Preferential Expression of OVOL1 in Inner Root Sheath of Hair, Sebaceous Gland, Eccrine Duct and their Neoplasms in Human Skin

Mitoma, Chikage
Department of Dermatology, Graduate School of Medical Sciences, Kyushu University

Nakahara, Takeshi
Department of Dermatology, Graduate School of Medical Sciences, Kyushu University

Uchi, Hiroshi
Department of Dermatology, Graduate School of Medical Sciences, Kyushu University

Ito, Takamichi
Department of Dermatology, Graduate School of Medical Sciences, Kyushu University

他

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Original Article

Preferential Expression of OVOL1 in Inner Root Sheath of Hair, Sebaceous Gland, Eccrine Duct and their Neoplasms in Human Skin

Chikage Mitoma[1)], Takeshi Nakahara[1)], Hiroshi Uchi[1)], Takamichi Ito[1)], Yusuke Inatomi[1)], Takatoshi Ide[1)], Shunichi Jinnai[1)], Naomi Jinnai[1)], Nahoko Iwasaki[1)], Keiko Sakamoto[1)], Nanae Kimura[1)], Ayami Maeda[1)], Yuki Kuma[1)], Eriko Maehara[1)], Midori Tsutsumi[1)], Makiko Kido-Nakahara[1)], Tomomitsu Hirota[2)], Mayumi Tamari[2)] and Masutaka Furue[1)]

[1)]*Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*

[2)]*Laboratory for Respiratory Diseases, Center for Genomic Medicine, RIKEN, Yokohama, Japan*

Abstract

OVOL1 is an important transcription factor for epidermal keratinization, which suppresses proliferation and switches on the differentiation of keratinocytes. A recent genome-wide association study has revealed that *OVOL1* is one of the genes associated with susceptibility to atopic dermatitis. Although it is known to be expressed in murine skin and hair follicles, no investigations have focused on its localization in human skin. In the present study, we thus immunolocalized the expression of OVOL1 in normal and diseased human skin. In normal human skin, OVOL1 was preferentially expressed in the suprabasal layer of the epidermis, inner root sheath of hair, mature sebocytes and the ductal portion of the eccrine glands. Compared to this, no remarkable change in the expression of OVOL1 was observed among inflammatory skin diseases. The expression of OVOL1 was evident in eccrine poroma and hidradenoma. Moreover, it was overexpressed in Bowen's disease and sebaceous adenoma, in sharp contrast to its downregulation in their more malignant counterparts, squamous cell carcinoma and sebaceous carcinoma. OVOL1 may play an important role in human skin morphogenesis and tumorigenesis.

Key words : OVOL1・Inner root sheath・Hair・Eccrine gland・Terminal differentiation

Introduction

The epidermis, mainly composed of keratinocytes, is a highly sophisticated barrier tissue that protects the interior of the body against continuous external injuries. In the human epidermis, basal cells are capable of self-renewal and of producing transiently amplifying progenitor cells, which subsequently exit the cell cycle and embark on a terminal differentiation pathway as they migrate toward the skin surface[1)]. *Ovol1* is an evolutionarily conserved family of genes encoding C2H2 zinc finger transcription factors in animals. Functional studies in *Caenorhabditis elegans, Drosophila melanogaster* and mice suggest that this gene family plays important roles in the development of epithelial tissues and germ cells[2)~4)]. *Ovol1* is expressed in multiple somatic

Corresponding author :
Masutaka Furue, M.D., Ph.D., Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashiku, Fukuoka 812-8582, Japan
Tel. : + 81-92-642-5581, Fax : + 81-92-642-5600
E-mail : furue@dermatol.med.kyushu-u.ac.jp

epithelial tissues, including skin[2]. Recent studies have revealed that it plays a key role in switching off proliferation and turning on terminal differentiation[4]. In keeping with this notion, Dai et al. have demonstrated that murine Ovol1 is expressed in the suprabasal layer of the epidermis and the inner root sheath of hair[2]. However, no studies have demonstrated its localization in human skin. In this study, we immunolocalized OVOL1 expression in normal and diseased human skin.

