

Expression of Drebrin, an actin binding protein, in basal cell carcinoma, trichoblastoma and trichoepithelioma

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Summary. Drebrin, an F-actin binding protein, is known to play important roles in cell migration, synaptogenesis and neural plasticity. Although drebrin was long thought to be specific for neuronal cells, its expression has recently been reported in non-neuronal cells. As for skin-derived cells, drebrin was shown to be enriched at adhering junctions (AJs) in cultured primary keratinocytes and also be highly expressed in basal cell carcinoma (BCC) cells. Since BCC and two types of benign neoplasm, trichoblastoma and trichoepithelioma, are considered to derive from the same origin, follicular germinative cells, it is sometimes difficult to morphologically distinguish BCC from trichoblastoma and trichoepithelioma. In this study, we performed immunohistochemical staining of drebrin in BCC, trichoblastoma and trichoepithelioma, to examine whether drebrin could serve as a biomarker for BCC diagnosis. In western blotting, drebrin was detected highly and moderately in the lysates from a squamous cell carcinoma cell line, DJM-1, and normal human epidermis, respectively. In immunofluorescence analyses, drebrin was colocalized with markers of AJs and tight junctions in DJM-1 cells and detected at cell-cell junction areas of human normal epidermis tissue. We then examined the distribution patterns of drebrin in BCC, trichoblastoma and trichoepithelioma. In BCC

tissues, intense and homogeneous drebrin expression was observed mainly at tumor cell-cell boundaries. In contrast, drebrin was stained only weakly and non-homogeneously in trichoblastoma and trichoepithelioma tissue samples. For differential diagnosis of BCC, drebrin may be a novel and useful marker.

Key words: Drebrin, Basal cell carcinoma, Trichoblastoma, Trichoepithelioma

Introduction

Drebrin is an F-actin binding protein originally isolated from brains of chick embryos (Shirao and Obata, 1985). There are 4 splicing isoforms (E1, E2, A and sA) which have been reported to play important roles in neural cell migration, synaptogenesis and neural plasticity (Kojima et al., 1993; Shirao, 1995).

Although drebrin was originally thought to be specific for neuronal cells, it has also been detected in non-neuronal cells (Peitsch et al., 1999, 2001; Keon et al., 2000). Drebrin has been detected in mesangial cells and podocytes of renal glomeruli, where large complexes contain drebrin and actin, suggesting that this protein is involved in the regulation of actin dynamics (Peitsch et al., 2003). Drebrin has been detected in gastric parietal cells (Keon et al., 2000) and plays a role in the regulation of parietal cell maturation and function (Chew et al., 2005). The protein is also expressed in T

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Abbreviations. BCC, basal cell carcinoma; SCC, squamous cell carcinoma; AJ, adherens junction; TJ, tight junction

cells where it interacts with the cytoplasmic tail of chemokine receptor CXCR4 (C-X-C chemokine receptor type 4, also known as fusin or CD184, cluster of differentiation 184) in the immune synapse, functions as a regulator for actin polymerization and is required for IL-2 production (Pérez-Martínez et al., 2010). In addition, it is possible that drebrin is involved in tumorigenesis, since the protein level was significantly increased in acute lymphoblastic leukemia, although a relationship between the expression level and the pathophysiology remains to be elucidated (Vascova et al., 2011).

In the dermatology field, Peitsch et al. showed drebrin expression in normal human skin, epithelial skin cancer tissues and cultured keratinocytes. While drebrin was enriched at adherens junctions (AJs) in cultured primary keratinocytes, it was abundant in basal cell carcinoma (BCC) compared to squamous cell carcinoma (SCC) and epidermal precancer (Peitsch et al., 2005). Histologically, BCC tends to share the common features of a predominant basal cell type, peripheral palisading of lesional cell nuclei and specialized stroma. There are also variable degrees of cytologic atypia and mitotic activity. It is notable that BCC shares a common origin, follicular germinative cells, with 2 types of benign neoplasm, trichoblastoma and trichoepithelioma. Trichoblastoma is comprised of islands of basaloid cells occupying the dermis with occasional extension into the subcutaneous fat. These basaloid islands exhibit peripheral palisading and fibrocellular stroma similar to that surrounding follicles, and mitotic activity can be observed. On the other hand, trichoepithelioma is comprised of tumor islands containing of basophilic cells with the same appearance as epidermal or skin appendage basal cells, which are typically arranged in a lacelike or adenoid network but occasionally form solid aggregates. Horn cysts are also common in trichoepithelioma, although they may be absent in some lesions (Elder, 2008).

Because of the histological similarity among BCC, trichoblastoma and trichoepithelioma, it is frequently difficult to distinguish BCC from trichoblastoma and trichoepithelioma in small biopsy specimens, particularly nodular BCC and nodular trichoblastoma (Ackerman et al., 1993; Elder, 2008). In this study, we produced and characterized a specific antibody against drebrin, performed immunohistochemical staining analyses of BCC, trichoblastoma and trichoepithelioma together with some other skin tumors, and found that drebrin could serve as a useful marker for BCC differential diagnosis.

Materials and methods

Antibodies

Using a drebrin E1 fragment (aa472-707) expressed in *E. coli* as an antigen, a rabbit polyclonal antibody

specific for drebrin was generated and affinity-purified. Characterization of the antibody is described elsewhere. Monoclonal anti-occludin (Chemicon, Temecula, CA, USA), anti-E-cadherin (BD Biosciences, Franklin Lakes, NJ, USA), and anti- β -catenin (BD Biosciences) were also used.

Western blotting

Concentrations of protein were estimated with a micro bicinchoninic acid (BCA) protein assay reagent kit (Thermo Scientific, Rockford, IL, USA). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10% gel) and western blot analyses were performed and immunoreactive bands were visualized as described (Nagata et al., 2009).

Cell culture and tissue specimens

Human squamous cell carcinoma cell line DJM1 cells, which we first isolated and established as a model for transformed keratinocytes, were grown as described (Kitajima et al., 1988). Typical cell-cell contact formation was observed in DJM-1 cells in protein kinase C- and calcium-dependent manners (Kitajima et al., 1992). Rat insulinoma Ins-1 and mouse neuroblastoma Neuro2A cells were cultured in 10% fetal bovine serum (FBS)/5mM D-glucose/RPMI-1640 and 10% fetal calf serum/RPMI-1640, respectively (Hanai et al., 2004). Human colorectal adenocarcinoma Caco2, canine kidney epithelial cell MDCK, human cervix cancer HeLa and human alveolar adenocarcinoma A549 were cultured in 10% FBS/Dulbecco's Modified Eagle Medium (DMEM) (Hanai et al., 2004; Nagata et al., 2004). All lines were maintained at 37°C and 5% CO₂ in a humidified atmosphere. Tissue samples were obtained during surgical resection from unrelated Japanese patients who were treated at Gifu University Hospital. Tumors and patient-matched normal epithelium were obtained intraoperatively after the patients provided informed consent for a protocol approved by the institutional review board of Gifu University. Diagnosis was made by 3 dermatologists.

