Localization of S100A2, S100A4, S100A6, S100A7, and S100P in the Human Hair Follicle

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Original Article

Localization of S100A2, S100A4, S100A6, S100A7, and S100P in the Human Hair Follicle

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Abstract

The hair follicle is a highly differentiated structure. In this study, we examined immunohistological localization of S100A2, S100A4, S100A6, S100A7, and S100P using specific monoclonal antibodies. S100A2 was strongly expressed in the entire outer-root sheath (ORS), but more weakly in cuticle and medulla in the bulb. S100A6, S100A7, and S100P were expressed in the innermost cells of ORS. The cuticular area was weakly positive for S100A2, S100A6, S100A7, and S100P. S100A4 was expressed in dendritic Langerhans cells and melanocytes. Sebaceous cells were variably immunopositive for S100A2, S100A6, and S100A7. A subset of dermal papilla cells expressed S100A4 and S100A6. None of the antibodies labeled the inner-root sheath. The distinct spatiostructural distributions of the S100 family proteins suggest that each protein is differentially involved in the physiological function of normal hair follicles.

Key words : S100 protein・$Ca2^{+}$-binding protein・Hair follicle・Immunohistochemistry

Introduction

S100 proteins are a group of small acidic proteins of 10-12 kDa[1]. Via binding with different proteins such as annexins, cytoskeletal proteins, p53, and pattern-recognition receptors, S100 proteins are involved in numerous intra-and extracellular functions including protein phosphorylation, enzyme activation, interactions with cytoskeletal components, and calcium homeostasis. With more than 25 members identified to date, the S100 proteins constitute the largest family of calcium binding proteins. These proteins share amino-acid sequence homology and similar structures : in particular, each family member binds calcium via two EF-hand calcium-binding domains[1)~4)].

The expression of S100 family proteins is regulated coordinately with differentiation. As reviewed by Eckert et al., human skin harbors at least thirteen S100 proteins, including S100A2, S100A4, S100A6, S100A7, and S100P[1]. In normal epidermis, S100A2 is compartmentalized in the basal layer, whereas S100A7 (also called psoriasin) is confined to the uppermost granular layer, and its expression is markedly up-regulated in the spinous layer of inflammatory skin diseases such as psoriasis[5)~8)]. The existence of S100A4 and S100A6 has been documented in murine and human hair ; however, the spatial localization of

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these and other S100 family proteins remains unclear. The hair follicle is a highly differentiated structure consisting of eight concentrically arranged autonomous tissue compartments of different sizes. The external compartment, the outer-root sheath (ORS), is a continuation of the epidermis through the infundibulum. The adjacent inner layers comprise the companion layer and the inner-root sheath (IRS), which itself consists of three individual layers (Henle, Huxley, and IRS cuticle). Internal to the IRS is the hair-forming compartment, encompassing the hair cuticle and cortex. The eighth compartment, the central hair medulla, is not present in all hair types[9]. The purpose of this study was to describe the spatial distribution of S100A2, S100A4, S100A6, S100A7, and S100P in the anatomical structures of human hair follicles.

Materials and methods

1. Tissue samples

Formalin-fixed and paraffin-embedded specimens from 15 cases each of squamous cell carcinoma, Bowen's disease, actinic keratosis, seborrheic keratosis, basal cell carcinoma, keratoacanthoma, extramammary Paget's disease, and normal control skin were immunostained with commercially available monoclonal antibodies against S100A2, S100A4, S100A6, S100A7, and S100P. Normal hairs were identified in the normal skin adjacent to the tumors (or anywhere in the normal control), and the distribution pattern of the aforementioned S100 family proteins was determined in the follicles. The study was approved by the institutional ethical committee of Faculty of Medical Sciences, Kyushu University.