Materials and Methods

Tissue samples

We examined 41 diseased skin samples, including 3 psoriasis, 3 chronic eczema, 3 atopic dermatitis, 3 systemic sclerosis, 2 Bowen's disease, 3 basal cell carcinoma, 2 squamous cell carcinoma, 2 Paget's disease, 2 malignant melanoma, 3 basal cell carcinoma, 3 pilomatricoma, 2 trichilemmal cyst, 2 sebaceous adenoma, 2 sebaceous carcinoma, 3 eccrine poroma and 3 eccrine hidradenoma. We also immunostained 5 normal skin samples. All formalin-fixed and paraffin-embedded tissues were obtained from the archives of the Department of Dermatology of Kyushu University Hospital, Japan. Clinical and demographic data were retrieved from the patient files.

Immunohistochemical analysis

All samples were fixed with 10% buffered formalin. The archival paraffin-embedded tissue blocks were cut into 4-μm-thick tissue sections. The sections were deparaffinized with xylene for 10 min and rehydrated through a graded ethanol series. Antigen retrieval was performed using Heat Processor Solution pH6 (Nichirei Biosciences Inc., Tokyo, Japan) at 100 °C for 40 min, and endogenous peroxidase was blocked by incubating the sections with 3 % H_2O_2 (Nichirei Biosciences Inc., Tokyo, Japan). The sections were then incubated with rabbit antibody against human OVOL1 (1 : 200, LifeSpan BioSciences, Inc., Seattle, WA, U.S.A.) at 4 °C overnight, followed by incubation with secondary antibody, N-Histofine® Simple Stain MAX-PO MULTI (Nichirei Biosciences Inc.). Immunodetection was conducted with 3,3-diaminobenzidine as a chromogen, followed by light counterstaining with hematoxylin. Sections stained without primary antibody served as a negative control.

Results

Expression of Ovol1 in normal skin and inflammatory skin diseases

In normal epidermis, positive signals for OVOL1 were detected in the suprabasal keratinocytes, with mainly a cytoplasmic (partially nuclear) dotted pattern (Fig. 1A). In the pilosebaceous unit, the expression of OVOL1 was exclusively confined to the inner root sheath of the hair follicle (Fig. 1B, 1C), lipid-containing mature sebocytes (Fig. 2A) and erector pili muscle (Fig. 2A). The staining intensity was especially strong in the inner root sheath (Fig. 2A). The expression of OVOL1 was also observed in the ductal portion of the eccrine glands (Fig. 2B), with much less positive staining in the secretory portion (Fig. 2C). The epidermal staining intensity and pattern of OVOL1 were quite similar among samples from cases of psoriasis, chronic eczema, atopic dermatitis and systemic sclerosis, as well as from normal skin (data not shown).

Expression of OVOL1 in skin tumors

Since no apparent differences in OVOL1 expression were observed in inflammatory skin diseases, we next examined its expression in various skin tumors. In this case, we evaluated the intensity of OVOL1 expression in tumor cells by comparison with that of adjacent normal skin. As shown in Table 1, the expression levels of OVOL1 in basal cell carcinoma, squamous cell carcinoma, extramammary Paget's disease and maligant melanoma were similar to or lower than those of adjacent normal skin. Interestingly, OVOL1 expression was increased in Bowen's disease (Fig.