Immunofluorescence

Immunofluorescence analysis was conducted as described (Nagata et al., 2004). Briefly, sections were embedded in Tissue Tek OCT compound (Miles, Elkhart, IN, USA), frozen in liquid nitrogen and stored at -80°C, and then sectioned at 4 μ m thickness, using a cryostat (Leica CM1850), air-dried and fixed with 3.7% formaldehyde. Alexa Fluor 488- or 568-labeled IgG (Molecular Probes, Eugene, OR, USA) was used as a secondary antibody. Rhodamine-phalloidin was from Cytoskeleton Inc. (Denver, CO, USA). Fluorescent images were obtained using FV-1000 confocal laser scanning microscope (Olympus, Tokyo, Japan).

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Immunohistochemistry

Immunohistochemical staining was performed on 4- μ m sections of paraffin-embedded specimens with the use of rabbit polyclonal anti-drebrin antibody. Briefly, after deparaffinization and hydration, slides were treated with methanol and H₂O₂ for 10 min. Sections were blocked with Super Block (ScyTek Laboratories, Logan, Utah, USA) for 20 min before reacting with anti-drebrin antibody (1:100 dilution) at room temperature for 1 h. Then specimens were then washed 3 times in phosphate buffered saline and treated with Envision reagent (DAKO, Copenhagen, Denmark). Finally, slides were visualized by horseradish peroxidase/diaminobenzidine (HRP/DAB) or hematoxylin eosin (H&E) staining and mounted. As a negative control, duplicate sections were immunostained without exposure to the primary antibody.

Evaluation of immunohistochemistry and statistics

To evaluate drebrin expression, immunohistochemistry score was used (Tanaka et al., 2003; Shimada et al., 2005; Kato et al., 2007). The mean percentage of positive tumor cells was determined in at least 5 random fields at magnification x400 in each section. The intensity of drebrin-immunoreaction was scored as

follows: 1+, weak; 2+, moderate; 3+, intense. Drebrin-immunohistochemistry scores were calculated as follows; the score = (% of positive tumor cells) x (the staining intensity). Statistical significance was evaluated by the Mann-Whitney U test.

Results

Antibody characterization and western blot analyses of drebrin

To study the pathophysiological significance of drebrin in skin tumors, we first generated a specific antibody. After characterization of the antibody, we profiled drebrin protein expression in rat tissues by western blotting (Fig. 1A). While drebrin with a molecular mass of ~120 kDa was expressed strongly in both soluble and membrane fractions of cerebrum, ~95 kDa protein band was detected in the soluble fraction of cerebellum, heart, kidney adrenal gland, small intestine and long extensor muscle (EDL) (Fig. 1A, left). It is possible that 2 isoforms are expressed in rat tissues although the possibility of protein degradation during sample preparation cannot be ruled out. In some tissues such as lung, liver, kidney and small intestine, protein bands with ~47 kDa, ~42 kDa and ~38 kDa were detected. Protein identities of these bands are not known

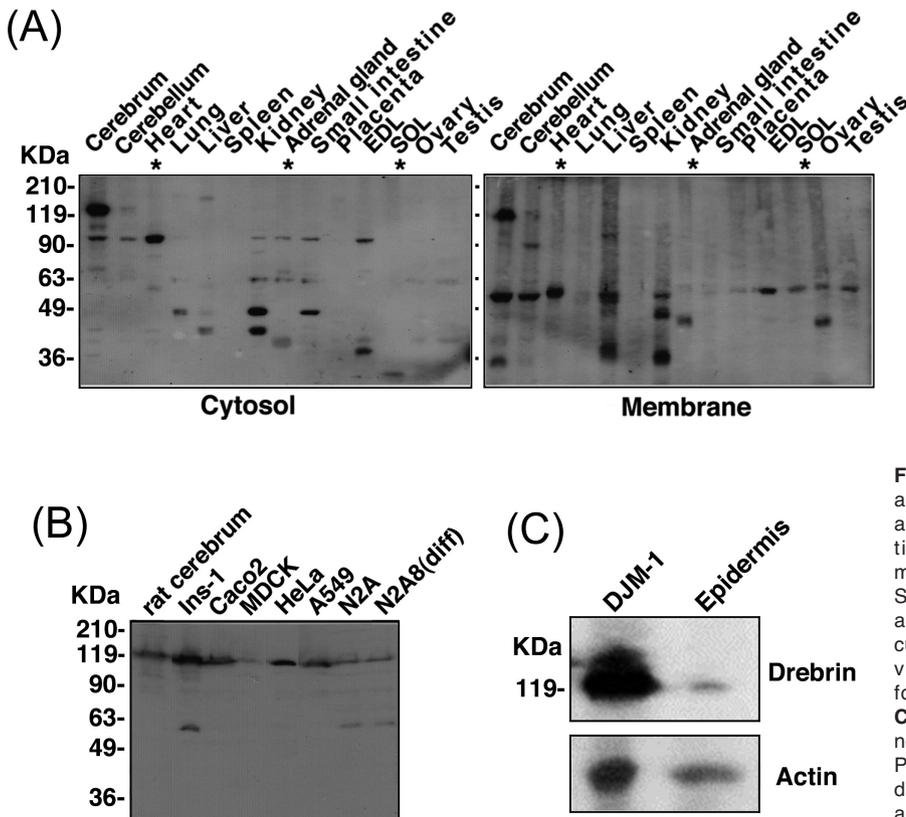


Fig. 1. Characterization of anti-drebrin antibody and detection of drebrin in normal human epidermis and DJM-1 cells. **A.** Distribution of drebrin in rat tissues. Cytosolic (20 μ g of proteins) and membrane (30 μ g) fractions were separated by SDS-PAGE and subjected to western blotting using anti-drebrin. **B.** Detection of endogenous drebrin in cultured cell lines. Lysates (30 μ g of proteins) of various lines were subjected to SDS-PAGE followed by western blotting with anti-drebrin. **C.** Lysates (30 μ g proteins) of DJM-1 cells and normal human epidermis were subjected to SDS-PAGE followed by western blotting using anti-drebrin. The blot was re-probed with anti-actin antibody.

