# A preliminary study of markers for human hair follicle melanin stem cell

**Xing-Yu Mei, Zhou-Wei Wu, Cheng-Zhong Zhang, Yue Sun, Wei-Min Shi**

Department of Dermatology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200080, China.

*To the Editor:* Melanocyte stem cells (MSCs), derived from the neural crest, function as the repository of melanocytes (MCs). Currently, most scholars suggest that MSCs mainly exist in the bulge of hair follicles.[1] At the end of the embryo, the neural crest cells differentiate into melanoblasts (MBs), which migrate through the dermis to the epidermis and into the developing hair follicles.[2] When the MBs enter the hair follicles, some of them migrate to the hairy mother region and differentiate into mature MCs, producing the pigment and passing it to the keratinocytes (KCs). The others are settled in the follicular and become MSCs, which are responsible for the regeneration of MCs.[3] The labelled marker of MSCs includes paired box gene 3 (*PAX3*) and dopachrome tautomerase.[4]*PAX3*, as a kind of important transcription factor and belonging to the paired box (PAX) family, plays a key role in the differentiation, migration, and proliferation of MB and MCs.[5] Some scholars consider that *PAX3* plays a key role in maintaining the undifferentiated molecular genetic mechanism of MSCs.[6] So *PAX3* could be used as a marker of MSCs. In addition to MSC, another kind of stem cell called hair follicle stem cell (HFSC) is also found in hair follicles. HFSCs, derived from ectodermal epithelium, have the potential of differentiation in multiple directions; they could accept melanin from mature MCs and generate hair shaft which contains melanin.[7] HFSCs have various molecular markers, including CK15, CK19, CD34, and others.[8] As a kind of adhesion molecule, CD34 is selectively expressed on the surface of stem cell of human and other mammalian, and gradually diminishes with the maturity of the cell.[9] In 2010, some scholars used immunohistochemical staining of CD34 to label hair follicle skin tissue and demonstrated that CD34 had a positive expression with good specificity in the bulge of human hair follicle.[10] Therefore, in this study, CD34 was used as the marker of HFSCs.

This study was approved by the Institutional Research Ethics Committee of Shanghai General Hospital. Informed consents were obtained from all patients before the enrollment in this study. As foreskin tissue contains more MCs,[11] we separated the skin cells from normal human foreskin by enzymatic digestion in this study. In theory, these cells include large numbers of KCs, MCs, partial fibroblasts, and a small amount of skin stem cells from basal layer of the skin and hair follicles. *PAX3* and CD34 were regarded as markers of MSCs and HFSCs, respectively, and then we detected whether MSCs and HFSCs exist in the original mixed skin cells by flow cytometry (FCM). In view of the study that the hair follicle melanin stem cells and the HFSCs share a cell nest,[12] we tried to detect whether there was a certain kind of cell in the original hybrid cells that expressed markers of HFSCs and MSCs at the same time. If it exists, does the cell express the specific markers of MCs such as tyrosinase-2 (TYR-2), Melan-A, and microphthalmia-associated transcription factor (MITF), and whether they have the expression characteristics of MCs or MSCs?

The percentage of cells was detected by FCM. The result showed the percentage of *PAX3*+, CD34+ and *PAX3*+/CD34+ was $4.82 \pm 0.15\%$, $5.38 \pm 0.21\%$, and $0.58 \pm 0.05\%$, respectively [Figure 1]. After digesting normal human foreskin tissues ($n = 9$), the original skin mixed cell samples were obtained, and the *PAX3*+/CD34+ cells in these samples were selected by FCM. The result of FCM revealed total number of cells in these samples was about $(8.50 \pm 0.65) \times 10^7$, the percentage of *PAX3*+/CD34+ cells in these samples was about $0.67 \pm 0.15\%$, and the number of *PAX3*+/CD34+ cells in these samples was about $(5.70 \pm 0.40) \times 10^5$. The original MCs from human were set as control group ($n = 9$), *PAX3*+/CD34+ cells detected by FCM in the original skin mixed cells were set as *PAX3*+/ CD34+ group. The relative expression of tyrosinase related *protein-2* (TRP-2), MITF, Melan-A, TYR, TYR-1, and SRY-related HMG-box 10 (SOX10) were detected in *PAX3*+/ CD34+ and control groups. The result showed that there was no significant difference in

