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Letter to the Editor

Human and mouse hair follicles contain both multipotent and monopotent stem cells

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Our previous studies have demonstrated pluripotent nestin-positive, keratin (K15)-negative stem cells in the mouse hair follicle which can differentiate into neurons and many other cell types. In the present study, we have now observed that the intact anagen hair follicle of the mouse and the human scalp contain both nestin-positive, K15-negative stem cells as well as nestin-negative, K15-positive stem cells.

The hair follicle undergoes repeated cycles of periods of growth (anagen), regression (catagen), and rest (telogen) throughout the life of mammals.[1] The follicle bulge region was shown to contain progenitor (stem) cells that could give rise to the hair follicle. During the anagen phase of the hair growth cycle, the bulge stem cells periodically differentiate into all of the follicle cell types including the outer-root sheath, hair matrix cells and inner-root sheath as well as sebaceous-gland basal cells, and epidermis.[2,3] In response to wounding, some cells exit the follicle, migrate and proliferate to repopulate the infundibulum and epidermis.[4] Morris et al.,[5] used a keratinocyte promoter to drive green fluorescent protein (GFP) expression in the hair-follicle bulge cells. They showed that bulge cells in adult mice generate all epithelial cell types within the intact follicle and hair during normal hair-follicle cycling.

Toma et al.[6] reported that multipotent adult stem cells isolated from mammalian dermis, termed skin-derived precursors, can proliferate and differentiate in culture to produce neurons, glia, smooth muscle cells and adipocytes. However, the exact location of the skin-derived precursors was not identified, since they were obtained from enzymatic digestion of the skin.

We have previously reported that nestin, a marker for neural progenitor cells, is also expressed in stem cells of the hair follicle using nestin-driven green fluorescent protein (ND-GFP) transgenic nude mice.

We isolated and characterized hair follicle stem cells expressing the ND-GFP marker.[7] These cells appear primitive in that they express the stem cell marker CD34 as well as nestin, but do not express the keratinocyte marker keratin-15. We showed that these ND-GFP stem cells can differentiate into neurons, glia, keratinocytes, smooth muscle cells and melanocytes in vitro.[7] These results were confirmed by Mignone et al.[9]

In subsequent studies, we showed that transplanted hair follicle stem cells can enhance the regrowth and functional rejoining of severed sciatic and tibial nerves,[8] as well as the severed spinal cord,[10] in immunocompetent mice.

Sieber-Blum et al.,[11] showed that neural crest cells grew out when the hair follicle was explanted, resulting in differentiation to a variety of cell types including neurons, smooth muscle cells, rare Schwann cells and melanocytes. The original location of these cells in the follicle was not demonstrated. Yu et al.,[12] isolated a population of stem cells from human hair follicles that express nestin, proliferate as spheres and can differentiate into multiple lineages.

In the mouse, ND-GFP expressing stem cells are located in the upper hair follicle immediately below the sebaceous glands just above the hair follicle bulge area (Fig. 1A). The ND-GFP stem cells are K15-negative (Fig. 1). The nestin-positive, K15-negative stem cells in the mouse hair follicle are pluripotent and can differentiate to neurons, glial cells, keratinocytes and other cell types.[8] Nestin-negative, K15-positive cells, on the other hand, are located in the bulge area of the mouse hair follicle and can differentiate only to keratinocytes (Fig. 1A).

In the intact human hair follicle dissected from the scalp, the cells immediately below the sebaceous glands just above the bulge area were observed to be nestin-positive, K15-negative. In contrast, the hair follicle bulge area contained nestin-negative, K15-positive cells (Fig. 1A).

The intact human scalp hair follicle was divided in three parts (upper, middle and lower). Hair follicle cells that were located immediately below the sebaceous glands just above the bulge area in the upper section of the intact scalp hair follicle were isolated. The isolated stem cells were suspended in DMEM-F12 containing B-27 supplemented with bFGF every two days. These nestin-positive keratin-negative cells formed spherical colonies in this medium termed hair spheres by Yu et al.[12] Ten days after switching the medium to RPMI 1640 containing 10% FBS, differentiating cells were observed migrating away from the colony. These differentiated cells included β3-tubulin-positive neurons, S-100-positive and GFAP-positive glial cells, K15-positive keratinocytes, and SMA-positive smooth muscle cells (Fig. 1B).

The plucked scalp hair follicle, which did not contain sebaceous glands or nestin-positive, K15-negative cells above the bulge, was divided to three parts (upper, middle and lower). The upper part of the plucked scalp hair follicle, which contained the bulge area and nestin-negative, keratin-positive cells was suspended in DMEM-F12 containing B-27 supplemented with bFGF every two days. Ten days later, the upper part of the plucked hair formed hair spheres. The proliferating cells were identified as keratin 15-positive keratinocytes. After switching the medium to RPMI 1640 containing 10% FBS, keratinocystes were formed but neurons and other non-follicular differentiating cell types were not observed. The middle and lower parts of the plucked hair follicle did not proliferate in DMEM-F12 containing B-27 and bFGF (Fig. 1C).

Thus, the hair follicles of mice and men appear to have two populations of stem cells: a pluripotent type and an apparent unipotent type, the former above the bulge and the latter in the bulge, both in a "hair follicle stem cell zone." These stem cells have important potential for regenerative medicine and hair growth, respectively. Thus, the hair follicle may be the most useful of adult stem cells due to easy access and pluripotent differentiation potential.