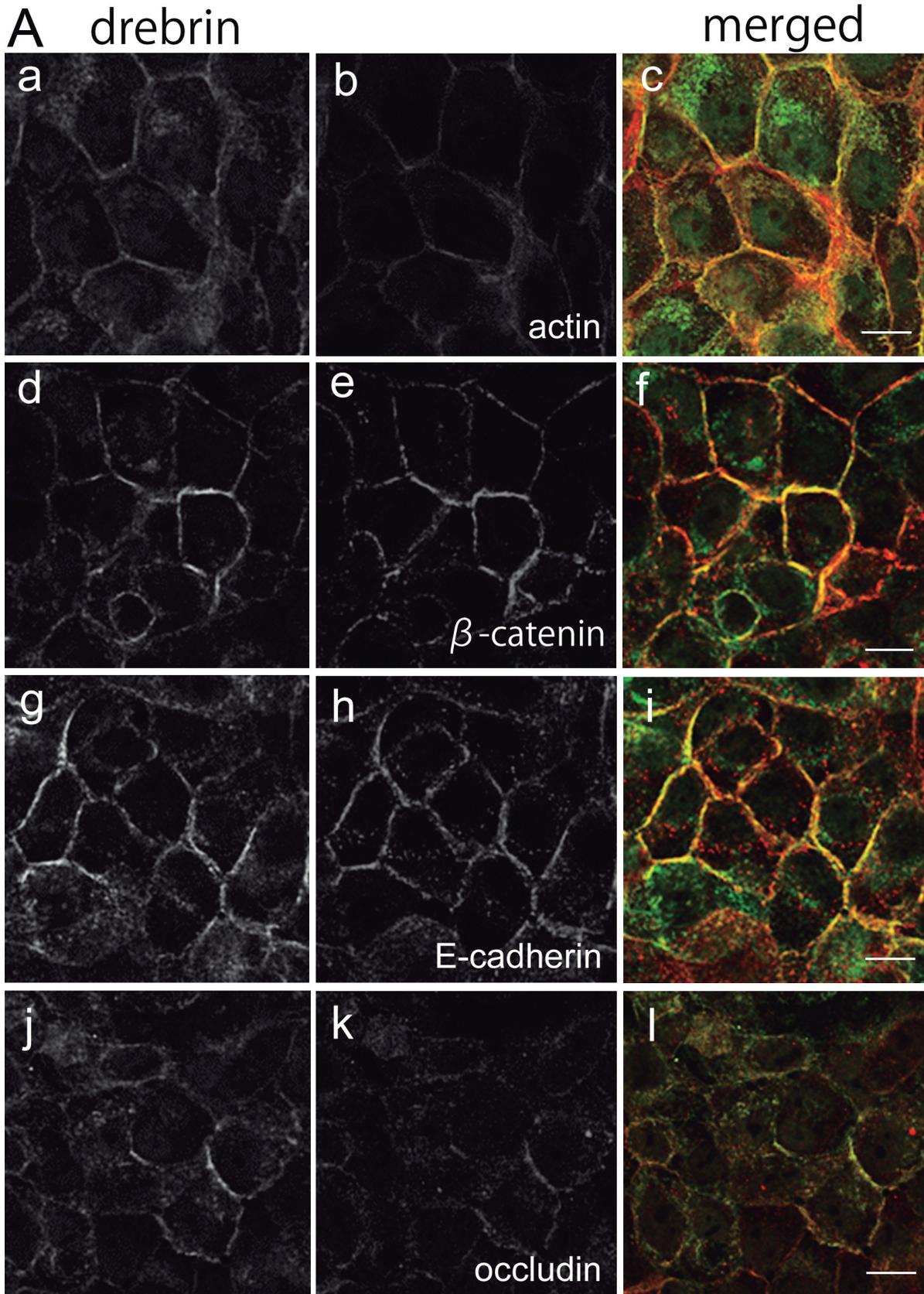
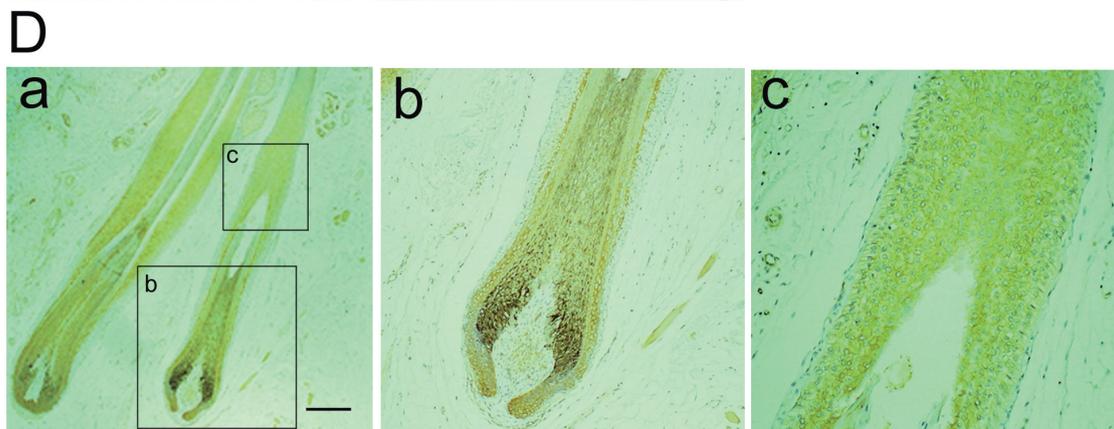
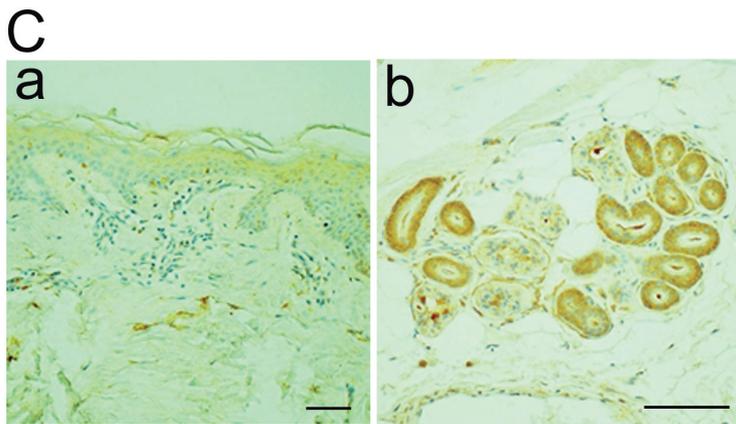
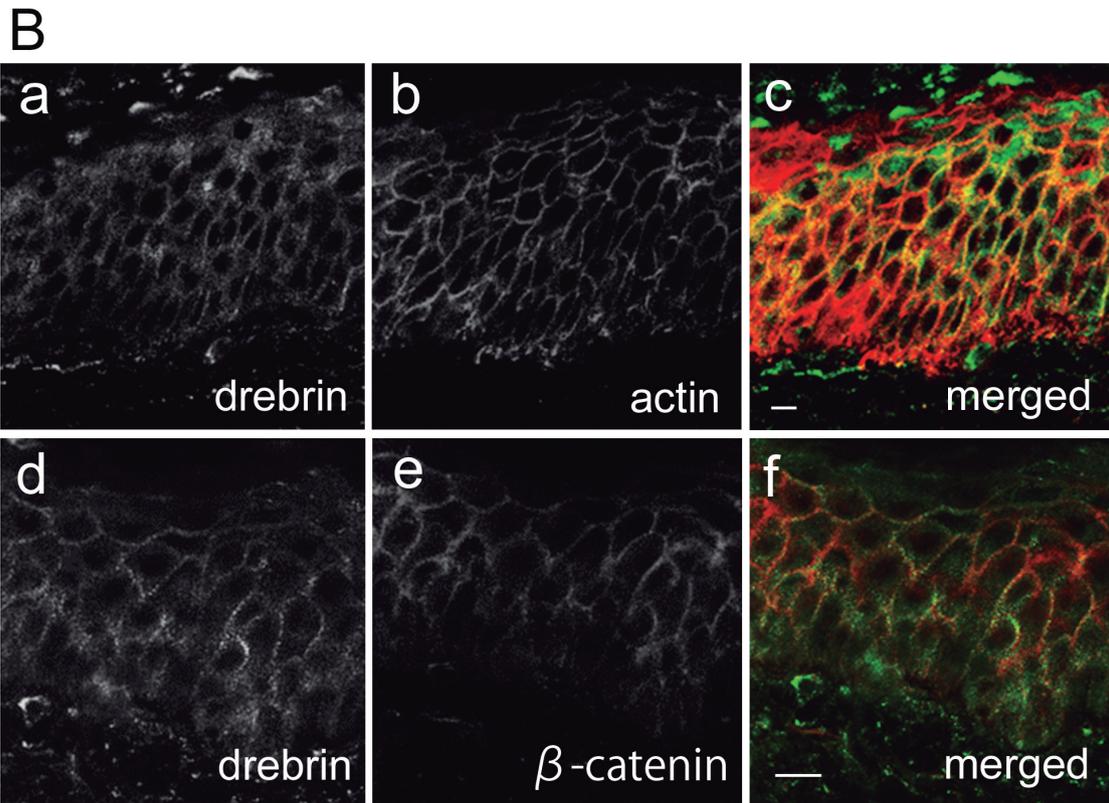