2. Immunohistochemical analysis

All samples were fixed with 10% buffered formalin. Archival paraffin-embedded tissue blocks were cut into 4-μm-thick tissue sections. The sections were deparaffinized with xylene for 10 min and dehydrated through a graded ethanol series, followed by blocking of endogenous peroxidase activity in 0.3% H_2O_2 in methanol for 30 min. Antibody-binding epitopes were retrieved by pressure-cooking the tissue sections in 10 mmol L^{-1} sodium citrate buffer, pH 7.0 (Yatoron, Tokyo, Japan) for 10 min, and nonspecific binding was blocked with 10% goat serum. The sections were then incubated at 4℃ overnight with antibodies against S100A2 (NBP1-95671 ; 1 : 250), S100A4 (NBP1-95606 ; 1 : 500), S100A6 (H00006277-M10 ; 1 : 400), S100A7 (NBP1-39963 ; 1 : 50), or S100P (EPR6142 ; 1 : 1000) ; all antibodies were obtained from Novus Biologicals LLC (U.S.A.). Immunodetection was performed using the avidin-biotin horseradish peroxidase method with 3, 3-diaminobenzidine as the chromogen, followed by light counterstaining with hematoxylin. Washes with Tris-buffered saline or phosphate-buffered saline were performed between each step, according to the manufacturer's protocols. Appropriate positive and negative controls were included in each assay. For immunostaining of each protein, we observed 178 hairs for S100A2, 179 for S100A4, 184 for S100A6, 199 for S100A7, and 104 for S100P.

Results

In the normal epidermis, nuclear and cytoplasmic staining of S100A2 was confined to the lower epidermis, especially in the basal layer (Fig. 1A and 1B). In the hair follicle, ORS cells were strongly immunolabeled by anti-S100A2 antibody, from the hair bulb to the infundibular orifice (Fig. 1A, 1C, 1D, 1E and 1F). The hair bulb, hair matrix cells, hair medulla, and cuticles were weakly positive for S100A2 (Fig. 1C and 1D). Throughout the hair structure, IRS cells were negative for S100A2 (Fig. 1C, 1D and 1E). The companion layer located between the ORS and IRS also stained positively for S100A2, appearing as one thin continuous layer (Fig. 1D). The infundibular cells and sebaceous basal cells, as well as the intralobular spindle cells, were positive for S100A2 (Fig. 1F). Cells in the hair papilla were negative for S100A2.

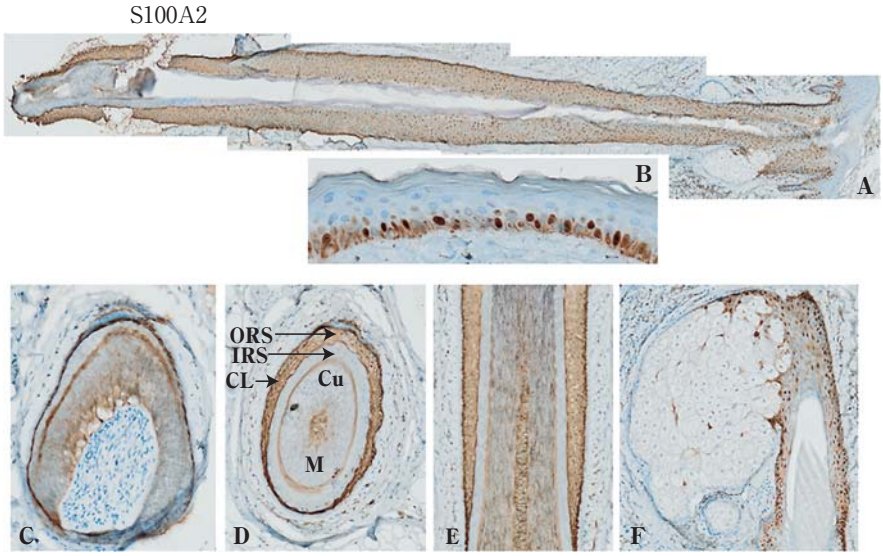

Fig. 1 S100A2 staining. A: entire hair follicle. B: normal epidermis. C: hair bulb and dermal papilla. D: lower portion of hair follicle, ORS: outer-root sheath, IRS: inner-root sheath, CL: companion layer, Cu: cuticular area, M: medulla. E: middle portion of hair follicle. F: infundibulum and sebaceous gland.

