Developmental biology

A 4D road map for the formation of hair follicles

Nivedita Saxena & Michael Rendl

Combined imaging and gene-expression analyses reveal that the arrangement of cells in concentric rings in the disc-like structures that give rise to hair follicles predetermines their eventual fate and location in mature follicles. **See p.547**

As a child, you might dream one day of becoming an astronaut and, the next day, of becoming a ballet dancer – the possibilities are endless. Eventually, this wealth of choice is whittled down by external circumstances and internal interests. Similarly, precursor cells in early embryos make a series of stepwise 'decisions' governed by external forces and internal factors to generate the diverse array of cell types present in adult organisms[1]. On page 547, Morita *et al.*[2] show that the fate of cells that eventually make up mature hair follicles is determined by their positioning in concentric rings of nascent follicle structures during embryonic skin development. In this case, how and where cells begin their developmental journey ultimately decides their destination.

Hair-follicle development begins with a clean slate. The skin comprises two major components: the epidermis, a layer of epithelial cells that form a protective barrier against external insults; and the dermis, which contains cells called fibroblasts that support the skin[3]. Epithelial cells and fibroblasts seem to be equally able to contribute to and regulate the formation of hair follicles. Dermal fibroblasts induce the formation of placodes – cellular thickenings in the epithelial layer that eventually give rise to hair follicles[4,5]. Placodes develop as flat circles on the surface of the skin. How these flat disc structures eventually transform into a long, cylindrical 3D shape is a question of great interest not only to skin and hair researchers, but also to scientists investigating potential hair-replacement therapies that work through developmental mechanisms[6,7].

This is where Morita and colleagues step in. The authors optimized a technique called live imaging to record the movement of cells in cultured skin tissue taken from mouse embryos, beginning at 11.5 days after conception. They imaged the skin samples for three to five days, in three dimensions and at a microscopic level, to produce time-lapse videos of hair-follicle development[8]. By playing these videos in reverse, the authors traced cells in fully grown hair follicles back to their embryonic placodal origins. They discovered that cells in early placode structures are organized into concentric zones, like the bullseye and surrounding rings of a target, such that the centre-most cells give rise to the bottom of the hair follicle, and cells at the outer edge of the placode remain at the surface of the skin, between follicles (Fig. 1). The downward growth from placode to fully developed hair follicle, which is enabled by the proliferation and movement of these cells over time, resembles the elongation of an extendable telescope; thus, the authors propose referring to this type of development as the "telescope model".

Although this model is not familiar to hair biologists, the formation of cylindrical or other 3D-shaped appendages, such as legs and antennae, from flat surfaces was first described in the fruit fly *Drosophila melanogaster*[9]. Studies in flies have long informed biological principles in more-complex mammalian species; thus, although perhaps unsurprising, it is exciting that this telescope mechanism is evolutionarily conserved across species.

Adding further complexity to this system is the appearance of stem cells in the hair follicle as it develops. In adult animals, a reservoir of such cells resides in a part of the upper hair follicle called the bulge[10,11] and is crucial for enabling the follicle to expand during the growth phase of the hair cycle, as part of normal tissue turnover throughout an animal's life[12]. These stem cells can even repair injury to the rest of the epidermis[12]. The hair-follicle placode harbours predecessors to bulge stem cells, and the stem cells' origin in the early placode was previously demonstrated by using protein markers expressed by the cells, such as the transcription factor SOX9, to track them. This approach suggested that, as the placode begins to extend downwards, bulge stem-cell precursors are born and remain superficial to the placode, before ultimately colonizing the bulge region[13].

Morita and colleagues selectively labelled and isolated cells from early mouse hair follicles at the same time points as those