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**Correspondence to:** Dr. Wei-Min Shi, Department of Dermatology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, No. 100 Haining Rd, Shanghai 200080, China
E-Mail: shiyipifu@163.com



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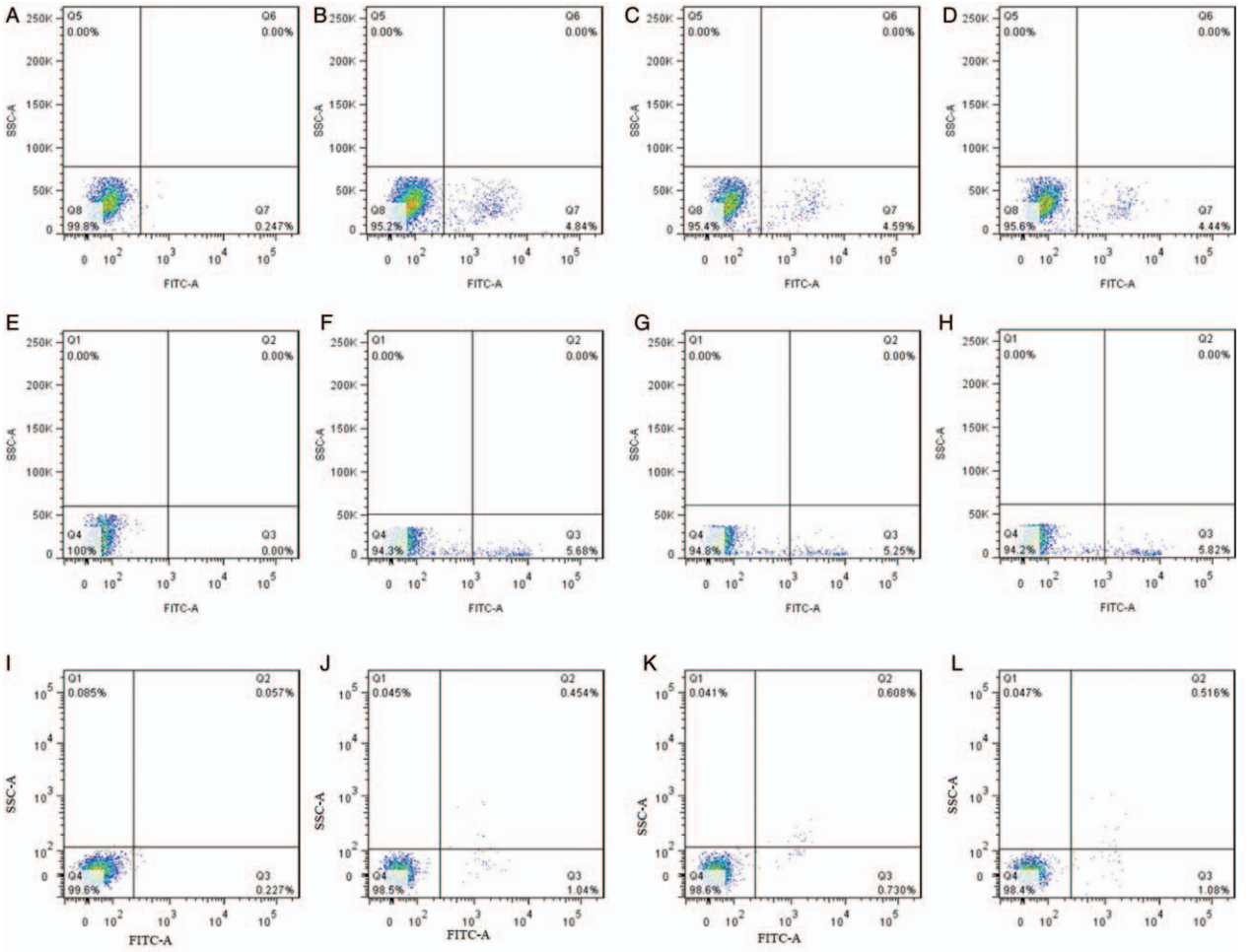


