Correspondence

Scleroedema adultorum associated with sarcoidosis

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Sarcoidosis is a systemic granulomatous disease of unknown aetiology that displays a wide variety of skin features including maculopapules, nodules, plaques, subcutaneous nodules, infiltrative scars, and lupus pernio.[1] We report a case of sarcoidosis with subcutaneous induration of the neck.

A 62-year-old Japanese man presented with a 6-month history of asymptomatic, firm indurations on the neck. He had first noticed these skin lesions after bilateral symmetrical hilar lymph-node enlargement was found during routine chest radiography. Transbronchial biopsies resulted in the histological identification of non-caseating granulomas compatible with sarcoidosis. The patient had no history of diabetes mellitus or preceding infection.

On physical examination, symmetrical, hard, nonpitting indurations of the skin were found on the posterior neck (Fig. 1a). The patient's general health was good.

Results of routine laboratory studies including angiotensin-converting enzyme and tuberculin response gave normal results, and there was no evidence of monoclonal proteinaemia. Computed tomography scans showed bilateral hilar lymphadenopathy but there was no other lymphadenopathy noted.

Histological examination of skin-biopsy specimens taken from the posterior neck revealed swelling of the dermal collagen bundles without increase in fibroblast numbers, and the subcutaneous fat had been replaced by collagen fibres (Fig. 1b). A diagnosis of SA was made. Treatment was started with steroid ointment or 9 months, but without evident improvement.

SA is a rare disorder of unknown cause, but often complicates diabetes mellitus. In such cases, the lesions are usually limited to neck and upper back, and tend to be persistent.[2] In contrast, in SA not associated with diabetes mellitus, the lesions often spread to the face, trunk and upper arms, but may spontaneously subside.[3,4] However, in spite of no obvious association with diabetes mellitus, our patient had intractable induration distributed over a localized area. Interestingly, in this case, development of the skin lesion was coincidental with the diagnosis of sarcoidosis. The clinical appearance was indicative of scleroedema. There have been no previous reports of any association between SA and sarcoidosis. Therefore, we first suspected a subcutaneous form of sarcoidosis rather than scleroedema. However, the histopathological findings confirmed a diagnosis of scleroedema.

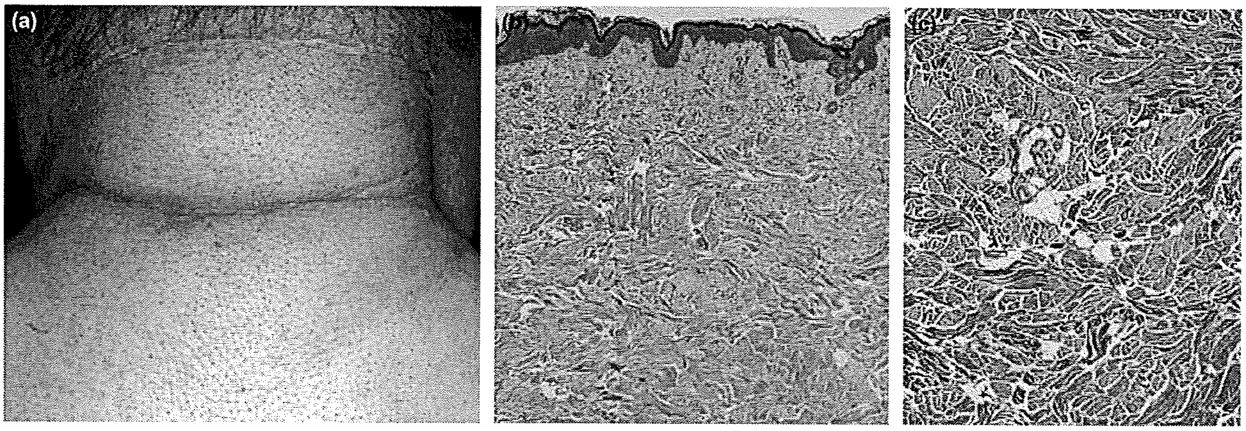

Figure 1 (a) Symmetrical, hard, nonpitting induration on the posterior side of the neck; (b) inflammatory cell infiltration in the upper dermis and swelling of collagen bundles in the lower dermis; (c) swelling of the dermal collagen bundles without any increase in fibroblast numbers, and the replacing of subcutaneous fatty tissues by collagen fibres.

