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Not Follicular but Dermal Melanocyte Precursors Are Histopathologically Retained in Vitiligo

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Abstract

Although perifollicular repigmentation in the vitiligo lesions is owing to activation of follicular melanocyte stem cells and/or precursor cells followed by supplying matured melanocytes, the underlying mechanism of diffuse repigmentation on the whole vitiligo surface remains still unknown. In addition to the presence of remaining melanocytes, it is conceivable that dermal melanocyte precursor cells contribute to induce diffuse repigmentation after treatment. Therefore, we investigated here whether dermal and follicular melanocyte precursor cells were reduced or not in vitiligo lesions. We performed an immunostaining for Nestin and p75NGFR as dermal melanocyte precursor cells and MITF/Fzd4 as follicular melanocyte precursor cells and compared the positive cells number between lesions and non-lesions (n = 11). Although $MITF^+/Fzd4^+$ cells in the hair follicle were significantly decreased in number in the lesions, $Nestin^+$ and $p75NGFR^+$ cells were not. This result indicates that dermal precursor cells could be retained in the vitiligo lesions but be disturbed to differentiate into matured melanocytes.

Keywords

Melanocyte Precursor Cells, p75NGFR, Nestin, Frizzled4, Vitiligo

1. Introduction

Vitiligo is an acquired pigmentary disorder caused by the failure of epidermal melanocytes and the pathological autoimmunity and oxidative stress responses

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to melanocytes are thought to eliminate mature melanocytes [1]. Melanocyte precursor cells located in not only the hair follicle but also dermis potentially supply epidermal melanocytes when repigmentation takes place [2] [3]. In clinical aspect, there are three repigmentation patterns after proper vitiligo treatment: perifollicular; marginal; and diffuse. In the perifollicular pattern, melanocytes are believed to migrate from the pores to the epidermis, supplying mature melanocytes. The marginal pattern is believed to be the mechanism by which melanocytes migrate from the marginal region. The diffuse pattern is believed to take place under the mechanism by which inactivated melanocytes and melanocyte precursor cells in the vitiligo region are activated [4]. Melanocyte precursor cells in not only hair follicles but also in the dermis are considered essential for supplying mature melanocytes that produce melanosome, especially in hairless vitiligo regions. Therefore, in this study, we performed immunostaining of the skin of vitiligo patients using Nestin and p75NGFR, which are the neural stem cell markers, MITF, which is a melanocyte transcription factor marker, and frizzled 4 (Fzd4), which is a Wnt signal receptor, in order to analyze how many dermal melanocyte precursor cells and follicular melanocyte precursor cells existed.

2. Material and Methods

Table 1 summarizes clinical data of 11 vitiligo patients enrolled in this study. The study was approved by the research ethics committee of the graduate school of medicine, Osaka University (approval number: 13421-14, approval date: 2022.01.21). After obtaining the patient's written consent, skin biopsy specimens from vitiligo lesions, non-lesions, and repigmented lesions obtained from 2019 to 2021 were immunohistochemically stained with Nestin, p75NGFR, and MITF/Fzd4. Representative positive staining for each marker is shown in Figure 1. The detail information of antibodies used in this study was summarized in Table 1. Using Nestin and p75NGFR as markers for dermal melanocyte precursor cells as well as MITF/Fzd4 as markers for follicular melanocyte precursor cells, the number of positive or double positive cells for each marker was significantly compared by a paired t-test. The <0.05 of p-value was defined significant. Three independent dermatologists counted the positive cells number and those averages were used for statistical analysis.

Table 1. The antibodies' information used for immunohistochemistry.

Marker	Clonality	Antibody clone	Bland/#	Marker description
Nestin	Mouse mono	2C1.3A11	Abcam/ab18102	Neural stem cell
NGFRp75	Rabbit mono	EP1039Y	Abcam/ab252987	Nerve growth factor
MITF	Rabbit poly	-	Sigma-Aldrich/ HPA003259	Melanocyte transcription factor
Fzd4	Rabbit poly	-	Millipore/07-2166	Wnt signal receptor