**Figure 1:** Cells detected by FCM in mixed skin cells *in vitro*. The percentage of *PAX3*-positive cells detected: the negative control (A), and three patients' samples (B–D; the percentage of *PAX3*-positive cells was 4.84%, 4.59%, and 4.44%, respectively). The percentage of CD34-positive cells detected: the negative control (E), and three patients' samples (F–H; the percentage of CD34-positive cells was 5.68%, 5.25%, and 5.82%, respectively). The percentage of *PAX3*+/CD34+ cells detected: the negative control (I), and three patients' samples (J–L; the percentage of *PAX3*+/CD34+ cells was 1.04%, 0.73%, and 1.08%, respectively). FCM: flow cytometry; *PAX3*: paired box gene 3.

expression of TRP-2 between *PAX3*+/CD34+ and control groups ($1.06 \pm 0.20$ and $0.91 \pm 0.11$, $P > 0.05$). The expression of MITF in *PAX3*+/CD34+ group was $0.15 \pm 0.03$, which was obvious lower than control group ($0.93 \pm 0.10$, $P < 0.05$). Similarly, there were significant differences in the expression of Melan-A, TYR, TYR-1, and SOX10 between *PAX3*+/CD34+ and control groups ($0.13 \pm 0.04$ *vs.* $0.83 \pm 0.16$, $0.16 \pm 0.02$ *vs.* $0.90 \pm 0.15$, $0.15 \pm 0.03$ *vs.* $0.87 \pm 0.20$, $0.10 \pm 0.03$ *vs.* $0.92 \pm 0.20$, all $P < 0.05$). Therefore, the expression of TRP-2 in *PAX3*+/ CD34+ group was similar to the control group, while the expression levels of the MITF, Melan-A, TYR, TYR-1, and SOX10 were almost less than 20% of the control group. Immunofluorescence showed that TRP-2 in *PAX3*+/ CD34+ cells was stained red positive. Melan-A and MITF in *PAX3*+/CD34+ cells were stained negative and no fluorescence.

In this study, we examined the *PAX3*+ cells and CD34+ cells in the original hybrid cells by FCM, namely MSCs and HFSCs. And CD34+/*PAX3*+ cells also did exist in the primary skin mixture, accounting for 0.53%, which was about one eighth of the *PAX3*+ cells and one tenth of the CD34+ cells. In addition, we selected *PAX3*+/CD34+ cells in original skin mixed cells, which accounted for $0.67 \pm 0.15\%$ of the mixed cells. We hypothesized that this part of the cells expressed both the marker of MSCs and HFSCs, CD34+ cells may be hair follicle melanin stem cells [Table 1].

In this study, we further examined whether *PAX3*+/CD34+ cells in the original skin cells expressed specific markers of MCs. Results showed that the expression of TRP-2 in *PAX3*+/CD34+ cells was similar to that of control group, while the MITF, Melan-A, TYR, TYR-1, and SOX10 were rarely expressed, which meant that these *PAX3*+/CD34+ might not have the similar characters as mature MCs. By immunofluorescence assay, we confirmed that the TRP-2 was expressed on the surface of *PAX3*+/CD34+ cells. Nevertheless, MITF and Melan-A were hardly ever expressed on cells' surface. Therefore, we deemed that this kind of cell has the particular expression characteristics of MSCs. Accordingly, we considered that *PAX3*+/CD34+/TRP-2+/MITF–/Melan-A– could be the candidate marker for human hair follicle melanin stem cells.

**Table 1: The primers and products of real-time polymerase chain reaction.**

| Primers | Sequence | Product size (bp) |
|---|---|---|
| GAPDH | Forward: 5′-GGAGCGAGATCCCTCCAAAAT-3′<br>Reverse: 5′-GGCTGTTGTCATACTTCTCATGG-3′ | 197 |
| TRP-2 | Forward:5′-CTGCATGTGCTGGTTCTTCAT-3′<br>Reverse: 5′-TTGTGACCAATAGGGGCCAG-3′ | 113 |
| MITF | Forward: 5′-GCCTGTCTCGGGAAACTTGA-3′<br>Reverse: 5′-ACGCTGTGAGCTCCCTTTTT-3′ | 119 |
| Melan-A | Forward: 5′-CTGCTCATCGGCTGTTGGTA-3′<br>Reverse: 5′-GAGACACTTTGCTGTCCCGA-3′ | 142 |
| TYR | Forward: 5′-CAGCTTTCAGGCAGAGGTTC-3′<br>Reverse: 5′-GCTTCATGGGCAAAATCAAT-3′ | 133 |
| TYR-1 | Forward: 5′-GCAGAATGAGTGCTCCTAAACTCC-3′<br>Reverse: 5′-CCTGATGATGAGCCACAGCG-3′ | 121 |
| SOX10 | Forward: 5′-TGCCAGCCGTCCCAGATGT-3′<br>Reverse: 5′-CGACTGGACTCTCGTGCCCAT-3′ | 148 |