Fig. 2. Localization of drebrin in DJM-1 cells and normal human epidermis. **A.** DJM-1 cells were double-stained using anti-drebrin with rhodamin-phalloidin (**a-c**), anti- β -catenin (**d-f**), anti-E-cadherin (**g-i**) or anti-occludin (**j-l**). Merged images were also shown (**c, f, i and l**). Bar: 10 μ m. **B.** Sections of normal human epidermis were double-stained for drebrin (**a and d**) together with F-actin (**b**) or β -catenin (**e**). Merged images were also shown (**c and f**). Bar: 10 μ m. **C.** Drebrin was stained with HRP/DAB in sections of normal epidermis (**a**) and eccrine glands (**b**). Bar: 100 μ m. **D.** Drebrin was stained with HRP/DAB in sections of hair follicles. Indicated areas in (**a**) were magnified in (**b**) and (**c**). Bar: 100 μ m.



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but they may be degradation products. As for the membrane fractions, a protein band with an appropriate molecular mass (~120 kDa) was only detected in cerebrum (Fig. 1A, right). Proteins with 36~55 kDa in

various tissues including cerebrum may be degradation products (Fig. 1A).

We next conducted western blot analyses to examine drebrin expression in lysates of various mammalian

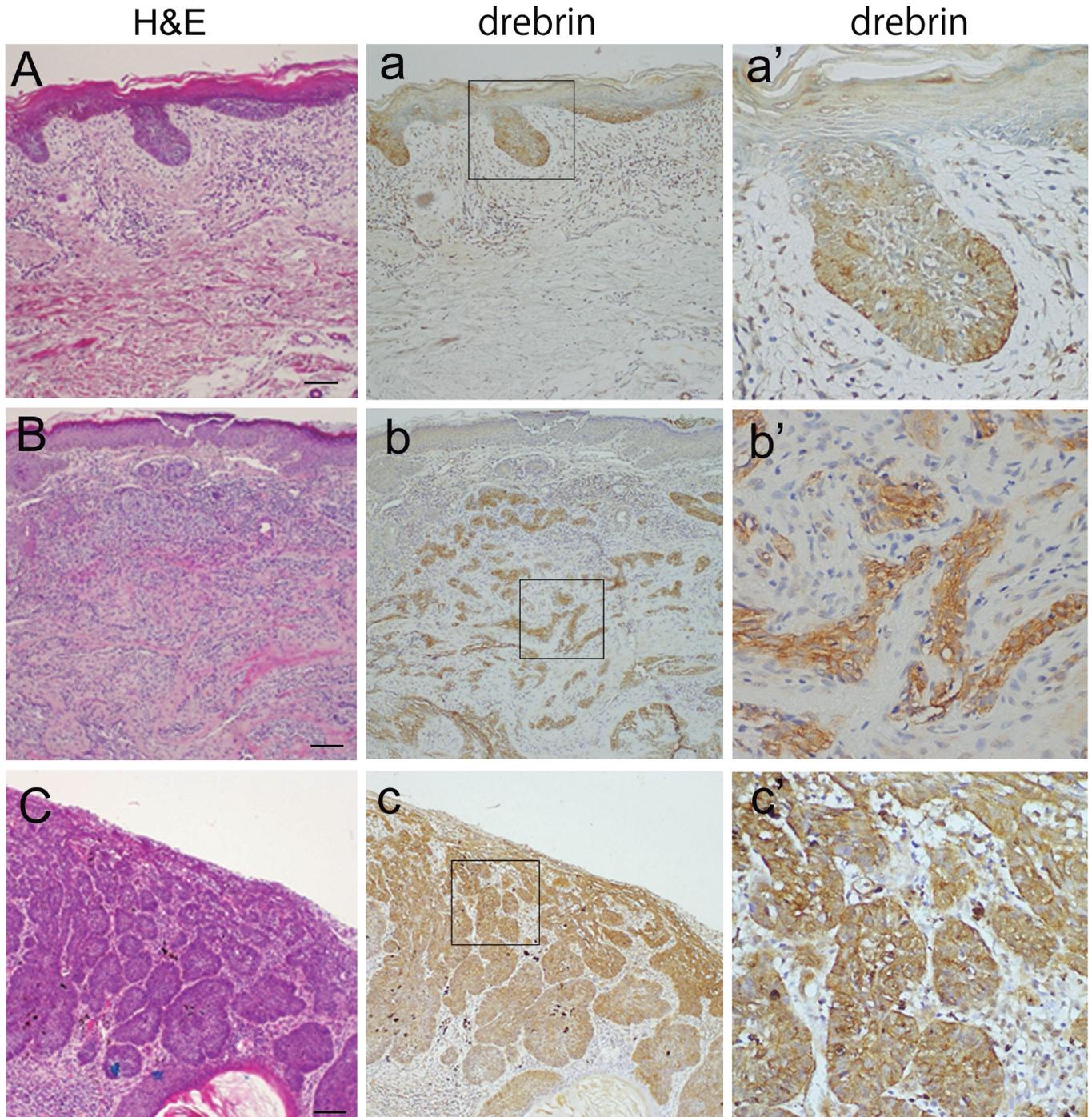
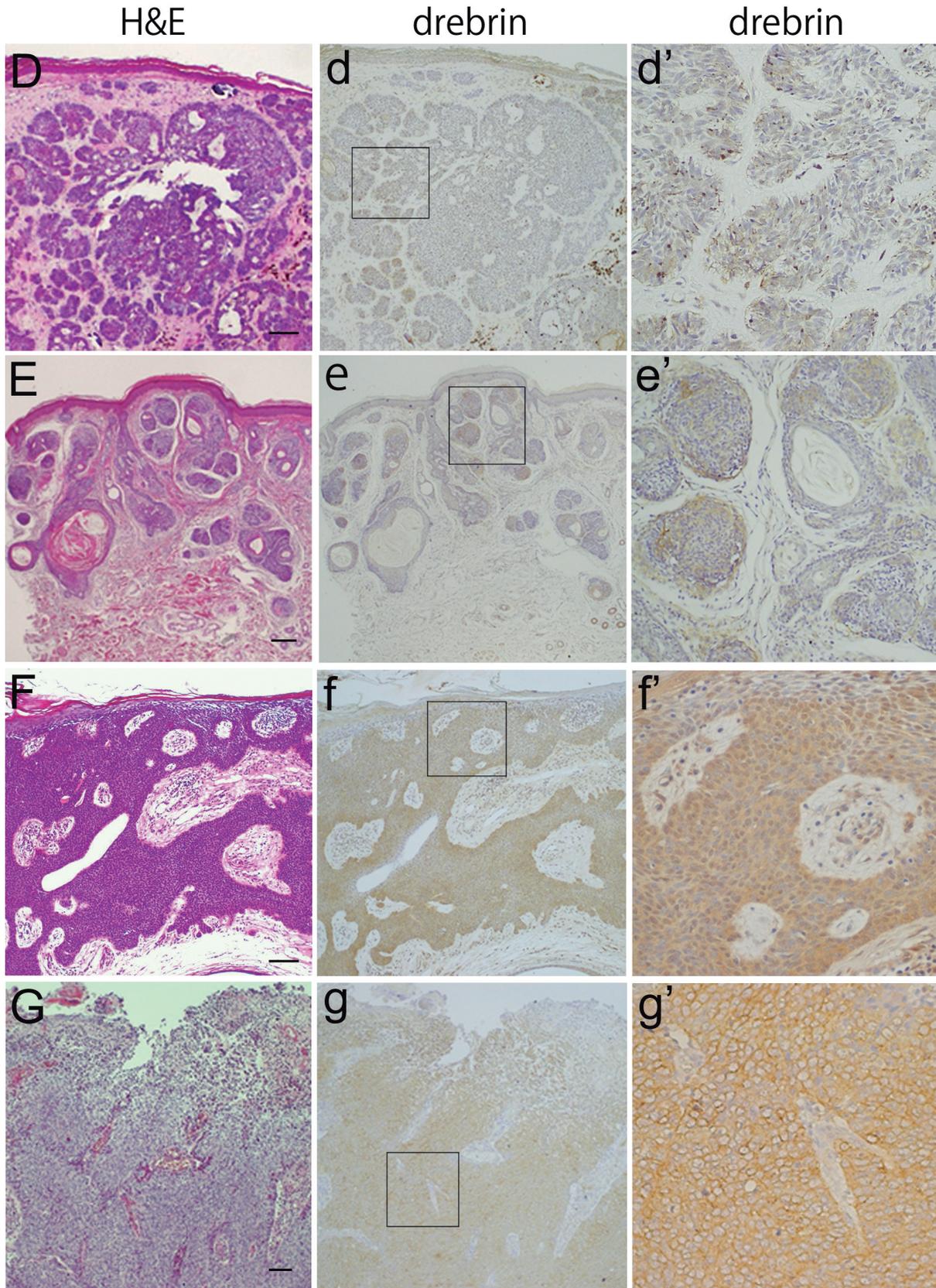


Fig. 3. Drebrin expression in BCC, trichoblastoma, trichoepithelioma, eccrine poroma and eccrine porocarcinoma tissue samples. Drebrin was stained with H&E (left panels) and HRP/DAB (middle and right panels) in sections of BCC (superficial, **A**, **a** and **a'**), BCC (fibrosing, **B**, **b** and **b'**), BCC (solid, **C**, **c** and **c'**), trichoblastoma (**D**, **d** and **d'**), trichoepithelioma (**E**, **e** and **e'**), eccrine poroma (**F**, **f** and **f'**) and eccrine porocarcinoma (**G**, **g** and **g'**). The boxed areas in **a-g** are magnified in **a'-g'**, respectively. Bars: 100 μ m.

