RESEARCH ARTICLE

# Depletion of CD200+ Hair Follicle Stem Cells in Human Prematurely Gray Hair Follicles

**Introduction:** Melanocyte stem cells (MelSCs) are known to be depleted in gray hair follicles. Hair follicle stem cells (HFSCs) are important for maintenance of stemness of MelSCs. **Methods:** We compared the proportion of CD200+ (Cluster of Differentiation 200 positive) stem cells in the outer root sheath cell suspension of gray and pigmented hair follicles of three patients with the premature graying of hair. In addition, explants culture for HFSCs was also carried out from gray and pigmented hair follicles. Cultured HFSCs were also differentiated into melanocytes. **Results:** The mean ± SD CD200+ HFSCs population were 9.4 ± 1.4% and 3.5 ± 0.5% for pigmented and gray hair follicles, respectively ($P = 0.002$). In explants culture, the growth of HFSCs from the gray hair follicle stopped at around day 20-22, whereas the growth of the cells from the pigmented follicle continued. **Conclusion:** CD200+ HFSCs are depleted in prematurely gray hair in the humans. CD200+ hair follicle stem cell yield is poorer in gray hair explant culture than pigmented hair explant culture.

**KEYWORDS:** Hair follicle, stem cells, CD200

## INTRODUCTION

The hairs in all species are important, not only from the aesthetic angle, but also as a protective barrier from environment. Colour of skin and hair is due to synthesis of melanin by melanocytes.[1,2] Human hair follicles stem cells (HFSCs) are primarily CD200+ (Cluster of Differentiation 200 positive) keratinocyte stem cells. In close proximity to these, melanocyte stem cells (MelSCs) are located. It is now well documented that MelSCs are located in the lower permanent portion of the hair follicle to replenish the pool of melanocytes and loss of MelSCs is thought to be responsible for hair graying.[3-6] Herewith, we present data on comparison of CD200+ HFSCs of keratinocyte lineage in pigmented and prematurely gray human hair follicle.

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Quick Response Code:

Website: www.jcasonline.com

DOI: 10.4103/0974-2077.112669

## METHODS

### Human scalp gray and pigmented hair sample collection

Both gray and pigmented hair follicles were obtained using the follicular unit extraction method from three patients with premature graying of hair after taking their informed consent as recommended by the Institutional Ethics Committee and Institutional Committee for Stem Cell Research and Therapy. Hair was trimmed to a length of approximately 2 mm. Field block anaesthesia was given with 2% lignocaine. To obtain follicular units, 1-mm punch was rotated till mid-dermis in the direction of the hair follicle. Then follicular unit was then pulled out gently using the hair follicle holding forceps. Approximately 10-20 pigmented and gray follicles were extracted per subject and collected separately in collection media containing Dulbecco's Modified Eagle's medium (DMEM) (Sigma, St. Louis, MO, USA), supplemented with penicillin, streptomycin and amphotericin-B (Gibco, Gaithersburg, MD, USA).

### Preparation of single cell suspension and flow cytometry

Preparation of single cell suspension was carried out by a method described by us previously.[7] For flow

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cytometry, cells were labelled with PE (phycoerythrin) mouse anti-human CD200 antibody (BD Biosciences, CA, USA). Marker for the negative population was set with the use of PE mouse IgG1κ antibody (BD Biosciences, CA, USA) and unstained cell population. After labelling, cells were acquired on a Becton Dickinson LSRII (BD LSRII™) flow cytometer and analysed by BD FACSDiva™ software version 6.1.2. For each sample, at least duplicate acquisitions were performed.

### Whole hair follicle culture

One explants/sq cm area with a total of five follicle of each group, gray and pigmented hair, were cultured according to the modified Rheinwald-Green system[8] consisting of 3:1, DMEM and Ham's F12 nutrient mix (Sigma, MO, USA), supplemented with 10% fetal bovine serum (Hyclone, NJ, USA), 10 ng/ml epidermal growth factor (PeproTech, NJ, USA), 2.8 μg/ml hydrocortisone, 5 μg/ml insulin, 10 μg/ml transferrin (Sigma, MO, USA), 10 ng/ml cholera toxin (Millipore, MA, USA) and 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco BRL, Gaithersburg, MD, USA) over fibronectin (Sigma, MO, USA) coated culture dish (BD Falcon, MD, USA).

Cells grown over the coverslips or cytospin preparations were taken for the immunofluoresence. For keratinocyte stemness, K19 and β1-Integrin, (Millipore, MA, USA) antibodies were used. Texas red (BD Biosciences, CA, USA) conjugated secondary antibodies were used to develop fluorescence signal.

## RESULTS

The mean ± SD CD200+ HFSCs population were 9.4 ± 1.4% and 3.5 ± 0.5% for pigmented and gray hair follicles, respectively ($P$ = 0.002) [Table 1]. There was a marked visible difference in the spread of cell sheet in culture from the pigmented and gray hair follicle. The growth of the cells from the gray hair follicle stopped at around day 20-22, whereas the growth of the cells continued from the pigmented follicle. At day 35, only few cells could be observed in the gray hair follicle culture. A sheet of cells was observed from the pigmented follicle culture at day 35 [Figure 1a and b]. Because of poor cell number, we could not characterize the cells from the gray hair follicle.