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; MITF: Microphthalmia-associated transcription factor; SOX10: SRY-related HMG-box 10; TRP-2: Tyrosinase related protein-2; TYR: Tyrosinase.

We suggested that this kind of cell might belong to the transition cells of MSCs and HFSCs.


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## Conflicts of interest

None.

## References


1. Chou WC, Takeo M, Rabbani P, Hu H, Lee W, Chung YR, *et al*. Direct migration of follicular melanocyte stem cells to the epidermis after wounding or UVB irradiation is dependent on Mc1r signaling. Nat Med 2013;19:924–929. doi: 10.1038/nm.3194.
2. Nishimura EK. Melanocyte stem cells: a melanocyte reservoir in hair follicles for hair and skin pigmentation. Pigment Cell Melanoma Res 2011;24:401–410. doi: 10.1111/j.1755-148X.2011.00855.x.
3. Jang YH, Kim SL, Lee JS, Kwon KY, Lee SJ, Kim DW, *et al*. Possible existence of melanocytes or melanoblasts in human sebaceous glands. Ann Dermatol 2014;26:469–473. doi: 10.5021/ad.2014.26.4.469.
4. Delfino-Machín M, Chipperfield TR, Rodrigues FS, Kelsh RN. The proliferating field of neural crest stem cells. Dev Dyn 2007;236:3242–3254. doi: 10.1002/dvdy.21314.
5. Medic S, Rizos H, Ziman M. Differential PAX3 functions in normal skin melanocytes and melanoma cells. Biochem Biophys Res Commun 2011;411:832–837. doi: 10.1016/j.bbrc.2011.07.053.
6. Lang D, Lu MM, Huang L, Engleka KA, Zhang M, Chu EY, *et al*. Pax3 functions at a nodal point in melanocyte stem cell differentiation. Nature 2005;433:884–887. doi: 10.1038/nature03292.
7. Coelho de Oliveira VC, Silva Dos Santos D, Vairo L, Kasai Brunswick TH, Pimentel LAS, Carvalho AB, *et al*. Hair follicle-derived mesenchymal cells support undifferentiated growth of embryonic stem cells. Exp Ther Med 2017;13:1779–1788. doi: 10.3892/etm.2017.4195.
8. Tiede S, Kloepper JE, Bodo E, Tiwari S, Kruse C, Paus R. Hair follicle stem cells: walking the maze. Eur J Cell Biol 2007;86:355–376. doi: 10.1016/j.ejcb.2007.03.006.
9. Yoshida S, Lee JO, Nakamura K, Suzuki S, Hendon DN, Kobayashi M, *et al*. Lineage-CD34+CD31+ cells that appear in association with severe burn injury are inhibitory on the production of antimicrobial peptides by epidermal keratinocytes. PLoS One 2014;9:e82926. doi: 10.1371/journal.pone.0082926.
10. Pascucci L, Mercati F, Gargiulo AM, Pedini V, Sorbolini S, Ceccarelli P. CD34 glycoprotein identifies putative stem cells located in the isthmic region of canine hair follicles. Vet Dermatol 2006;17:244–251. doi: 10.1111/j.1365-3164.2006.00527.x.
11. Bandarchi B, Jabbari CA, Vedadi A, Navab R. Molecular biology of normal melanocytes and melanoma cells. J Clin Pathol 2013;66:644–648. doi: 10.1136/jclinpath-2013-201471.
12. Rabbani P, Takeo M, Chou W, Myung P, Bosenberg M, Chin L, *et al*. Coordinated activation of Wnt in epithelial and melanocyte stem cells initiates pigmented hair regeneration. Cell 2011;145:941–955. doi: 10.1016/j.cell.2011.05.004.


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