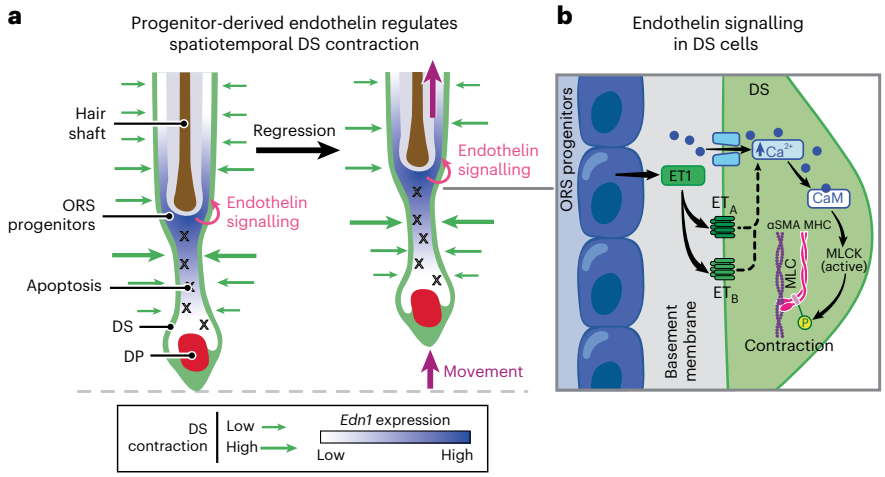

Fig. 1 | Endothelin signalling controls dermal sheath contraction and follicle regression. a,**b**, Schematic of spatiotemporal contraction regulation and molecular mechanism of endothelin signalling. **a**, High production of the endothelin ligand ET-1 by progenitors at the follicle bottleneck (dark blue) between the epithelial strand undergoing apoptosis and the club hair shaft activates endothelin signalling and contraction in the neighbouring dermal sheath (green). **b**, ET-1 binding to the endothelin receptors ET_A and ET_B activates Ca^{2+} influx and Ca^{2+}–calmodulin–MLCK signalling, resulting in dermal sheath cell contraction. DP, dermal papilla; DS, dermal sheath; MLCK, myosin light-chain kinase; ORS, outer root sheath. © 2023, Martino, P. et al.

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BEHIND THE PAPER

After the recent discovery that the follicle-lining dermal sheath is a smooth muscle that is essential for relocating the inductive dermal papilla to the stem-cell reservoir during hair regression, the next logical step for us was to dissect how the critical contraction is spatiotemporally activated. A fortunate early 'eureka' moment came from analysing our transcriptome data, which revealed the expression of only a single contraction-regulating pathway, endothelin signalling, with a rarely found clear-cut ligand and receptor distribution. Having seemingly struck gold, we were lucky to team up with endothelin experts Donald Kohan and Masashi Yanagisawa for functional mouse genetic experiments. After several swift and informative contraction induction and chemical blocking experiments, the (sometimes unbearable) year-long wait for the multiple crosses to generate ET_A–ET_B double-knockout mice was a nail-biter before we succeeded in proving the functional requirement of this pathway. **M.R.**

FROM THE EDITOR

"The work by Martino, Sunkara et al. beautifully combines transcriptomics, genetic and functional studies to reveal a major mechanism through which the hair follicle stem-cell niche coordinates sheath contraction and regression. The study stood out to us because, by dissecting the regulation of dermal sheath smooth muscle contraction during regression, it answers a long-standing question in the field." **Editorial Team, *Nature Cell Biology.***