

The Localization of Cytokeratin 19 and Vimentin in Sprague Dawley Albino Rat Skin Tissue

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Cytokeratin 19 (CK19) expressed in epidermis of skin, bulge region of hair follicle, outermost layer of outer root sheath and proximal and distal to bulge. Vimentin is a fibrous protein that localized in cytoplasm of fibroblast and forms cytoskeleton to maintain shape of cell and nucleus. In this study, CK19 and vimentin in skin were confirmed with light, fluorescence and transmission electron microscope. As a result, CK19 was localized epidermis, hair follicles, outer root sheath and nucleus of Merkel's cell. However, vimentin was localized some epidermis, dermis, hypodermis and nucleus of Merkel's cell. The role of CK19 is self-renewal and homeostasis in skin. Also, hair follicle regeneration and hair growth is known to be related. It is supposed that required of structural proteins that make up cytoskeleton is increased. Thereby, expression of CK19 is increased. It is considered that vimentin localized in order to stabilize structure of cell and cytoskeleton of fibroblasts. Also, CK19 and vimentin present in nuclei of Merkel's cell, and to act as a fibrous protein that make up end of a nerve fiber present in Merkel's cell and paracrine function of Merkel's cell.

Key Words: Cytokeratin 19, Vimentin, Skin

INTRODUCTION

Skin is an organ that maintains the homeostasis of the body and self-renewal from injury. Self-renewal of skin is possible due to skin stem cell present throughout life. The variation in the number of skin stem cell after birth remains to be determined (Lajtha, 1979; Leblond, 1981; Lavker & Sun, 1982; Hall & Watt, 1989; Potten & Loeffler, 1990). Cytokeratin 19 (CK19) has been proposed as a marker for stem cells in the skin hair follicle and for proliferative keratinocytes in the basal layer (Lyle et al., 1998). Expression of CK19 was first thought to be restricted to the bulge region of the hair follicle, but studies have shown up-regulated expression of CK19 in the outer root sheath of the hair follicle as well as the outer root sheath proximal and distal to the bulge (Michel et al., 1996; Lyle et al., 1999; Kloepper et al., 2008; Abbas & Mahalingam, 2009).

Cytokeratins are intermediate filaments with diverse functions. Several types of cytokeratin play roles in the tissue differentiation, growth and morphogenesis of the epithelium (Kirkel et al., 2003). CK19 is mostly localized in the skin (Bartek et al., 1985; Heid et al., 1988), especially in the stratum corneum of epithelial tissue (Moll et al., 1982; Stasiak et al., 1989; Su et al., 1993). CK19 protein reported to be localized as the structural proteins in Merkel's cells in the basal layer (Fradette et al., 1995) and cells of the hair follicle (Moll, 1994; Michel et al., 1996).

Vimentin is a type-III intermediate filament normally expressed in cells of mesenchymal origin (Steinert & Roop, 1988). It maintains the shape of the cell and nucleus (Goldman & Knipe 1973; Goldman et al., 1996; Gonzales et al., 2001; Lammerding et al., 2005; Tolstonog et al., 2005), the mechanical integrity of the cytoplasm (Wang et al., 1993) and cell-substrate adhesions (Goldman et al., 1996). Vimentin is

highly abundant in the cytoplasm and nucleus (Lazarides, 1980; Herrmann et al., 2007).

Recently, CK19 protein was used to label stem cells from invasive in skin tissue (Abbas et al., 2011), squamous cell carcinoma in lung tumor cells and epidermal tissue (Nadia et al., 2010). Epithelial to mesenchymal transition is carried out in the embryonic development process. Changes vimentin during adhesion and motility of cell was studied (Mendez et al., 2010) suggesting the relation of CK19 to the stem cell of the skin and of vimentin protein specifically to fibroblast.

In this study, localization of CK19 and vimentin proteins in rat skin tissue were examined with light, fluorescence and transmission electron microscope (TEM) using immunohistochemistry and immunogold labeling method.

MATERIALS AND METHODS

Experimental Animals

Four weeks-old female rat (Sprague Dawley Albino Rat; Hallym University Experimental Animal Center, Korea) skin was used after removing hairs.