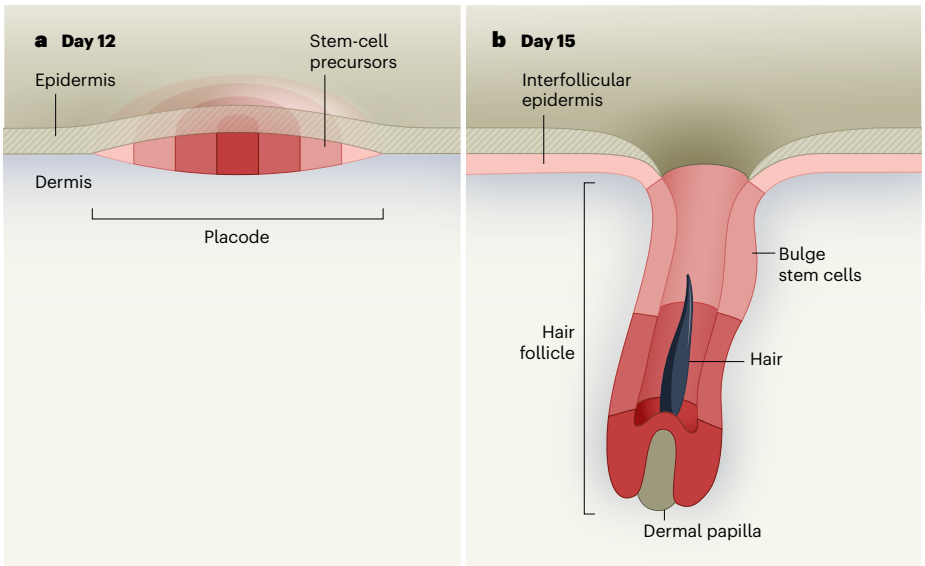

Figure 1 | Early placode arrangement determines final cell position in the hair follicle. Hair follicles develop from flat, disc-like structures called placodes in the epidermis – the surface layer of skin cells above the dermis. Morita *et al.*[2] used time-lapse live imaging and profiled the gene expression of cells in developing hair follicles from mouse embryos during days 12–15 after conception. Cells in the placodes were found to be arranged in concentric rings that define the final cell position in fully grown hair follicles. The innermost circle gives rise to the lowest portion of the hair follicle (adjacent to the dermal papilla, a cluster of specialized dermal cells), and the next ring out contributes to the next portion. Hair-follicle stem cells born in the peripheral ring of the placode colonize the future bulge region of mature hair follicles, and the outermost area gives rise to interfollicular epidermal cells, which are not part of the hair follicle but form part of the epidermis between follicles.

analysed in their live-imaging experiments, to perform single-cell transcriptomics — gene-expression profiling of individual cells. Because single-cell transcriptomics allows a broad and unbiased survey of the genes and proteins expressed by each cell, it can be highly informative for ascribing cellular identity and for characterizing cellular dynamics during development[2,8]. Using this approach, Morita and colleagues identified a population of cells expressing bulge stem-cell markers at the earliest stages of placode development. Through mathematical modelling of cells at all assessed time points, the authors determined that these marker-expressing cells differentiate into bulge stem cells in the early placode[6], long before the time point at which such differentiation was previously reported to occur[12].

These stem-cell precursors expressed the gene that encodes SOX9, consistent with previous reports[13]. However, through further cell-lineage tracing and live imaging, these precursors were unequivocally demonstrated to originate from the previously unidentified peripheral ring zone of the early placode. This newly posited spatial localization will probably require a re-examination of previous findings related to bulge stem-cell precursors and reopen questions of the regulatory conditions necessary for the induction of this cell population.

Although the authors' study makes huge strides in dissecting how different cell populations contribute to the mature hair follicle, it also invites many questions, the most compelling of which is how the concentric zones in the placode are established. It is likely that various elements contribute to the formation of these zones, such as diffusible factors secreted by other epidermal and dermal cells (including specialized dermal cells clustered directly below where placodes form[14]). By improving our understanding of hair-follicle development, future studies might unveil ways of generating hair follicles *de novo* that could eventually be used in hair-replacement therapies. However, unlike the routes taken by cells on their developmental journeys to their final destinations in the mature hair follicle, the pathway to such applications is still unclear.

Nivedita Saxena and **Michael Rendl** are at the Black Family Stem Cell Institute and in the Department of Cell, Developmental and Regenerative Biology, Department of Dermatology and the Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA.
e-mail: michael.rendl@mssm.edu