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cultured cell lines. Consequently, drebrin with ~120 kDa was detected moderately in Ins-1, Caco2, HeLa and A549 cells, and weakly in differentiated and undifferentiated Neuro2A cells (Fig. 1B). The ~60 kDa proteins detected in Ins-1 and Neuro2a cells may be degradation products. These results show that drebrin is present not only in neuronal cells and but also in various non-neuronal cultured cells. We then found that drebrin with ~120 kDa was expressed strongly and weakly in a SCC cell line, DJM-1, and normal human epidermis, respectively (Fig. 1C).

Localization of drebrin in DJM-1 cells and normal human skin

We examined intracellular localization of drebrin in DJM-1 cells by immunofluorescence. As shown in Fig. 2A, drebrin was well colocalized with actin cytoskeleton at cell-cell junction sites although it was also distributed in the cytoplasm. Double immunostaining with drebrin and an AJ marker, β -catenin or E-cadherin, revealed their colocalization at AJs. Occuldin, a tight junction (TJ) marker, was also colocalized with drebrin to some extent. These results strongly suggest that drebrin is localized at AJs and TJs.

We next analyzed drebrin distribution in frozen sections of normal human epidermis, and double-stained for drebrin with actin or β -catenin. Consequently, drebrin was found to accumulate at cell-cell boundaries and was colocalized with actin cytoskeleton and β -catenin in keratinocytes in all the living layers of the epidermis (Fig. 2B). We next analyzed drebrin expression in normal skin tissue by immunohistochemistry with HRP/DAB. Consistent with the western blotting result (Fig. 1C), drebrin was very weakly

expressed in the epidermis (Fig. 2Ca), but it was visualized in eccrine sweat glands, hair matrix, and the inner and outer sheath of hair follicle (Fig. 2Cb, Da-c). These results indicate that drebrin is mainly expressed in skin appendages.

Drebrin expression in human skin tumors

We then looked into drebrin expression profiles in various follicular and eccrine tumors such as BCC, trichoblastoma, trichoepithelioma, eccrine poroma and eccrine porocarcinoma. Immunohistochemical analyses were performed for 25 BCC, 6 trichoblastoma, 9 trichoepithelioma, 10 eccrine poroma (benign) and 10 eccrine porocarcinoma (malignant) tissue samples. The correlation between the clinicopathologic characteristics and the drebrin-immunohistochemistry score in BCC, trichoblastoma and trichoepithelioma samples are shown

Table 1. Drebrin-immunohistochemistry score in tissue samples from BCC, trichoblastoma and trichoepithelioma.

	Staining intensity	Score
Basal cell carcinoma		
solid (n=20)	Weak (4) Moderate (5) Intense (11)	181.7
fibrosing (n=2)	Weak (0) Moderate (0) Intense (2)	242.0
superficial (n=3)	Weak (0) Moderate (2) Intense (1)	184.3
Trichoblastoma (n=6)	Weak (4) Moderate (2) Intense (0)	81.7
Trichoepithelioma (n=9)	Weak (7) Moderate (2) Intense (0)	49.1

Drebrin-immunohistochemistry score= (% of positive tumor cells) X (the staining intensity). The staining intensity: 1+, weak; 2+, moderate; 3+, intense

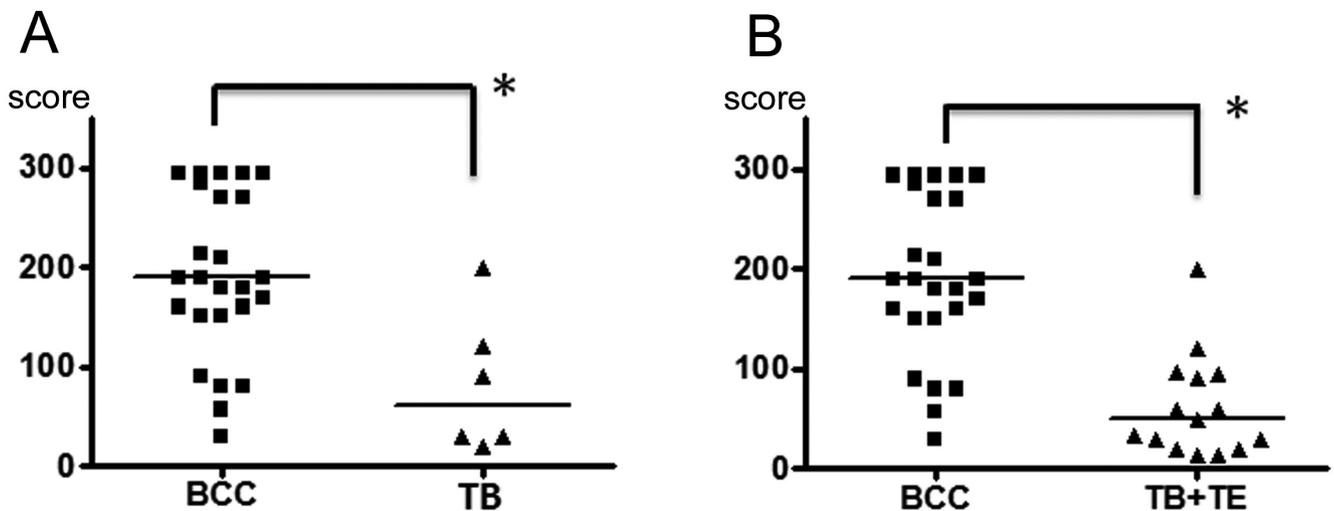


Fig. 4. Comparison of the drebrin expression level in BCC, trichoblastoma and trichoepithelioma tissue samples. Score was calculated as described in "Materials and methods". Bars indicate median. Statistical analyses were done using Mann-Whitney U test (* $p < 0.05$).

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in Table 1. In all the BCC samples, intense drebrin expression was homogeneously observed, especially at cell-cell boundaries (Fig. 3Aa - Cc'). In superficial BCC, drebrin staining was highlighted in the tumor area compared to the surrounding normal epidermal region (Fig. 3Aa'). In contrast, drebrin was heterogeneously and faintly expressed in trichoblastoma and trichoepithelioma samples (Fig. 3Dd' and Ee'). The mean drebrin-immunohistochemistry scores for BCC, trichoblastoma and trichoepithelioma samples were 186.8, 81.7 and 49.1 respectively (Table 1). In addition, drebrin expression level in BCC samples was significantly higher than in trichoblastoma and trichoepithelioma ones ($p < 0.05$, Mann-Whitney U test) (Fig. 4).