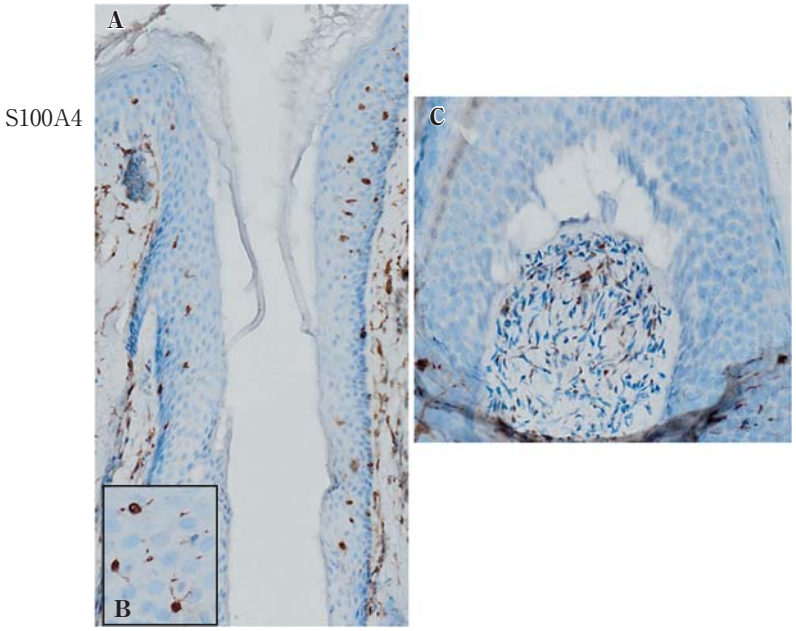

Fig. 2 S100A4 staining. A: infundibulum. B: high magnification of S100A4 + Langerhans cells. C: dermal papilla.

S100A4 staining sharply defined the dendritic shape of epidermal Langerhans cells and melanocytes, especially in the infundibulum (Fig. 2A and 2B). By contrast, no epithelial staining for S100A4 was observed throughout the entire normal epidermis and hair follicle. A subset of hair papilla cells stained positively for S100A4 in a dendritic or spindle shape (Fig. 2C).

Normal epidermis did not exhibit any S100A6 staining (Fig. 3A and 3B). In the lower hair follicle, the hair matrix cells in the hair bulb were not stained by anti-S100A6 antibody (Fig. 3C).

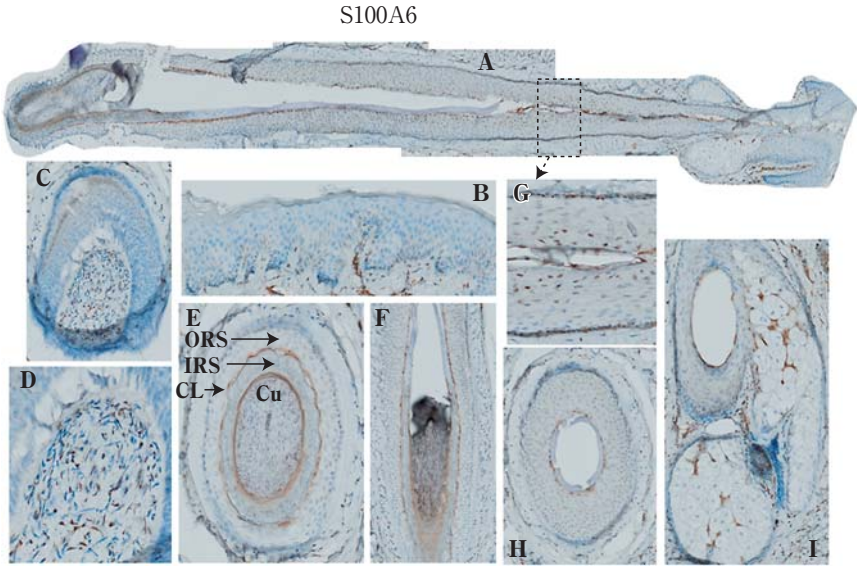

Fig. 3 S100A6 staining. A: entire hair follicle. B: normal epidermis. C: hair bulb and dermal papilla. D: dermal papilla. E: lower portion of hair follicle, ORS: outer-root sheath, IRS: inner-root sheath, CL: companion layer, Cu: cuticular area. F: middle portion of hair follicle. G: middle portion of hair follicle. H: middle portion of hair follicle. I: infundibulum and sebaceous gland.