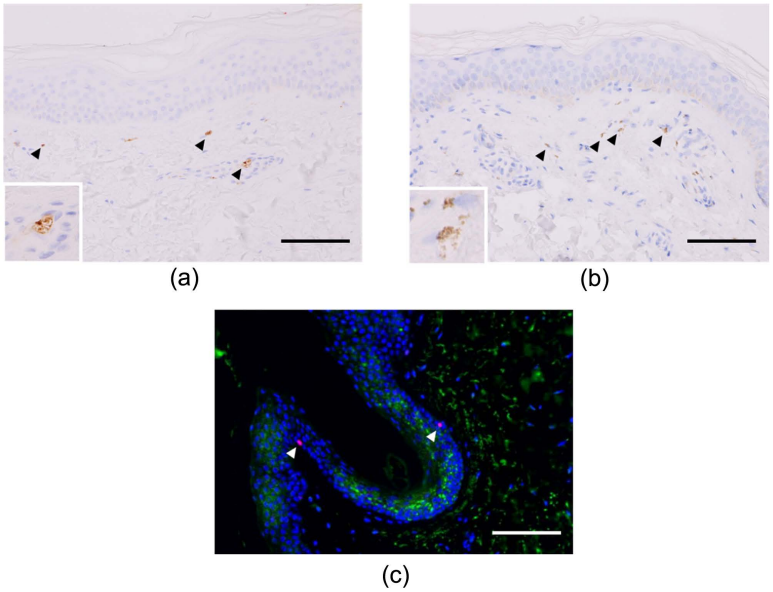

Figure 1. Melanocyte precursor cells were present in the vitiligo skin. (a) Immune staining for Nestin on the representative vitiligo lesion. The Nestin$^+$ cells were indicated by arrowheads and magnified in the left inner box. Scale bar denotes 100 μm. (b) Immune staining for p75NGFR on the representative vitiligo lesion. The p75NGFR $^+$ cells were indicated by arrowheads and magnified in the left inner box. Scale bar denotes 100 μm. (c) The double positive cells for MITF (red) and Fzd4 (green) notably located on the infundibular follicle in non-lesion. The double positive cells (pink) were indicated by arrowheads. Scale bar denotes 100 μm.

3. Results

The enrolled patients' age ranged from 8 - 79 years old with three females and eight males. Ten patients except one were nonsegmental type. Skin samples were collected from head and neck (n = 1), body trunk (n = 3), and limb (n = 7) while the non-lesions were considered to be pigmented skins a few centimeters away from the lesional border. Among patients in whom repigmentation was observed, two patients had the diffuse pattern while one patient had the perifollicular pattern (**Table 2**). Representative immune-staining for Nestin and p75NGFR in lesions supposed the positive cells were located on the upper dermis (**Figure 1(a)** and **Figure 1(b)**). MITF$^+$/Fzd4$^+$ cells were detected on the infundibular follicle in non-lesion (**Figure 1(c)**). We compared the number of positive cells from lesions and non-lesions. Although we expected that the number of Nestin$^+$ cells and p75$^+$ cells would decrease in the vitiligo lesions which lacked matured melanocytes, there was no significant difference in the number of positive cells for both markers. (**Figure 2(a)** and **Figure 2(b)**) This result suggested that dermal melanocyte precursor cells detected by Nestin or p75NGFR remained quiescent not deleted in vitiligo lesions. Subsequently, when MITF$^+$/Fzd4$^+$ cells in the hair follicle were compared, there was a significant decrease in the lesions compared with non-lesions. On the other hand, MITF$^+$/Fzd4$^+$ cells remained in 54.5% of the vitiligo lesions, supporting the fact that there is potential for repigmentation even if it seems to exhibit clinical aspect of com-

plete depigmentation (Figure 2(c)). The number of MITF$^+$/Fzd4$^+$ cells on the repigmented lesion was comparably recovered to the non-lesional level.