Microscopic Observation

Immunohistochemistry method

Prepared tissues were fixed with 4% paraformaldehyde in 0.1 mol/L phosphate buffered saline (PBS, pH 7.4), paraffin-embedded after dehydration, and then sectioned with microtome. For light microscopic observation, prepared samples were treated for antigen retrieval and reacted with anti-CK19 rabbit polyclonal antibody (Abcam, USA) and anti-vimentin rabbit polyclonal antibody (Abcam). Then samples were 3,3'-diaminobenzidine (DAB) stained with DAKO Envision kit (DAKO, USA) and counterstained with Mayer's Hematoxylin (DAKO). Prepared samples were observed with a light microscope (Axio Imager A1; Carl Zeiss, Germany).

For fluorescence microscopic observation, prepared samples were treated for antigen retrieval and reacted with anti-CK19 rabbit polyclonal antibody (Abcam) and anti-vimentin rabbit polyclonal antibody (Abcam). Then samples were reacted with Alexa Fluor 488 goat anti-rabbit immunoglobulin G (IgG) (H+L) (Invitrogen, USA) and counterstained with 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI). Prepared samples were observed with fluorescence microscope (DM2000; Leica, Germany).

Immunogold labeling method

For transmission electron microscopic observation, prepared tissues were fixed with 1% paraformaldehyde-1% glutaraldehyde in 0.1 mol/L PBS (pH 7.4) and 2% osmium tetroxide, then embedded with Lowicryl HM 20 (Polysciences Inc., USA).

Prepared samples were sectioned with ultramicrotome and reacted with anti-CK19 rabbit polyclonal antibody (Abcam) and anti-vimentin rabbit polyclonal antibody (Abcam). Then samples were reacted with protein A gold complex (15 nm) which mixed 1% HAuCl₄ (Sigma, USA), 1% tri-sodium citrate (Sigma), 1% tannic acid (EMS, USA), 25 mmol/L potassium carbonate (Sigma) and heated, then added to protein A (Sigma) after centrifugation. Prepared samples double-stained with uranyl acetate and lead citrate and were observed with TEM (EM109; Carl Zeiss, Germany).

RESULTS

Immunohistochemistry

CK19 was localized on the hair follicle and epidermis (Fig. 1A). In epidermis, CK19 was localized basal layer and around nucleus of the basal layer. Also, CK19 was observed around nucleus of the hair follicle and outer root sheath (Fig. 1B). However, CK19 specific reaction was not observed on dermis and hypodermis (Fig. 1B and C). Vimentin was observed on dermis, hypodermis and adipose tissue of subcutaneous layer (Fig. 1D). In dermis, vimentin was not localized on specific region, and it was observed on whole dermis irregularly. On the other hand, vimentin specific staining at epidermis and hair follicle was not observed (Fig. 1E). Also, vimentin was localized irregularly on around blood vessel in hypodermis (Fig. 1F).

The distribution of CK19 and vimentin were observed with fluorescence microscope. CK19 was localized on whole epidermis and hair follicle of skin tissue (Fig. 2A). And CK19 of hair follicle was localized on nucleus around the hair follicle and outer root sheath (Fig. 2B). However, CK19 was not observed on dermis and hypodermis (Fig. 2C). Vimentin was observed on a part of epidermis, dermis, hypodermis and adipose tissue of subcutaneous layer in skin (Fig. 2D). In dermis, vimentin was irregularly localized on the dermis, and the reaction did not appear on hair follicle (Fig. 2E). Vimentin was localized irregularly on the hypodermis, especially around blood vessels (Fig. 2F).

Immunogold Labeling

CK19 localized on the epidermis of skin tissue with CK19 antibody, but CK19 was not observed on the keratin (Fig. 3A). Also, CK19 was labelled on outer root sheath of hair follicle (Fig. 3B).

Vimentin was not observed on the epidermis, keratin and outer root sheath of hair follicle (Fig. 3C and D). In addition, the granules of secretory cells in the dermis were not labelled with CK19 (Fig. 4A); however, vimentin was distributed on the granules of secretory cells (Fig. 4C). CK19 and vimentin were labelled on the nucleus of Merkel's cell that present in the dermis adjacent to the basal layer of the epidermis (Fig. 4B and D).