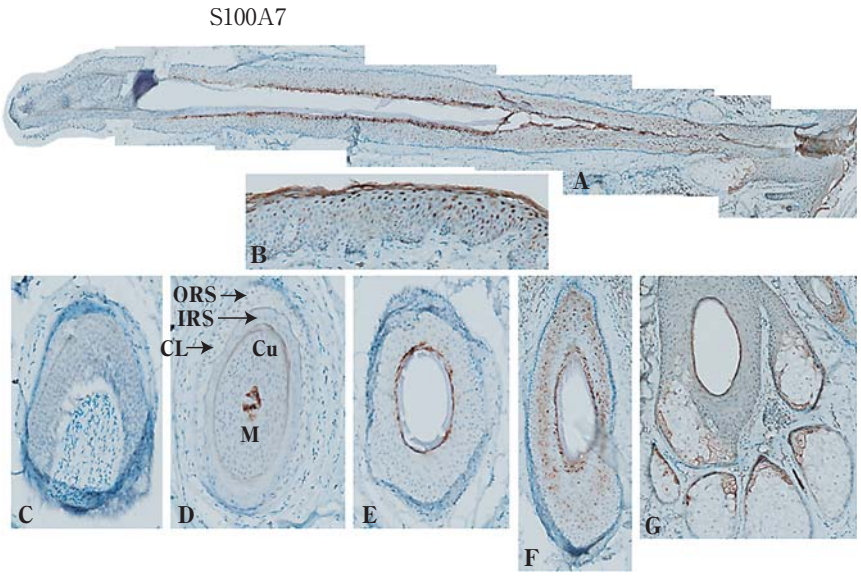

Fig. 4 S100A7 staining. A: entire hair follicle. B: normal epidermis. C: hair bulb and dermal papilla. D: lower portion of hair follicle, ORS: outer-root sheath, IRS: inner-root sheath, CL: companion layer, Cu: cuticular area, M: medulla. E: middle portion of hair follicle. F: suprainfundibular middle portion of hair follicle. G: infundibulum and sebaceous gland.

However, a subset of hair papilla cells were positive for S100A6 (Fig. 3C and 3D). The ORS and IRS cells were basically negative for S100A6. However, the innermost cells of the ORS, companion layer, as well as the cuticles, were positive for S100A6 staining (Fig. 3E, 3F, 3G and 3H). In the middle hair follicle, inner staining of the ORS widened, with moderate to weak basal-layer staining (Fig. 3G). In the upper hair follicle, basal-layer staining for S100A6 was no longer