Sarcoidosis is known to be complicated by a variety of immunological diseases including malignant lymphoma, autoimmune diseases and multiple myeloma, and scleroedema is associated with infections, paraproteinaemia and multiple myeloma. Some previous studies have shown an increase in amounts of proα1(I) collagen mRNA in both sarcoidosis and scleroedema lesions.[5] Some common factors in the pathogenesis of two diseases might therefore be involved in this patient.

D. Inokuma, D. Sawamura,* A. Shibaki, R. Abe and H. Shimizu
Department of Dermatology, Hokkaido University Graduate School of Medicine, North 15 West 7, Kita-ku, Sapporo, 060-8638, Japan; and
**Department of Dermatology, Hirosaki University Graduate School of Medicine, Hirosaki CIty, Japan*
E-mail: inokuma@med hokudai.ac.jp
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Table 2 Confounding factors in skin sensitivity testing

Menstrual cycle[4]
Concentration of test substance[4,6,10]
Dose of test substance[6,10]
Batch volume[6]
Purity of test substance[6,10]
Test substance vehicle[6]
Length of exposure[6]
Time of occlusion[6]
Release of test substance from chamber[6]
Enhancement of penetration[6]
Method of evaluation[4–7]
Time of evaluation after exposure[2,4,6,7]
Humidity[6,10]
Water vapour gradient[6]
Impairment of skin barrier[6]
Anatomical site[6]
Intrinsic level of skin sensitivity[6]
Booster effect[8]
Individual variation[8,10]
Skin temperature[6]
Ultraviolet exposure[6]
Age[6]
Sex[6]
Race[6]
Circadian rhythms[10]
Concomitant medications[10]

effect of reproductive hormones on skin disorders have been somewhat hampered, however, by the fact that individual patient sensitivity varies widely and by a body of research that has employed diverse research parameters, such as dosage, testing sites, concentration, vehicle of irritant delivery, and method of assessment. Further research, with strict attention to standardizing experimental parameters and limiting compounding factors, will help to elucidate the biochemical basis for the observed effects of progesterone and oestrogen levels on skin health. Dermatologists should be aware that the menstrual cycle can affect the sensitivity of women to primary irritants and can affect their allergic response.

Feminine Clinical Sciences,
The Procter and Gamble Company,
Winton Hill Business Centre,
6110 Centre Hill Road, PO Box 136,
Cincinnati, OH 45224, U.S.A.
**San Gallicano Dermatological Institute,*
Rome, Italy
†Department of Dermatology,
School of Medicine, University of California,
San Francisco, CA, U.S.A.
E-mail: farage.m@pg.com

M.A. FARAGE
E. BERARDESCA*
H.I. MAIBACH†

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Key words: *allergic response, atopic dermatitis, menstruation, oestrogen, progesterone, skin testing*

Conflicts of interest: none declared.

Autoantibodies against type XVII collagen C-terminal domain in a patient with bullous pemphigoid associated with psoriasis vulgaris

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SIR, Bullous pemphigoid (BP) has been reported to develop occasionally in patients with psoriasis vulgaris.[1,2] BP with psoriasis vulgaris is typically associated with autoantibodies against type XVII collagen (Col17) with the main antigenic site occurring within the noncollagenous 16a (NC16a) domain.[2] We report the first patient with BP associated with psoriasis vulgaris whose autoantibodies targeted the C-terminal domain of Col17. In addition, our patient also exhibited gastric carcinoma, similar to other patients with BP in whom internal malignancies have been reported.

A 64-year-old Japanese man with 6-year history of psoriasis vulgaris presented with an extensive eruption that had started 3 days previously with numerous pruritic, tense bullae on a background of psoriatic plaques over his entire body (Fig. 1a). Palmoplantar bullous lesions were also severe (Fig. 1b) and there were erosions on his lips (Fig. 1c) but not on the genital area nor on the oral mucosa. He had never received psoralen + ultraviolet (UV) A (PUVA) or narrowband UVB therapy.

Fig 1. Clinical features. (a) Tense bullae and psoriatic plaques on the back. (b) Bullae and oedematous erythema on the palms. (c) Erosion on the lips.