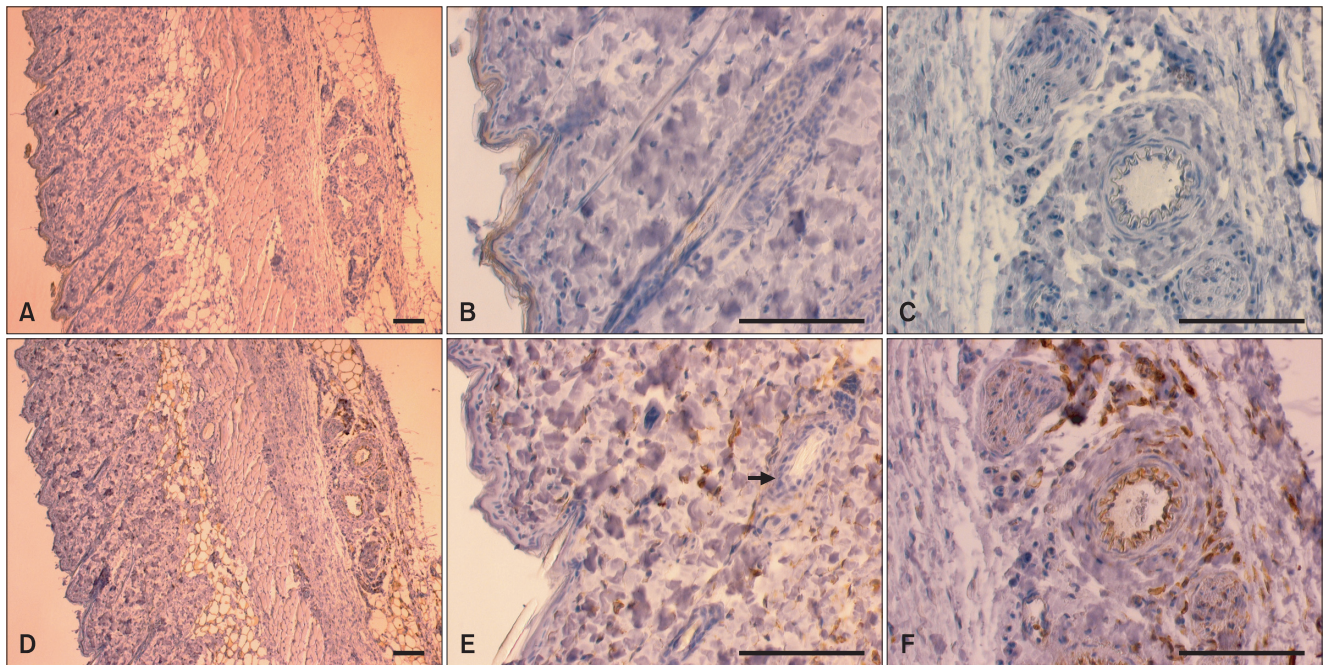


Fig. 1. (A) Light micrograph of the Sprague Dawley (SD) albino rat skin tissue which was reacted with cytokeratin 19 (CK19) (stained as brown color). (B) CK19 stained on epidermis, nucleus around the hair follicles. (C) On the other hand, the hypodermis was not stained. (D) SD rat skin tissue reacted with vimentin (stained as brown color). (E) Vimentin Irregularly stained on dermis but the hair follicle (arrow) was not stained. (F) It is also the hypodermis and around the blood vessels were stained. Scale bars=100 µm. H, hair follicles.