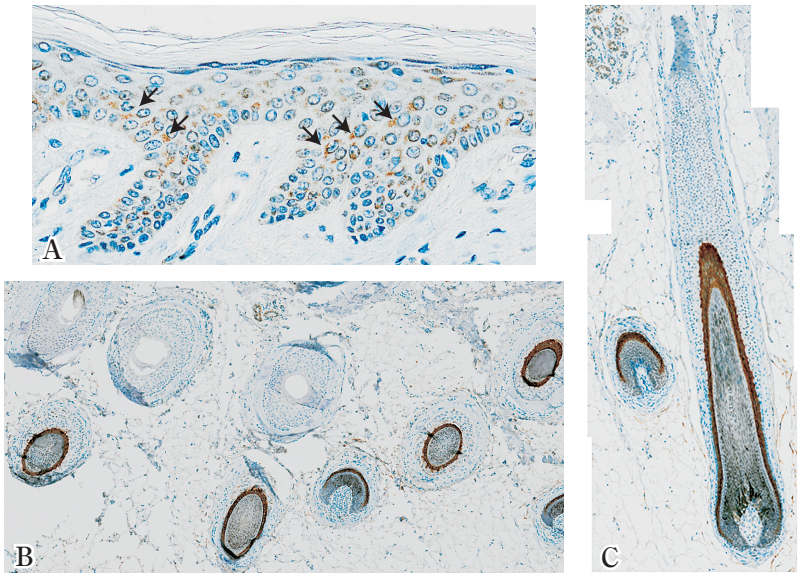

Fig. 1 A : Normal skin epidermis. The expression of OVOL1 was identified as a dotted pattern, mainly in the cytoplasm (arrow). B : Horizontal section of hair. C : Vertical section of hair. The expression of OVOL1 was confined to the inner root sheath.

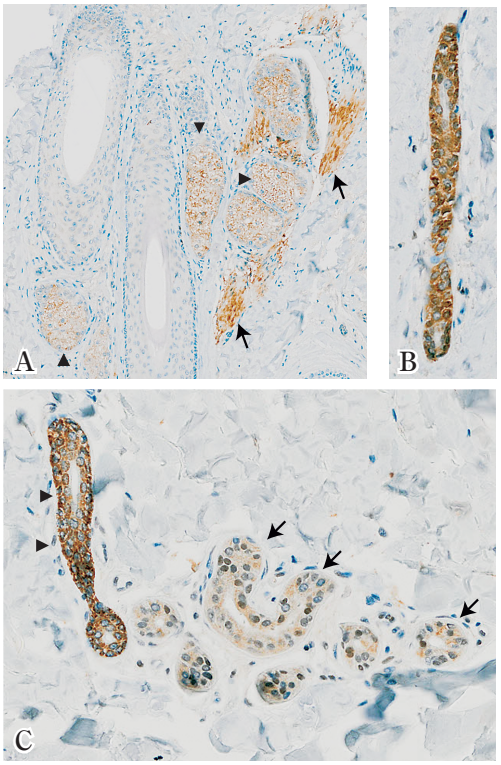

Fig. 2 A : The expression of OVOL1 was detected in sebaceous gland (arrowhead) and erector pili muscle (arrow). B : Ductal portion of eccrine gland. C : Ductal (arrowhead) and secretory (arrow) portions of eccrine gland.

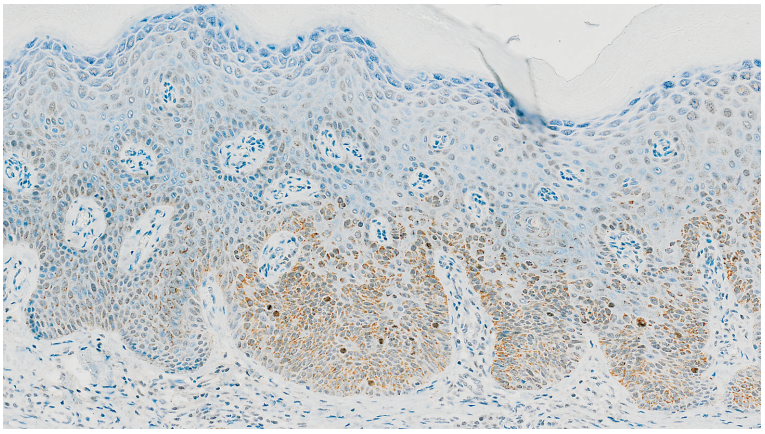

Fig. 3 Tumor cells in Bowen's disease showed upregulated expression of OVOL1 compared with adjacent normal keratinocytes.