1. Hadjantonakis, K. & Solnica-Krezel, L. *Dev. Biol.* **341,** 2–4 (2010).
2. Morita, R. *et al. Nature* **594**, 547–552 (2021).
3. Saxena, N., Mok, K.-W. & Rendl, M. *Exp. Dermatol.* **28**, 332–344 (2019).
4. Kollar, E. J. *J. Invest. Dermatol.* **55**, 374–378 (1970).
5. Millar, S. E. *J. Invest. Dermatol.* **118**, 216–225 (2002).
6. Ji, S., Zhu, Z., Sun, X. & Fu, X. *Signal Transduct. Target. Ther.* **6**, 66 (2021).
7. Lee, J. *et al. Nature* **582**, 399–404 (2020).
8. Heitman, N., Saxena, N. & Rendl, M. *Curr. Opin. Cell Biol.* **55**, 87–95 (2018).
9. Ruiz-Losada, M., Blom-Dahl, D., Córdoba, S. & Estella, C. *J. Dev. Biol.* **6**, 17 (2018).
10. Tumbar, T. *et al. Science* **303**, 359–363 (2004).
11. Morris, R. J. *et al. Nature Biotechnol.* **22**, 411–417 (2004).
12. Fuchs, E. & Blau, H. M. *Cell Stem Cell* **27**, 532–556 (2020).
13. Ouspenskaia, T., Matos, I., Mertz, A. F., Fiore, V. F. & Fuchs, E. *Cell* **164**, 156–169 (2016).
14. Mok, K.-W. *et al. Dev. Cell* **48**, 32–48 (2019).

The authors declare no competing interests.
This article was published online on 9 June 2021.

Cancer

Natural killer cells lull tumours into dormancy

Noella Lopes & Eric Vivier

Natural killer cells can drive spreading cancer cells to enter a state of dormancy. That finding, together with the discovery of a pathway that hinders this antitumour function, could spur the development of new treatments. **See p.566**

Efforts to treat tumours that have spread from their initial site in the body to grow elsewhere are often unsuccessful. Such tumours, called metastases, are the main cause of cancer-related deaths, so finding a way to control them is crucial to meeting this medical need. Before metastases begin to grow, cancer cells might have already migrated from the primary tumour to seed various other sites (a process called metastasis), where they can remain dormant for long periods of time. Surveillance by immune cells is known to help to maintain this dormancy[1], but the mechanisms involved in the switch from dormancy to the growth of metastases have been unclear — until now. On page 566, Correia *et al.*[2] report the pivotal role of natural killer (NK) cells in controlling the development of liver metastases arising from breast cancer.

NK cells are part of the innate branch of the immune system. They can kill other cells and produce soluble messenger molecules, called cytokines and chemokines, that regulate immune responses[3]. The ability of NK cells to detect and eliminate a wide array of tumour cells directly, and their capacity to shape antitumour immune responses by making cytokines or chemokines, have led to the development of clinical strategies that harness their anticancer functions[3–5].

Several studies have suggested that NK cells specialize in eliminating metastases rather than targeting tumour cells at their primary site of growth[6]. For some cancers, people who have more tumour-infiltrating NK cells seem to have fewer metastases, as seen in those with cancers such as gastrointestinal sarcoma, and gastric, colorectal, renal or prostate carcinoma[3,6]. The depletion or dysfunction of NK cells in mice also results in an increase in metastases[3]. By contrast, when their normal regulation is removed, NK cells protect against the spread of tumours to the liver and lungs[7]. Tumour cells entering dormancy downregulate their expression of ligand molecules that can activate NK cell receptors, and become resistant to killing mediated by NK cells[8].

Correia and colleagues decided to further investigate the composition and dynamics of tumour cells in dormancy. One approach they took was to study the gene-expression profile of human and mouse breast cancer cells transplanted into mice. These cells underwent metastasis to reach sites such as the liver, where they became dormant tumour cells. The authors assessed genes expressed by cells in the vicinity of the dormant tumour cells in the surrounding stromal tissue. These data revealed a gene signature associated with responses mediated by NK cells. Furthermore, Correia *et al.* compared the areas around dormant tumour cells with those in tumour-free livers, and found that NK cells were the only type of immune cell to increase in number during dormancy. This suggests that NK cells have a crucial role in events that block the reawakening of dormant tumour cells (Fig. 1).

Consistent with this hypothesis, the authors report that depleting NK cells in a mouse tumour model then led to higher levels of metastases in the liver. However, if NK cells were boosted using the cytokine IL-15, this prevented the formation of liver metastases and tumour cells remained dormant. The authors' results demonstrate that the size of the pool of NK cells in the liver environment determines whether dormancy occurs or metastases form.

The liver environment associated with