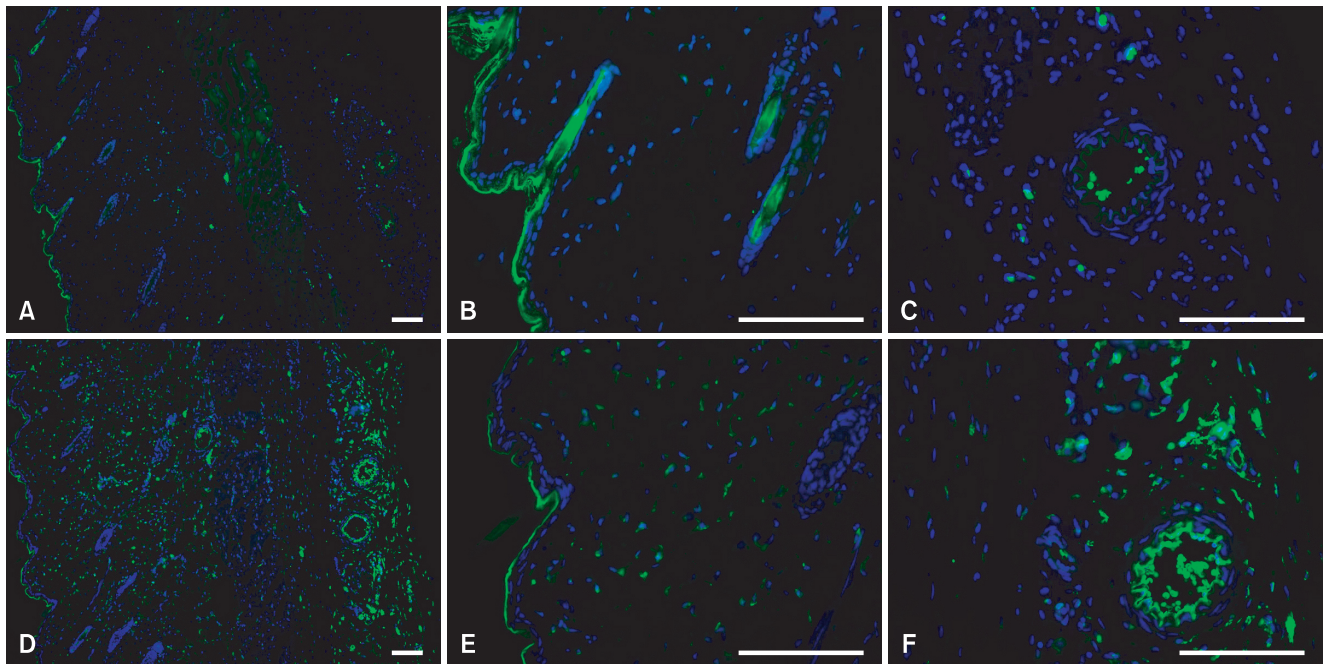


Fig. 2. (A) Fluorescence micrograph of the Sprague Dawley (SD) albino rat skin tissue which was labelled with cytokeratin 19 (CK19) (green color). CK19 labelled on whole epidermis, the hair follicles and outer root sheath (B), but which was not label on dermis and hypodermis (C). (D) SD rat skin tissue reacted with vimentin (green color). (E) Vimentin labelled on part of the epidermis and dermis otherwise was not label on the hair follicle. Also, it labelled hypodermis and around blood vessels (F). Scale bars=100 µm.