Skin biopsies were taken from two psoriatic plaques, one with and one without a blister. Histopathology of the first biopsy showed subepidermal blistering with an abundant infiltrate mainly of neutrophils and lymphocytes, and a small number of eosinophils. Parakeratosis and regular acanthosis were also seen around the roof of the blister (Fig. 2a). Histology of the typical psoriatic plaque without a blister demonstrated psoriatic-like regular acanthosis, parakeratosis, a subepidermal neutrophilic abscess and limited eosinophilic infiltration in the dermis (Fig. 2b).

Direct immunofluorescence (IF) from the specimen around the blister revealed linear C3 and IgG deposits along the basement membrane zone but no IgA or IgM deposits (Fig. 3a). Indirect IF (IIF), using normal human skin as substrate, demonstrated a high titre of circulating IgG autoantibodies against the dermal–epidermal junction (> 1 : 160). IIF on 1 mol L^{-1} NaCl-split skin revealed linear IgG deposition on both epidermal and dermal sides of the split (> 1 : 40) (Fig. 3b). Using an enzyme-linked immunosorbent assay (ELISA) to measure IgG against the NC16a domain of Col17, the index value was low at 12 IU (normal < 9). Immunoblot assays revealed that the patient sera reacted with the recombinant C-terminal domain of Col17[3] and with BP230 using human epidermal extracts (Fig. 3c). Autoantibodies against the 290-kDa epidermolysis bullosa acquisita (EBA) antigen, anti-p200 pemphigoid antigen, laminin 332 chains and recombinant Col17 NC16a domain were not detected by immunoblot.

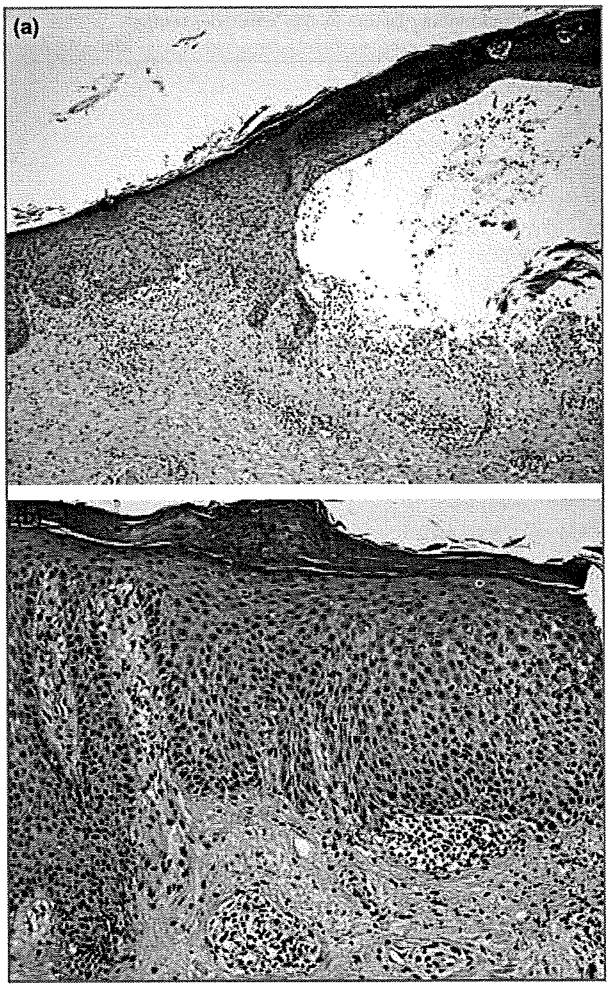

Fig 2. (a) Subepidermal blister with an abundant infiltrate of neutrophils and lymphocytes in a specimen from a bulla on the psoriatic plaque. (b) Subepidermal neutrophilic abscess with neutrophils in a specimen from a psoriatic plaque without bullae (haematoxylin and eosin).

The patient also had the complication of gastric cancer discovered through gastric fibrescope examination. Biopsy revealed a carcinoma *in situ*. Computed tomography did not show any metastases of this gastric cancer. We diagnosed our patient as having BP with antibodies positive for the anti-Col17 C-terminal domain, associated with psoriasis vulgaris and gastric carcinoma. Administration of oral prednisolone 35 mg daily (0·5 mg kg^{-1} daily) improved skin and oral involvement and he remained well controlled with prednisolone 10 mg daily. The gastric carcinoma *in situ* was removed endoscopically.