Next, we compared the staining patterns of drebrin in 10 cases each of eccrine poroma and eccrine porocarcinoma, because drebrin was enriched in eccrine gland in the normal human skin. As shown in Fig. 3Ff' and Gg', the expression level of drebrin was increased both in eccrine poroma and porocarcinoma at tumor cell borders and cytoplasm. Also, there was a tendency for drebrin to be more enriched at cell-cell contact sites in porocarcinomas than in poromas, in which drebrin was distributed throughout the cytoplasm.

Discussion

In the present study, we clarified that drebrin is localized at cell-cell junctional areas in SCC cell line (DJM-1), eccrine glands and hair follicles, with a homemade rabbit polyclonal antibody raised against the C-terminal 236 aa fragment (Fig. 2). Previously, Peitsch et al. performed morphological analyses of the skin tissue with a mouse monoclonal antibody raised against a cocktail of three different drebrin peptides (Peitsch et al., 2005). Our results are coincident with theirs except for hair matrix and the inner root sheath of hair follicles; drebrin was enriched in the ducts of eccrine sweat glands and the outer root sheath of hair follicles (Peitsch et al., 2005). The discrepancy between our results and theirs may be due to the antibodies used. Peitsch et al. also showed that drebrin was enriched in BCC compared to SCC, keratoacanthoma and precursor lesions such as actinic keratosis, and suggested a potential value of drebrin in diagnosis of BCC (Peitsch et al., 2005). We here confirmed the value they proposed and did further analyses by comparing drebrin expression patterns in BCC, trichoblastoma and trichoepithelioma. Consequently, we found that drebrin could serve as a useful marker for BCC differential diagnosis.

In spite of the importance of differential diagnosis of BCC, a malignant neoplasm, from benign neoplasm such as trichoblastoma and trichoepithelioma, it is often difficult since these neoplasms are all derived from the same origin, follicular germinative cells. Instead, there is considerable histopathological similarity among them, particularly between nodular BCC and nodular

trichoblastoma. For better differential diagnosis of BCC, intensive studies have been carried out. Recent investigations have shown that constitutive activation of the patched-hedgehog signaling pathway plays a central role in the development of BCC but not of trichoblastoma (Takata et al., 2001, 2002). Córdoba et al. reported immunohistochemical detection of Bcl-2 and CD10 in BCC and trichoblastoma (Córdoba et al., 2009). Based on the immunohistochemistry for CD10, they distinguished trichoblastoma with peritumoral stromal staining from BCC with epithelial staining. Sengul et al. also showed pathophysiological significance of CD10 in BCC and benign tumors of cutaneous appendages originating from the hair follicle (BTCOHF); they concluded that CD10 might be a useful marker for the differential diagnosis between these 2 tumors, particularly in the case of small and superficial biopsies (Sengul et al., 2010). On the other hand, Misago et al. demonstrated abnormal nestin expression in stromal cells of trichoblastoma and BCC. They claimed that the development of trichoblastoma incompletely recapitulated the epithelial-mesenchymal interaction in embryonic hair germs or early anagen hair follicles, whereas BCC fundamentally loses this ability (Misago et al., 2010). Hamasaki et al. did immunohistochemical analyses of laminin-5 γ 2 chain in BCC and trichoblastoma tissue samples, and clarified that 96.2% and 12% of BCC and trichoblastoma samples were positive for laminin-5, respectively (Hamasaki et al., 2011). Moreover, Wiedemeyer et al. reported that CK20 was positive in Merkel cells in the epidermal layer in trichoblastoma when compared to BCC (Wiedemeyer and Hartschuh, 2009).

As mentioned above, several proteins have so far been proposed for possible markers for the differential diagnosis between BCC and benign tumors, especially trichoblastoma. Although the pathophysiological relationship between drebrin and these possible marker proteins is still enigmatic, the results obtained in this study suggest that drebrin may be involved in BCC tumorigenesis. On the other hand, when expression patterns of drebrin were examined in other benign and malignant tumors with the same origin, eccrine poroma and porocarcinoma, there was a difference in localization patterns of drebrin; this protein was accumulated at cell-cell contact sites (membranes) in porocarcinoma while it showed cytoplasmic distribution in poroma. The different localization of drebrin might be involved in the malignancy of porocarcinoma, although further study is required to determine the pathophysiological role of drebrin.

In summary, our results show that drebrin protein expression was increased in BCC tissue samples with a homogeneous staining profile based on immunohistochemical analyses. In contrast, drebrin was weakly and heterogeneously expressed in trichoblastoma and trichoepithelioma tissues. Thus, in difficult cases of diagnosis between BCC and trichoblastoma, drebrin may

be a novel and useful marker.