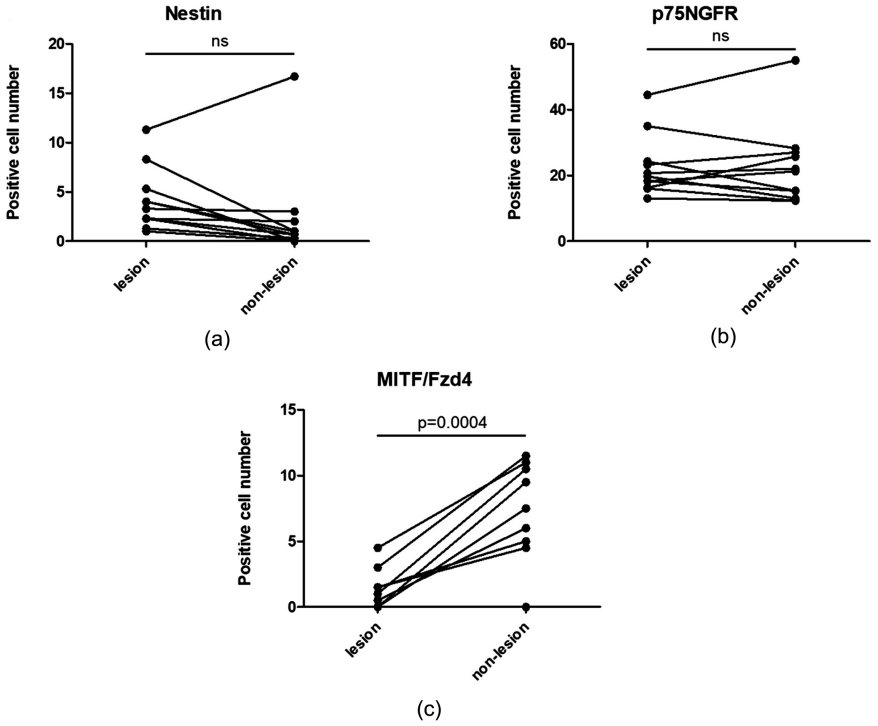

Figure 2. No statistical change in the number of Nestin and p75NGFR positive cells between vitiligo lesions and non-lesions. (a) The paired-comparison of the number of dermal Nestin$^+$ cells between vitiligo lesion and non-lesion. (b) The paired-comparison of the number of dermal p75NGFR$^+$ cells between vitiligo lesion and non-lesion. (c) The paired-comparison of the number of MITF$^+$/Fzd4$^+$ cells in follicular regions between vitiligo lesion and non-lesion.

Table 2. Patients' profile enrolled in this study.

Patient number (n)	11
Age (yrs)	8 - 79
Gender	
F	3
M	8
Vitiligo type	
Nonsegmental	10
Segmental	1
Biopsy site	
Head and neck	1
Trunk	3
Extremity	7
Acral	0

4. Discussion

In this study, we focused on the remaining and localized immature melanocytes which were essential for repigmentation from vitiligo lesions, and examined those markers reported in human skin. The importance of supplying mature melanocytes from follicular melanocyte stem cells or melanoblasts has been proposed as a mechanism for repigmentation. When these precursor reservoir cells are completely depleted, repigmentation cannot be expected with conservative treatments other than surgical supply. However, that does not sufficiently explain why repigmentation occurs in hairless regions and why diffuse repigmentation that does not match hair follicles occurs [5]. Therefore, it is possible that melanocytes may be supplied not only from the epidermis but also from the dermis. As a result of identifying and tracking p75NGFR$^+$ cells in the dermis using human artificial skin without hair follicles, it was reported that p75NGFR$^+$ cells could migrate to the epidermis and differentiate into mature melanocytes, suggesting the possibility of the presence of dermal melanocyte precursor cells in human skin [2]. In addition, it has been reported that one of the reasons for the presence of diffuse repigmentation is the presence of immature melanocytes in the vitiligo epidermis and that reactivation of the cells may also cause pigment regeneration [4]. More Nestin-positive melanocyte precursor cells infiltrated into the vitiligo dermis using both Narrowband UVB and tacrolimus [3]. Based on these reports, we used two markers, p75NGFR and Nestin, as markers for dermal melanocyte precursor cells. Comparing the number of positive cells of these two markers in the vitiligo and non-lesions, unexpectedly, there was no significant difference between the two markers, with many positive cells identified in the vitiligo region as well. This indicates that depletion of dermal melanocyte precursor cells in vitiligo lesions cannot be simple cause in which mature melanocytes are not supplied, but rather suggests the existence of unknown regulatory mechanism for maintaining the function of dermal melanocyte precursor cells. Moreover, the stimulation or differentiation of melanocyte precursor cells may accelerate repigmentation in vitiligo lesions.

Subsequently, Fzd markers were used to identify follicular melanocyte immature cells that mainly contribute to follicular repigmentation [6] [7]. Fzd is a Wnt pathway receptor that is more often expressed in immature melanocytes and is considered to be a signal serving in the maintenance of melanocyte stem cells [8]. Since MITF$^{+/}$Fzd4$^+$ cells were significantly more abundant in non-lesional hair follicles, it is believed that those cells were able to enrich immature melanocytes in the hair follicles. On the other hand, Nestin$^+$ cells and p75NGFR$^+$ cells in the dermis were not identical, therefore, we believe that it will be necessary to identify the more specific markers including Wnt signaling-related molecules [9] other than Nestin or p75NGFR for melanocyte precursor cells in the human skin. Although other specific markers for melanocyte are expected to be developed in further analysis, we demonstrated here that dermal precursor cells were retained in the vitiligo lesions. Clarifying the phenotypic characters of Nestin or

p75NGFR positive cells and dissolving the mechanism that controls the differentiation, function, and migration of dermal melanocyte precursors in vitiligo lesions would lead to develop novel therapies that promote diffuse repigmentation.