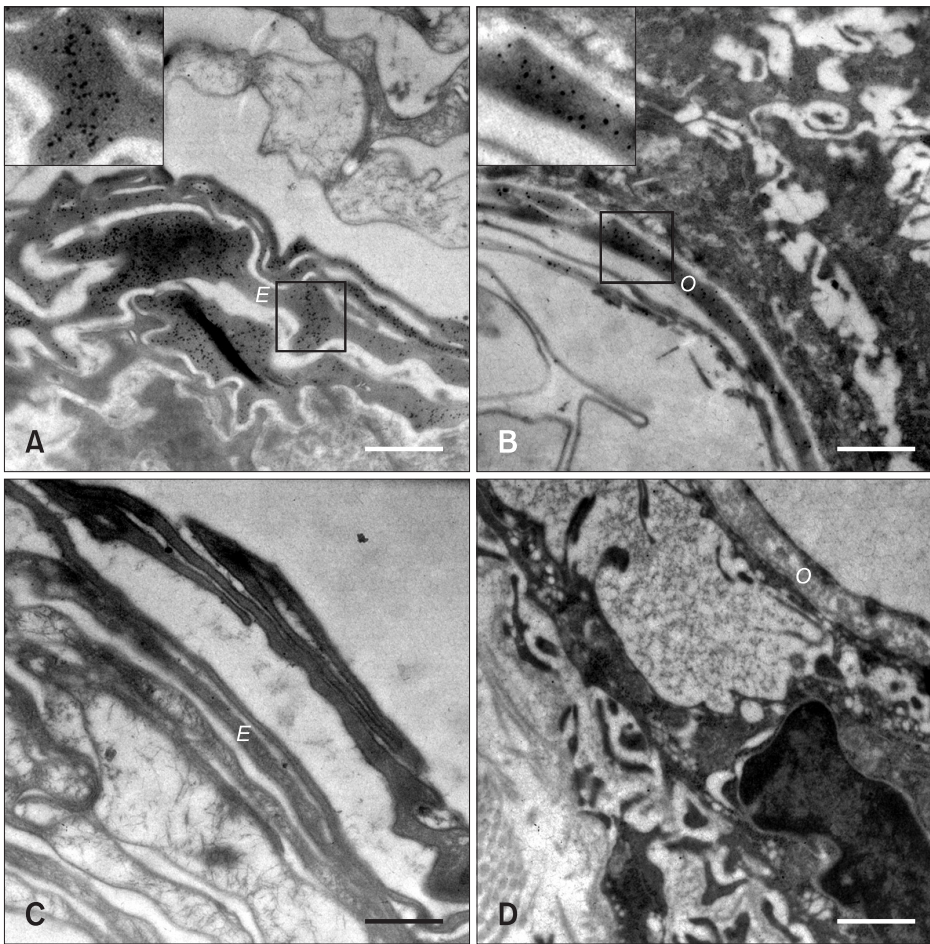


Fig. 3. (A) Transmission electron micrograph of the Sprague Dawley albino rat skin tissue. Gold particle was labelled with cytokeratin 19 which was localization on the epidermis, labelled only on the epidermis (enlarged inset) and (B) the outer root sheath of hair follicle (enlarged inset). Otherwise, gold particle was not labelled with vimentin on epidermis (C), and the hair follicles (D). Scale bars=1 μ m. E, epidermis; O, outer root sheath.

DISCUSSION

CK19 has been reported present as structural protein which was distributed in the basal layer of the epidermis, Merkel's cell of dermis and tissue cells of sensory cells and hair follicles (Moll, 1994; Fradette et al., 1995; Michel et al., 1996). In addition, Abbas & Mahalingam (2009), Kloepper et al. (2008) and Michel et al. (1996) were used as the marker of skin stem cells present in the skin to CK19 protein. In these studies, undifferentiated cells and skin stem cells that are involved in repair and self-renewal of the skin, and highly expressed $\alpha 3 \beta 1$ integrin and CK19 protein. Therefore, CK19 was confirmed on epidermis, hair follicle and outer root sheath of hair follicle of skin tissue. And this distribution consider with fibrous protein constitute the skin stem cell.

CK19 was usually used as a proliferation marker for stem cells on the hair follicles and keratinocytes on the basal layer in the skin (Lyle et al., 1998). And it was localized around nucleus of hair follicle which has important function of the growth, differentiation and reproduction. Thus, CK19 of the hair follicle consider in fibrous protein related to hair follicle stem cell.

Vimentin is a type-III intermediate filament, and it is normally expressed in mesenchymal origin cell (Steinert & Roop, 1988). Also, vimentin is involved in assembly and structure of fibroblast (Mucke et al., 2004). It is known as a protein that forms the cytoskeleton to maintain the shape of the cell and nucleus (Goldman & Knipe, 1973; Goldman et al., 1996; Gonzales et al., 2001; Lammerding et al., 2005; Tolstonog et al., 2005). Furthermore, vimentin is intermediate filament protein that maintains the mechanical integrity of the cytoplasm (Wang et al., 1993) and cell-substrate adhesions (Goldman et al., 1996). It was reported that vimentin is highly abundant proteins in the cytoplasm and nucleus (Lazarides, 1980; Herrmann et al., 2007).

In this study, vimentin localized irregularly on dermis and some of epidermis with fluorescence Microscope observation, but vimentin was distributed lower less than CK19 on epidermis. In addition, it was also present in around of subcutaneous adipose tissue of the dermis and hypodermis. Also, it is mainly localized on the nucleus of Merkel's cell. Vimentin is a protein that is distributed abundantly in fibroblasts, and fibroblast constitutes the connective tissue of dermis. Therefore, it is supposed that vimentin plays a