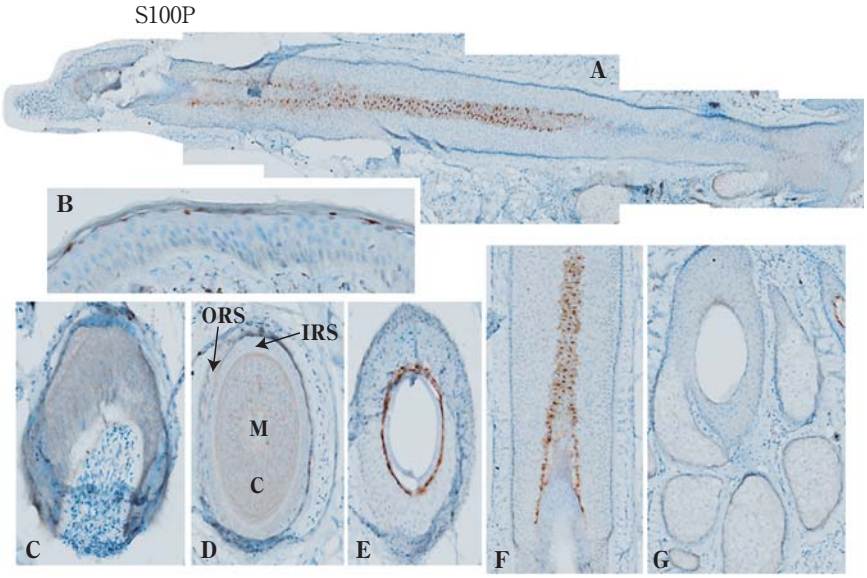

Fig. 5 S100P staining. A: entire hair follicle. B: normal epidermis. C: hair bulb and dermal papilla. D: lower portion of hair follicle, ORS: outer-root sheath, IRS: inner-root sheath, C: cortex, M: medulla. E: middle portion of hair follicle. F: middle portion of hair follicle. G: infundibulum and sebaceous gland.

observed, whereas positive staining was evident in the innermost cells of the infundibulum (Fig. 3I). Spindle or dendritic-shaped intralobular cells in the sebaceous glands were also stained positively by anti-S100A6 antibody (Fig. 3I).

In the normal epidermis, nuclear and cytoplasmic staining of S100A7 was evident in the spinous, granular, and cornified layers (Fig. 4A and 4B). In the lower hair follicle, neither the hair matrix cells nor the papilla cells in the hair bulb were stained by anti-S100A7 antibody (Fig. 4C). The hair medulla and cuticles were positive for S100A7, whereas the ORS, IRS, and companion layer were negative (Fig. 4D). In the middle hair follicle, the innermost cells of ORS exhibited strong S100A7 staining (Fig. 4E) ; this staining pattern widened, and the majority of suprabasal cells of the ORS expressed S100A7 in the subinfundibular area. This positive staining then became weaker in the suprainfundibular area, which connected to the strongly positive suprabasal epidermis (Fig. 4A and 4F). A subset of mature sebaceous cells located in the vicinity of sebaceous gland orifice were also strongly stained by anti-S100A7 antibody (Fig. 4G).

In the normal epidermis, S100P was expressed by a subset of the outermost cells of the granular layer (Fig. 5A and 5B). In the lower hair follicle, the hair matrix cells in the hair bulb were weakly stained by anti-S100P antibody, whereas the hair papilla cells did not express S100P (Fig. 5C). The ORS, IRS, companion layer, and medulla were negative for S100P, whereas cortex and cuticles weakly expressed S100P (Fig. 5D). In the middle hair follicle, intense S100P staining appeared in the innermost cells of the subinfundibular ORS (Fig. 5E and 5F), but in the upper hair follicle this staining was absent (Fig. 5A). Likewise, the infundibulum and sebaceous glands were devoid of S100P staining (Fig. 5G).

Discussion

The spatial distribution of S100 family proteins was indeed closely related to the anatomical structure of hair follicle. This study confirmed previous findings, with a few discordances, and also provided novel insights (Table 1).

Consistent with previous studies[10], diffuse

Table 1 Spatial distribution of S100 family proteins in hair follicle

Hair structure		S100A2	S100A4	S100A6	S100A7	S100P
Infundibulum			Langerhans cell Melanocyte	weak, inner-most	weak	
Sebaceous cells						
Subinfundibular and middle hair follicle	IRS					
	ORS			inner-most		inner-most
Lower hair follicle and Hair bulb	Matrix	weak		weak		weak
	Medulla	weak				weak
	Cortex			weak		
	Cuticular area	weak		weak	weak	
	IRS					
	Companion layer			weak	weak	
	ORS			inner-most	inner-most	
Dermal papilla cells						

immunostaining for S100A2 was present in the entire ORS throughout the hair structure, comparable to the sharp basal layer staining in the normal epidermis[5)10)11)]. In addition, hair matrix cells, hair medulla, and cuticles in the bulb were weakly positive for S100A2.