Limitation of this study is small sample number analyzed and skin biopsy from various not specific body sites which could cause unstable result. Further investigation is required to strengthen the presented conclusion with larger number and homogeneous patient cohort.

5. Significance

This is first report to demonstrate that dermal melanocyte precursor cells were retained even on the vitiligo lesions in stable state while follicular precursors were significantly reduced or depleted. Our unique observation and results suggest that the retaining dermal melanocyte precursor cells contribute diffuse pattern of repigmentation after treatment and that exploring a mechanism to induce migration and differentiation of melanocyte precursors leads to develop a unique treatment for intractable vitiligo.

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The patients provided written permission in IRB documents for publication.

IRB Approval Status

This research was approved by Osaka University Ethics Committee No. 13421-14.

Conflicts of Interest

The authors declare no conflicts of interest in association with the present study.

References

[1] Ezzedine, K., Lim, H.W., Suzuki, T., Katayama, I., Hamzavi, I., Lan, C.C., *et al.* (2012) Revised Classification/Nomenclature of Vitiligo and Related Issues: The Vitiligo Global Issues Consensus Conference. *Pigment Cell & Melanoma Research*, **25**, E1-13. https://doi.org/10.1111/j.1755-148X.2012.00997.x

[2] Li, L., Fukunaga-Kalabis, M., Yu, H., Xu, X., Kong, J., Lee, J.T., *et al.* (2010) Human Dermal Stem Cells Differentiate into Functional Epidermal Melanocytes. *Journal of Cell Science*, **123**, 853-860. https://doi.org/10.1242/jcs.061598

[3] Gauthier, Y., Almasi-Nasrabadi, M., Cario-André, M., Pain, C., Rakhshan, A., Ghalamkarpour, F. (2021) Tacrolimus (FK506) Ointment Combined with Nb-UVB Could Activate Both Hair Follicle (HF) and Dermal Melanocyte Precursors in Vitiligo: The First Histopathological and Clinical Study. *Archives of Dermatological Research*, **313**, 383-388. https://doi.org/10.1007/s00403-020-02068-z

[4] Yang, K., Xiong, X., Pallavi, G., Ling, Y., Ding, F., Duan, W., *et al.* (2018) The Early Repigmentation Pattern of Vitiligo Is Related to the Source of Melanocytes and by the Choice of Therapy: A Retrospective Cohort Study. *International Journal of Dermatology*, **57**, 324-331. https://doi.org/10.1111/ijd.13878

[5] Parsad, D., Pandhi, R., Dogra, S. and Kumar, B. (2004) Clinical Study of Repigmen-

tation Patterns with Different Treatment Modalities and Their Correlation with Speed and Stability of Repigmentation in 352 Vitiliginous Patches. *Journal of the American Academy of Dermatology*, **50**, 63-67. https://doi.org/10.1016/S0190-9622(03)00786-2

[6] Okamura, K., Ohe, R., Abe, Y., Ueki, M., Hozumi, Y., Tamiya, G., *et al.* (2015) Immunohistopathological Analysis of Frizzled-4 Positive Immature Melanocytes from Hair Follicles of Patients with Rhododenol-Induced Leukoderma. *Journal of Dermatological Science*, **60**, 156-158. https://doi.org/10.1016/j.jdermsci.2015.07.015

[7] Tanemura, A., Koguchi-Yoshioka, H., Yokoi, K., Kiyohara, E., *et al.* (2021) Immunohistopathological Observation of a Case Recovering from Leukotrichia after Suction Blister Transplantation. *The Journal of Dermatology*, **48**, e263-e264. https://doi.org/10.1111/1346-8138.15855

[8] Yamada, T., Akamatsu, H., Hasegawa, S., Inoue, Y., Date, Y., Mizutani, H., *et al.* (2010) Melanocyte Stem Cells Express Receptors for Canonical Wnt Signaling Pathway on Their Surface. *Biochemical and Biophysical Research Communications*, **396**, 837-842. https://doi.org/10.1016/j.bbrc.2010.04.167

[9] Regazzetti, C., Joly, F., Marty, C., Rivier, M., Mehul, B., Reiniche, P., *et al.* (2015) Transcriptional Analysis of Vitiligo Skin Reveals the Alteration of WNT Pathway: A Promising Target for Repigmenting Vitiligo Patients. *Journal of Investigative Dermatology*, **135**, 3105-3114. https://doi.org/10.1038/jid.2015.335

Abbreviation

Frizzled4: Fzd4.