Table 1: Percentage of ORS cells from gray and pigmented hair follicles positive for hair follicle stem cell marker CD200

| Gender | Age (years) | CD200% | |
|---|---|---|---|
| | | Pigmented follicle | Grey follicle |
| Male | 20 | 10 | 3.5 |
| Female | 22 | 10.6 | 4 |
| Male | 20 | 7.8 | 3 |

ORS: Outer root sheath, CD200: Cluster of differentiation 200 positive

Immunofluorescence studies were carried out for the HFSCs cultured from the pigmented hair follicle. Cells cultured from the pigmented hair follicle stained positive for HFSCs markers K19 and β1 integrin antibody [Figure 1c and d].

## DISCUSSION

Hair graying is the most obvious sign of aging in humans, yet its mechanism is largely unknown. Thumb rule of hair graying is that by 50 years of age, 50% of people have 50% gray hair.[9] Melanocytes in the hair matrix decrease in number as hair follicle is aged physiologically.[10] Analysis of MelSCs in mice at different ages has revealed that MelSCs population gets depleted with aging and this results in hair graying.[1,11] MelScs give rise to the progenitor cells, which differentiate into pigment-containing melanocytes. Melanoblasts in the human hair follicle bulge area also show a similar decrease in number and ectopic differentiation in the bulge-subbulge area with aging. MelSCs have direct contact with HFSCs marker expressing keratinocytes in the bulge area. Surrounding HFSCs of keratinocyte lineage secrete Transforming growth factor-β, which is critical for the maintenance of MelSCs by promoting their quiescence and immaturity. Therefore, HFSCs provide a functional niche with MelSCs.[11]

Our findings suggest that CD200+ HFSCs are depleted from outer root sheath (ORS) of gray phenotype of the hair follicle, which may result in secondary loss of melanocyte stem cell pool. The size of the cell sheet in culture was small in case of gray hair follicle as compared to pigmented follicle. Due to fewer cells obtained after *in vitro* expansion of gray hair follicle

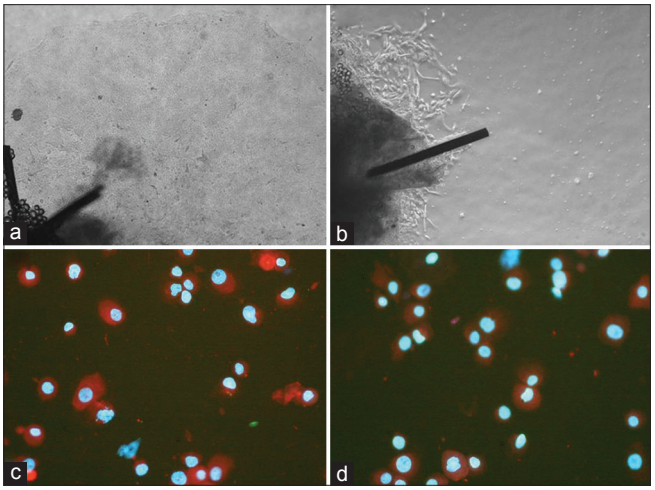


**Figure 1: Phase contrast photographs of cell sheet obtained from the pigmented (a) and gray (b) hair follicle explant culture. Photographs (c) and (d) depict the immunofluorescence images of cells obtained from the pigmented hair follicle culture stained with anti-K19 and anti-β1 integrin antibody respectively**

cells, we could not characterize cultured gray hair follicular cells. The depletion of HFSCs pool could be the reason behind the small size of the colony obtained from the culture of the gray hair follicle. However, the growth of the hair follicle is not affected in comparison to black follicle in real life. In *in vivo* condition, there may be other niches of the hair follicle stem cells such as sebaceous gland and hair bulb, which might be contributing towards the hair growth.

## CONCLUSIONS

The findings of our study are preliminary and carried out on a small sample size. Nevertheless, the decrease in HFSCs was clearly observed in grey hair follicle. Do HFSCs play any role in MelSCs maintenance needs to be evaluated.

## ACKNOWLEDGEMENT

This work was supported by the IADVL-L'Oreal Pigmentation Research Grant (IADVL/COMM/IADVL/L'Oreal Grant/09). Anil Kumar is a recipient of Research Fellowship from the University Grants Commission, Govt. of India. Authors gratefully acknowledge technical help from Stéphane COMMO, L'Oréal Recherche and Innovation.

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**How to cite this article:** Mohanty S, Kumar A, Dhawan J, Sharma VK, Gupta S. Depletion of CD200+ hair follicle stem cells in human prematurely gray hair follicles. J Cutan Aesthet Surg 2013;6:90-2.

**Source of Support:** This work was supported by the Indian Association of Dermatologists Venereologists and Leprologists-L'Oreal Pigmentation Research Grant. **Conflict of Interest:** None declared.