References

- Ackerman A.B., De Viragh P.A. and Chongchitnant N. (1993). Trichoblastoma. In: Neoplasms with follicular differentiation. 1st ed. Spahr J.F. and DiRienzi D.A. (eds). Lea & Febiger. Philadelphia. pp 359-422.
- Chew C.S., Okamoto C.T., Chen X. and Thomas R. (2005). Drebrin E2 is differentially expressed and phosphorylated in parietal cells in the gastric mucosa. *Am. J. Physiol. Gastrointest. Liver Physiol.* 289, 320-331.
- Córdoba A., Guerrero D., Larrinaga B., Iglesias M.E., Arrechea M.A. and Yanguas J.I. (2009). Bcl-2 and CD10 expression in the differential diagnosis of trichoblastoma, basal cell carcinoma, and basal cell carcinoma with follicular differentiation. *Int. J. Dermatol.* 48, 713-717.
- Elder D.E. (2008). Tumors of the epidermal appendages. In: Lever's histopathology of the skin. 10th ed. Elenitsas R., Johnson B.L., Murphy G.F. and Xu X. (eds). Lippincott Williams & Wilkins. Philadelphia. pp 851-909.
- Hamasaki H., Koga K., Aoki M., Hamasaki M., Koshikawa N., Seiki M., Iwasaki H., Nakayama J. and Nabeshima K. (2011). Immunohistochemical analysis of laminin 5- γ 2 chain expression for differentiation of basal cell carcinoma from trichoblastoma. *Histopathology* 59, 143-161.
- Hanai N., Nagata K., Kawajiri A., Shiromizu T., Saitoh N., Haegawa Y., Murakami S. and Inagaki M. (2004). Biochemical and cell biological characterization of a mammalian septin. *FEBS Lett.* 568, 83-88.
- Kato Y., Uzawa K., Yamamoto N., Kouzu Y., Koike Y., Shiiba M., Bukawa H., Yokoe H., Shibahara T. and Tanzawa H. (2007). Overexpression of Septin1: possible contribution to the development of oral cancer. *Int. J. Oncol.* 31, 1021-1028.
- Keon B.H., Jedrzejewski P.T., Paul D.L. and Goodenough D.A. (2000). Isoform specific expression of the neuronal F-actin binding protein, drebrin, in specialized cells of stomach and kidney epithelia. *J. Cell Sci.* 113, 325-336.
- Kitajima Y., Inoue S., Nagao S., Nagata K., Yaoita H. and Nozawa Y. (1988). Biphasic effects of 12-O-tetradecanoylphorbol-13-acetate on the cell morphology of low calcium-grown human epidermal carcinoma cells: involvement of translocation and down regulation of protein kinase C. *Cancer Res.* 8, 964-970.
- Kitajima Y., Owaribe K., Nishizawa Y., Jokura Y. and Yaoita H. (1992). Phorbol ester-and calcium-induced reorganization of 180-kDa bullous pemphigoid antigen on the ventral surface of cultured human keratinocytes as studied by immunofluorescence and immunoelectron microscopy. *Exp. Cell Res.* 203, 17-24.
- Kojima N., Shirao T. and Obata K. (1993). Molecular cloning of a developmentally regulated brain protein, chicken drebrin A and its expression by alternative splicing of the drebrin gene. *Brain Res. Mol. Brain Res.* 19, 101-114.
- Misago N., Mori T. and Narisawa Y. (2010). Nestin expression in stromal cells of trichoblastoma and basal cell carcinoma. *J. Eur. Acad. Venereol.* 24, 1354-1358.
- Nagata K., Asano T., Nozawa Y. and Inagaki M. (2004). Biochemical and cell biological analyses of a mammalian septin complex. *J. Biol. Chem.* 279, 55895-55904.
- Nagata K., Ito H., Iwamoto I., Morishita R. and Asano T. (2009). Interaction of a multi-domain adaptor protein, vinexin, with a Rho-effector, Rhotekin. *Med. Mol. Morphol.* 42, 9-15.
- Peitsch W.K., Grund C., Kuhn C., Schnölzer M., Spring H., Schmelz M. and Franke W.W. (1999). Drebrin is a widespread actin-associating protein enriched at junctional plaques, defining a specific microfilament anchorage system in polar epithelial cells. *Eur. J. Cell Biol.* 78, 767-778.
- Peitsch W.K., Hofmann I., Prätzel S., Grund C., Kuhn C., Moll I., Langbein L. and Franke W.W. (2001). Drebrin particles: components in the ensemble of proteins regulating actin dynamics of lamellipodia and filopodia. *Eur. J. Cell Biol.* 80, 567-579.
- Peitsch W.K., Hofmann I., Endlich N., Prätzel S., Kuhn C., Spring H., Gröne H.J., Kriz W. and Franke W.W. (2003). Cell biological and biochemical characterization of drebrin complexes in mesangial cells and podocytes of renal glomeruli. *J. Am. Soc. Nephrol.* 14, 1452-1463.
- Peitsch W.K., Hofmann I., Bulkescher J., Hergt M., Spring H., Bleyl U., Goerdts S. and Franke W.W. (2005). Drebrin, an actin-binding, cell-type characteristic protein: induction and localization in epithelial skin tumors and cultured keratinocytes. *J. Invest. Dermatol.* 125, 761-774.
- Pérez-Martínez M., Gordón-Alonso M., Cabrero J.R., Barrero-Villar M., Rey M., Mittelbrunn M., Lamana A., Morlino G., Calabia C., Yamazaki H., Shirao T., Vázquez J., González-Amaro R., Veiga E. and Sánchez-Madrid F. (2010). F-actin-binding protein drebrin regulates CXCR4 recruitment to the immune synapse. *J. Cell Sci.* 123, 1160-1170.
- Sengul D., Sengul I., Astarci M.H., Ustun H. and Mogan G. (2010). CD10 for the distinct differential diagnosis of basal cell carcinoma and benign tumours of cutaneous appendages originating from hair follicle. *Pol. J. Pathol.* 3, 140-146.
- Shimada K., Uzawa K., Kato M., Endo Y., Shiiba M., Bukawa H., Yokoe H., Seki N. and Tanzawa H. (2005). Aberrant expression of RAB1A in human tongue cancer. *Br. J. Cancer* 92, 1915-1921.
- Shirao T. (1995). The roles of microfilament-associated proteins, drebrins, in brain morphogenesis: a review. *J. Biochem.* 117, 231-236.
- Shirao T. and Obata K. (1985). Two acidic proteins associated with brain development in chick embryo. *J. Neurochem.* 44, 1210-1216.
- Tanaka C., Uzawa K., Shibahara T., Yokoe H., Noma H. and Tanzawa H. (2003). Expression of an inhibitor of apoptosis, survivin, in oral carcinogenesis. *J. Dent. Res.* 82, 607-611.
- Takata M., Tojo M., Hatta N., Ohara K., Yamada M. and Takehara K. (2001). No evidence of deregulated patched-hedgehog signaling pathway in trichoblastomas and other tumors arising within nevus sebaceous. *J. Invest. Dermatol.* 117, 1666-1670.
- Takata M., Tojo M., Hatta N., Yamada M. and Ohara K. (2002). Molecular biological differentiation between basal cell carcinoma and trichoblastoma. *Skin Cancer* 17, 21-25.
- Vaskova M., Kovac M. and Volna P. (2011). High expression of cytoskeletal protein drebrin in TEL/AML1pos B-cell precursor acute lymphoblastic leukemia identified by a novel monoclonal antibody. *Leuk. Res.* 35, 1111-1113.
- Wiedemeyer K. and Hartschuh W. (2009). Trichoblastomas with Merkel cell proliferation in nevi sebacei in Schimmelpenning-Feuerstein-Mims syndrome-histological differentiation between trichoblastomas and basal cell carcinomas. *J. Dtsch. Dermatol. Ges.* 7, 612-614.