S100A4 was present in the dendritic Langerhans cells and melanocytes, especially in the upper infundibular area. Although the epidermal Langerhans cells and melanocytes express S100B[11)], S100A4 also seems to be a useful marker for these dendritic cells. Previous studies showed that murine dendritic cells expressed immunodetectable levels of S100A4[12)], as do human melanocytes[13)].

Kizawa et al. reported that S100A6 is expressed in the inner root sheath and inner-ORS[10)14)]. However, in this study, the innermost cells of ORS, companion layer, and cuticles exhibited S100A6 staining, whereas the IRS was not stained. We attribute this discrepancy to the differences in the antibodies used : Kizawa et al. used polyclonal antibodies, whereas we used monoclonals.

Recently, S100A7 (psoriasin) has attracted a great deal of attention in the context of psoriasis as an inducible antimicrobial defense peptide[15)]. Using frozen sections, Reithmayer et al. showed that S100A7 is not expressed in the ORS, but is expressed abundantly in the epidermis[15)]. In our paraffin-embedded sections, the innermost cells of ORS exhibited strong S100A7 staining in the subinfundibular area, but the staining weakened in the suprainfundibular area. Again, this discrepancy may be due to differences in antibodies and fixing methods.

Hair-follicle expression of S100P has not been described in previous literature. We demonstrate here for the first time that the subinfundibular innermost ORS is positive for S100P. S100P is expressed in the secretory portion of glandular structures in normal gastric and intestinal mucosa[16)], as well as in the upper surface area of stratified esophagus[16)]. In the skin, similar localization is evident in sweat glands, and occasionally in the upper granular layer of the epidermis. The results of this and previous studies suggest that S100P is intimately involved in excretory functions.

Previous studies demonstrated that S100A2 is expressed in the sebaceous glands[5)]. In this study, we found that the sebaceous cells were positive for not only S100A2, but also S100A6 and

S100A7 ; however, the distribution pattern of positive cells in the sebaceous glands was different in each case. The fundamental roles of S100A4 (also called fibroblast-specific protein 1) and S100A6 (also called calcyclin) have been investigated in fibroblasts, in particular in regard to proliferation and the cancer-associated microenvironment[17)18)]. In accordance with this notion, we observed that dermal papilla cells were positive for S100A4 and S100A6.

In conclusion, the distinct spatiostructural distributions of S100 family proteins suggest that each protein is differentially involved in the physiological function of normal hair follicles.

Acknowledgements

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（和文抄録）

ヒト毛嚢組織における S100A2，S100A4，S100A6，S100A7，S100P の局在

九州大学大学院医学研究院　皮膚科学分野

三 苫 千 景，幸 田　 太，溝 手 政 博，見 明　 彰，伊地知亜矢子，河 原 紗 穂，
河 野 美 己，園 山 浩 子，三田村康貴，加来裕美子，井 上 寛 子，佐々木誉詩子，
大 野 文 嵩，岡 部 倫 子，武　 信 肇，溝 手 美 華，増田亜希子，古 江 増 隆

ヒト毛嚢はきわめて分化した組織であるが，表皮分化関連蛋白群に属する S100A2，S100A4，S100A6，S100A7，S100P の局在に関しては十分な解析がなされていない．我々はこれらの分子に対する特異抗体を用いて，その局在を免疫組織学的に明らかにした．S100A2 は外毛根鞘（ORS）の全域に強く染色された．毛髄質は S100A2 に弱陽性であった．S100A6，S100A7，S100P は ORS の最内層に陽性であった．cuticle は，S100A2，S100A6，S100A7，S100P で弱く染色された．S100A4 はランゲルハンス細胞と色素細胞を染色した．脂腺細胞は S100A2，S100A6，S100A7 で部分的に染色されたが，その染色パターンは異なっていた．毛乳頭細胞は部分的に S100A4，S100A6 に陽性であった．内毛根鞘はどの抗体でも染色されなかった．異なる局在を示すことから，これらの S100 蛋白は毛嚢脂腺系において異なった働きを担っていると考えられた．