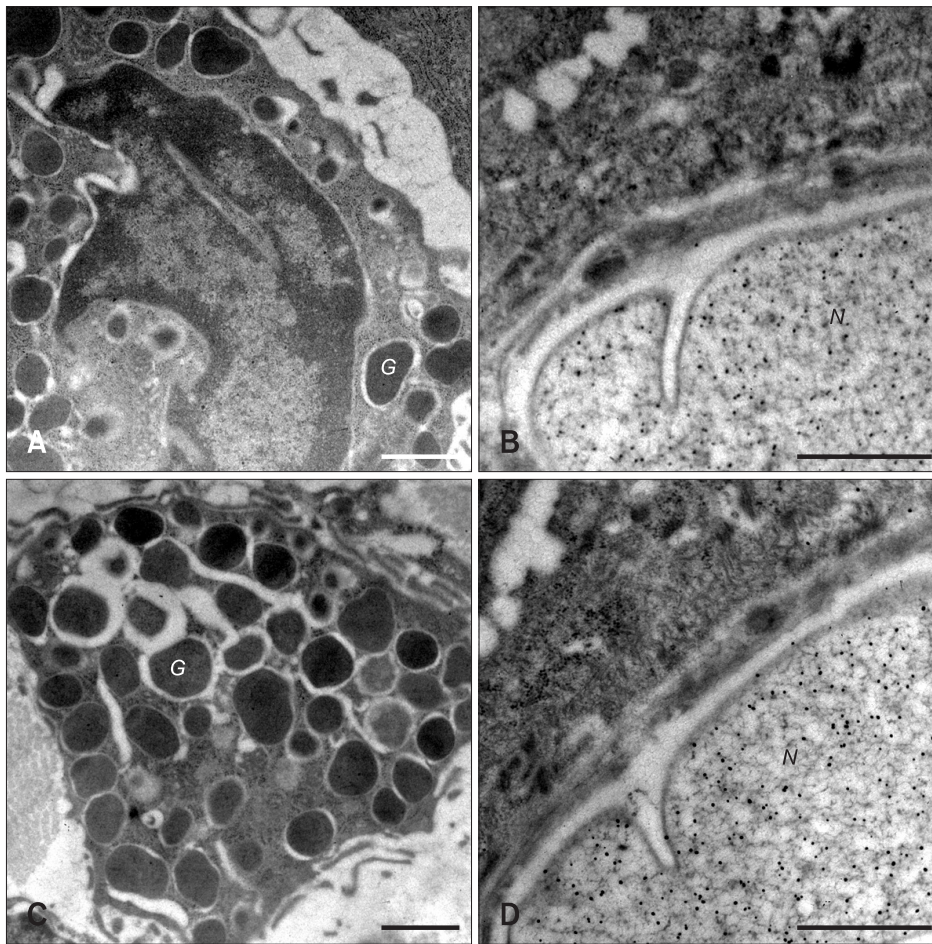


Fig. 4. (A) Transmission electron micrograph of the Sprague Dawley albino rat skin tissue. Gold particle was not labelled with cytokeratin 19 on the granules of secretory cells and (B) labelled on nucleus of Merkel's cells. Gold particle was labelled with vimentin on the granules of secretory cells (C), and nucleus of Merkel's cells (D). Scale bars=1 μ m. G, granule; N, nucleus of Merkel's cell.

function to form the cytoskeleton of fibroblast, and vimentin is distributed irregularly in dermis and hypodermis.

Merkel's cell is transformed keratinocyte, and Merkel's cell has a paracrine function of adjusting the activity of epidermal cell. CK19 and vimentin localized on nucleus of Merkel's cell, and they are fibrous protein that concerned in the secretion function of the Merkel's cells as a protein to perform the secretory function. Thus, it is considered to constitute the end of a nerve fiber present in the Merkel's cell. However, vimentin was distributed on granules of some secretory cell.

Homeostasis and self-renewal of skin occur by generation, differentiation and desquamation process of epidermal cell, and these processes is regulated by skin stem cell. Therefore, when homeostasis and self-renewal occur, demand for structural and fiber protein that form the cytoskeleton of skin stem cell is increases. Thereby, it is considered that CK19 was localized on whole epidermis. Also, CK19 of hair follicle assumed that with structural protein of hair follicle stem cell related to growth and regeneration of hair and paracrine function of Merkel's cell.

Vimentin was localized on the some epidermis, dermis, hypodermis, secretory cell and Merkel's cell as a protein that

is abundant in cytoplasm of fibroblast. Also, vimentin is a protein that is involved in structure and assembly of fibrous protein.

Dermis and hypodermis is composed of connective tissue and connective tissue is formed by fibroblast. Thus, it is assumed to localize in order to the structural stabilization and cytoskeleton of fibroblast that is present in the connective tissue which constituted the dermis and hypodermis.

CONCLUSIONS

CK19, a fibrous protein localized on epidermis and hair follicles, may be used as a marker for skin and hair follicle stem cell involved in the regeneration and restoration of skin and growth, regeneration and differentiation of hair. Vimentin involved in the structure and assembly of fibrous protein in the cytoplasm of fibroblasts was irregularly localized on dermis and hypodermis. CK19 and vimentin were localized on the nucleus of Merkel's cell. It is the fibrous protein involved in the formation of nerve fiber endings, and paracrine secretory function of the Merkel's cell.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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