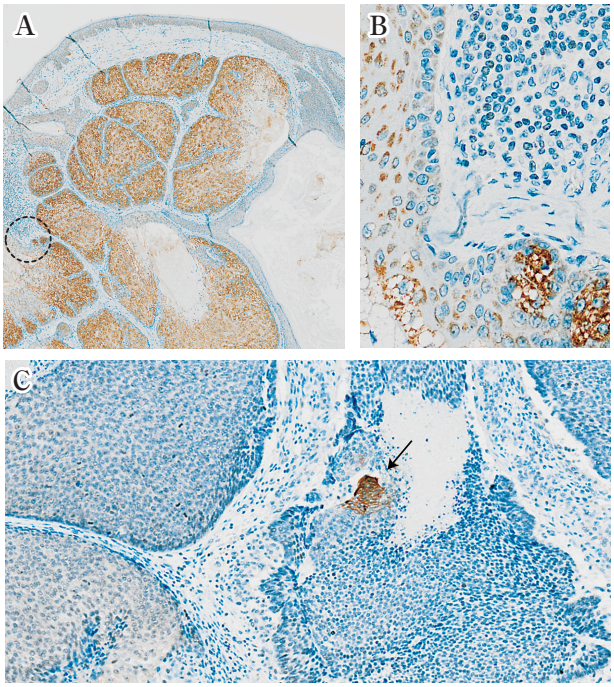

Fig. 4 A : Upregulated expression of OVOL1 in sebaceous adenoma. B : High-power view of encircled area in Fig. 4A. The expression of OVOL1 was noted in the mature sebocytes and sebaceous ductal cells as a dotted cytoplasmic and nuclear pattern. C : In pilomatricoma, the strongly positive area was scattered (arrow). This area was considered to represent differentiation into inner root sheath.

Table 1 Expression of OVOL1 in benign and malignant skin tumors

	Overall expression level compared with adjacent normal epidermis		
	Decreased	Similar	Increased
Basal cell carcinoma (n=3)	3	0	0
Bowen's disease (n=2)	0	0	2
Squamous cell carcinoma (n=2)	1	1	0
Extramammary Paget's disease (n=2)	1	1	0
Malignant melanoma (n=2)	1	1	0
Pilomatricoma (n=3)	3[A]	0	0
Trichilemmal cyst (n=2)	2	0	0
Sebaceous adenoma (n=2)	0	0	2
Sebaceous carcinoma (n=2)	2	0	0
Eccrine poroma (n=3)	0	1	2
Hidroadenoma (n=3)	0	1	2

A : very focally, probably in areas with inner root sheath differentiation

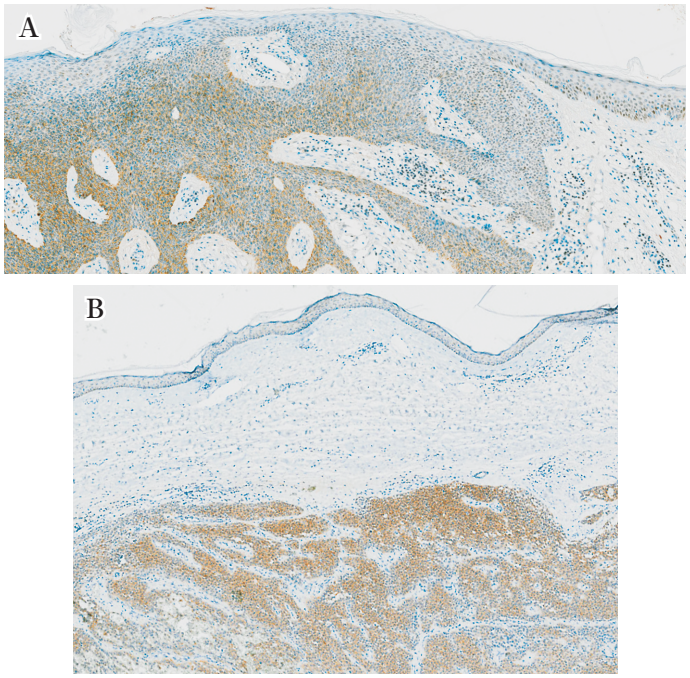

Fig. 5 Expression of OVOL1 in eccrine poroma (A) and hidradenoma (B).

3). In sharp contrast to the decreased expression of OVOL1 in sebaceous carcinoma, sebaceous adenomas exhibited high OVOL1 expression (Fig. 4A, 4B). Both trichilemmal cyst and pilomatricoma were negatively or weakly stained with OVOL1, whereas strongly positive areas were scattered in pilomatricoma, probably indicating that these areas had differentiated into the inner root sheath (Fig. 4C). The expression of OVOL1 was observed in eccrine poroma (Fig. 5A) and hidradenoma (Fig. 5B) as similar levels of normal ductal portion of eccrine glands.