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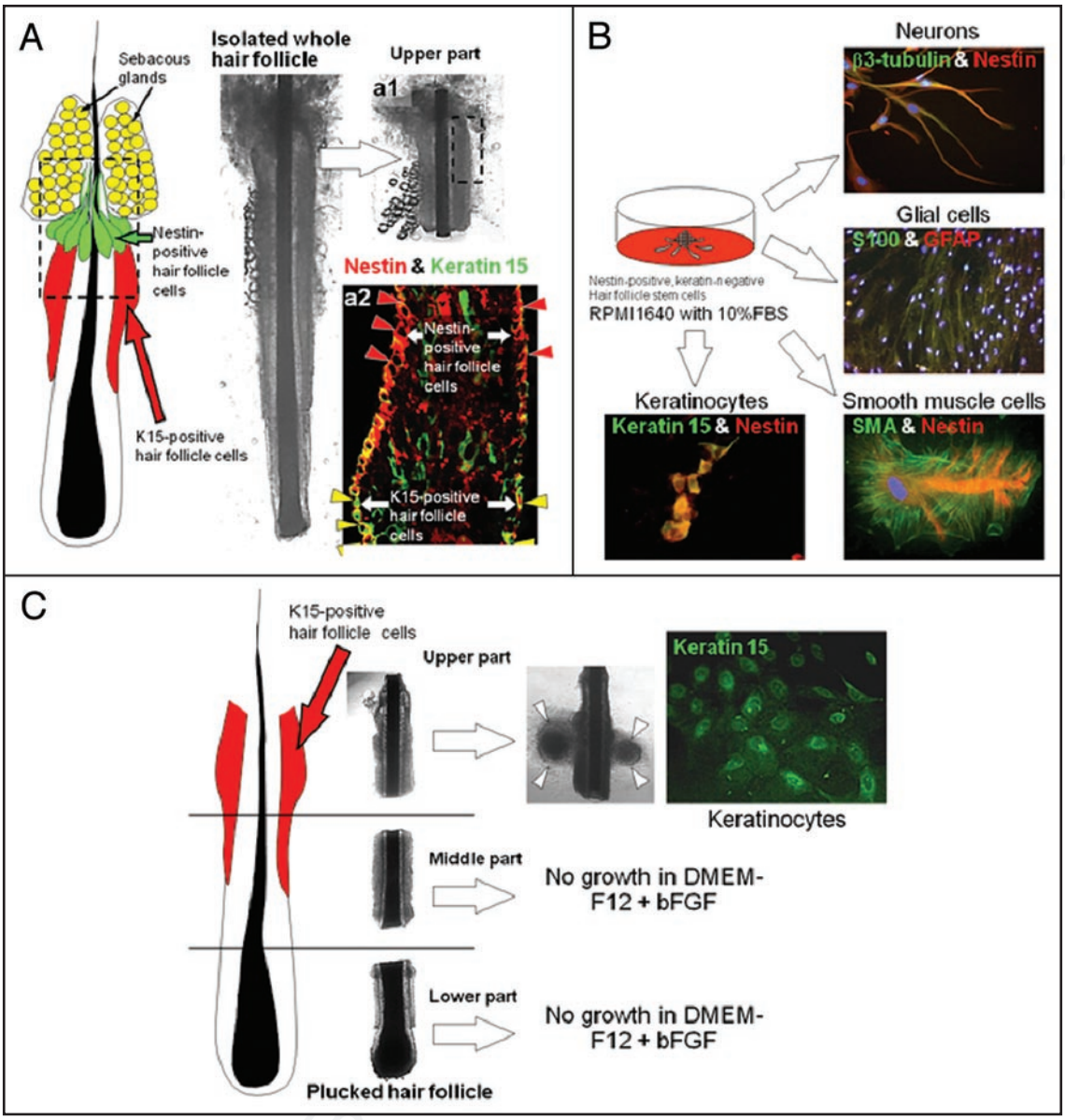

Figure 1. (A) Schema of intact human hair follicle. Intact hair follicles were dissected from the scalp and were divided into three parts (upper, middle and lower parts). (A1) The hair follicle stem cells that are located immediately below the sebaceous glands just above the hair follicle bulge area in the upper part of the sectioned hair follicle were isolated. The cells were suspended in DMEM-F12 containing B-27 supplemented with bFGF every two days. (A2) Immunofluorescence staining. Nestin-positive, K15-negative cells can be seen (red arrowheads). The hair follicle bulge area was only weakly positive for nestin and highly positive for K15 (yellow arrowheads). (B) A nestin-expressing colony formed from human hair follicle stem cells that were located immediately below the sebaceous glands just above the hair follicle bulge area in the upper part of the sectioned hair follicle which was dissected from the human scalp. The colony was cultured in DMEM-F12 containing B-27 and bFGF and then switched to RPMI 1640 containing 10% FBS. Ten days after the medium switch, differentiating cells migrated away from the colony. The nestin-positive, K15-negative cells differentiated into β3-tubulin-positive neurons, S 100- and GFAP-positive glial cells, K15-positive keratinocytes, and SMA-positive smooth muscle cells. (C) Schema of plucked scalp hair follicle. The plucked scalp hair follicle, which did not contain the sebaceous glands or nestin-positive, K15-negative stem-cell niche, was divided into three parts (upper, middle and lower parts). The upper part of the divided, plucked, scalp hair follicle was suspended in DMEM-F12 containing B-27 supplemented with bFGF every two days. Ten days later, colonies of K15-positive keratinocytes are seen (white arrowheads). The middle and lower parts of the divided plucked hair follicle did not proliferate in DMEM-F12 containing B-27 and bFGF. K15-positive keratinocytes grew from the upper part of the divided follicle.

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