Discussion

A previous study revealed that mRNAs of murine *Ovol1* are first detected at embryonic day 14.5 in the suprabasal layers of developing epidermis[2]. This is the earliest embryonic stage at which signs of epidermal differentiation are observed, with early-differentiation-specific markers appearing in suprabasal layers[5]. As development proceeds, the expression of murine Ovol1 becomes restricted to the inner root sheath[2]. These findings indicate that the expression of murine Ovol1 occurs concomitantly with the onset of terminal differentiation in the epidermis and its appendages. In keeping with these findings, *Ovol1*-deficient mice show fuzzy and ruffled hair[2]. A recent study by Shin et al. proved that *Ovol1* gene expression accelerates hair follicle neogenesis[6].

It has been shown that the c-myc proto-oncoprotein plays a pivotal role in keratinocyte proliferation[7]. Nair et al. have demonstrated that c-myc expression is upregulated in Ovol1-deficient suprabasal cells and that Ovol1 suppresses c-myc transcription by directly binding to its promoter[4]. Taking these findings together, Ovol1 accelerates epidermal differentiation by suppressing c-myc transcription. Another important line of evidence has been reported by Hirota et al., who showed that *OVOL1* is one of the genes associated with susceptibility to atopic dermatitis, although the precise role of *OVOL1* in the pathogenesis of this disease remains largely unknown[8].

In this study, we demonstrated the immunolocalization of OVOL1 in human skin. As in murine skin, the expression of OVOL1 was preferentially detected in the suprabasal keratinocytes, the inner root sheath of hair and mature sebocytes. In addition, its expression was also detected in the ductal portion of the eccrine gland. Although the epidermal expression of OVOL1 was unchanged among various inflammatory skin diseases, it was altered in skin tumors originating from the epidermis and skin appendages. As expected, its expression was observed in eccrine gland tumors (eccrine poroma and hidradenoma). Some area of the pilomatricoma lesion was strongly positive for OVOL1, which was considered to represent differentiation into the inner root sheath. The overexpression of OVOL1 in Bowen's disease and sebaceous adenoma was in sharp contrast to its downregulation in their more malignant counterparts, squamous cell carcinoma and sebaceous carcinoma. However, the present preliminary study on a small number of samples needs to be confirmed by further investigation. In conclusion, the expression of OVOL1 may play critical roles in human skin by regulating epidermal terminal differentiation and hair generation.

Conflict of Interest

The authors have no conflicts of interest to declare.

Acknowledgments

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（和文抄録）

OVOL1 はヒト皮膚の内毛根鞘，脂腺，汗腺とその腫瘍に発現している

[1)]九州大学大学院医学研究院皮膚科学分野
[2)]理化学研究所　呼吸器疾患研究チーム

三苫千景[1)]，中原剛士[1)]，内　博史[1)]，伊東孝通[1)]，稲富裕佑[1)]，井手豪俊[1)]，陣内駿一[1)]，陣内奈緒美[1)]，岩﨑菜保子[1)]，坂本佳子[1)]，木村七絵[1)]，前田彩未[1)]，隈　有希[1)]，前原恵里子[1)]，堤　碧[1)]，中原真希子[1)]，広田朝光[2)]，玉利真由美[2)]，古江増隆[1)]

OVOL1 は表皮細胞の増殖を抑制し角化を推進させる転写因子と考えられている．最近の genome-wide association study では，アトピー性皮膚炎の疾患感受性遺伝子の一つとしても注目を浴びている．マウスでは *Ovol1* は皮膚及び毛嚢に発現していることが報告されているがヒトでの研究はこれまで報告されていない．本研究では，ヒト健常皮膚および皮膚疾患で，OVOL1 の発現を免疫組織学的に明らかにした．健常皮膚では，OVOL1 は基底層上層の表皮細胞，毛嚢の内毛根鞘，成熟した脂腺細胞，エクリン汗腺の導管部に優位に発現していた．炎症性皮膚疾患の OVOL1 の発現は健常皮膚に比べ変化を認めなかった．OVOL1 の発現は eccrine poroma と hidradenoma で亢進していた．ボーエン病と脂腺腫では OVOL1 の発現は亢進していたが，有棘細胞癌や脂腺癌ではむしろ減少していた．OVOL1 はヒト皮膚の器官形成や腫瘍発生に重要な役割をになっているのではないかと考えた．