It is widely thought that BP is a subepidermal autoimmune blistering disease with autoantibodies against Col17 involving the skin and mucosa. BP is particularly associated with autoantibodies to Col17, with the major antigenic site being within the extracellular NC16a domain. ELISA using NC16a recombinant protein fragments is a valuable tool for the definite diagnosis of BP and for repeated, consistent monitoring of disease activity. Recent studies, however, have demonstrated

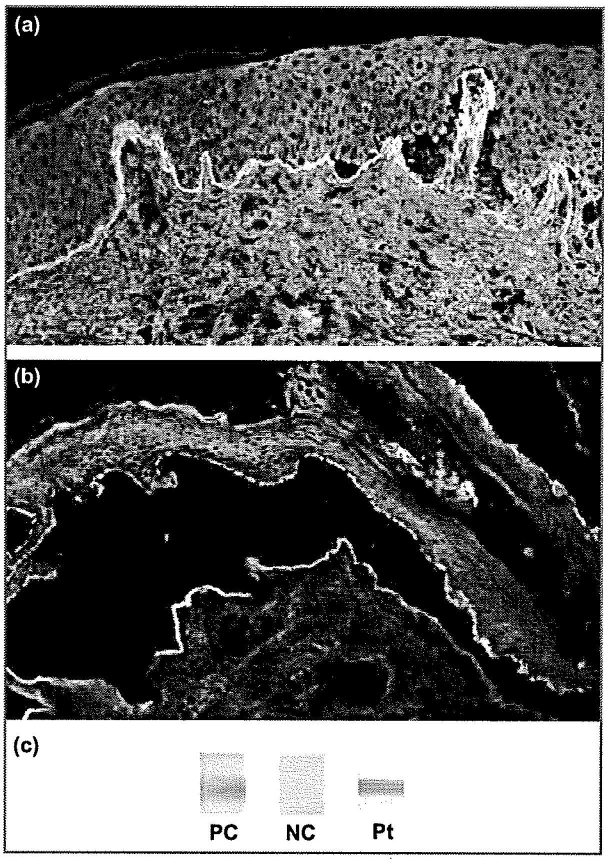

Fig 3. (a) Direct immunofluorescence from a specimen around the blister revealed a linear IgG deposition along the basement membrane zone. (b) Indirect immunofluorescence on 1 mol L^{-1} NaCl-split skin revealed a linear IgG deposition on both sides of the epidermal and the dermal separation. (c) Immunoblot analysis using Col17 C-terminal recombinant protein. Lane 1, serum from a patient with mucous membrane pemphigoid (PC); lane 2, normal control serum (NC); lane 3, the patient's serum (Pt).

that the reactivity of BP sera is not restricted to the NC16a domain.[4] It has been reported that 9% of BP sera do not react with the NC16a domain in immunoblot analysis. Moreover, in 20 cases of mucous membrane pemphigoid, nine sera recognized the NC16a domain, and three of the 20 sera reacted with epitopes in either the mid-portion or the C-terminus of Col17.[5]

IIF on 1 mol L^{-1} NaCl-split skin revealed linear deposition of antibodies to the C-terminal domain of Col17 on only the epidermal side but, in our case, IIF revealed deposition on both epidermal and dermal sides of the split. We performed immunoblot assays of autoantibodies against the 290-kDa EBA antigen, anti-p200 pemphigoid antigen, laminin 332 chains and recombinant Col17 NC16a domain, but all were negative. In a previous report, antibasement membrane zone antibodies reacting to both the dermal and epidermal sides were demonstrated by IIF in 2% of cases of BP, and the C-terminal domain of Col17 is present in the lamina densa under the lamina lucida, so it is possible for antibodies to the C-terminal to react to both epidermal and dermal sides in split-skin IIF.

The combination of psoriasis with autoimmune subepidermal bullous disease, for instance BP,[1,2] linear IgA bullous dermatosis[6,7] or EBA,[8] has been reported. Recently, especially in the Japanese population, several cases of the combination of psoriasis with anti-p200 pemphigoid have been reported.[1,2,9] Autoantibodies to p200 protein were not detected in the present case by immunoblot assay. To the best of our knowledge there have not been any previous reports of patients with BP associated with psoriasis vulgaris whose sera reacted with the C-terminal domain of Col17. There have been several reports on the association between BP and psoriasis vulgaris but the pathogenic significance of this relationship is unknown. There have been no reports which showed the association of other diseases with antibodies to the C-terminal domain, and the causal relationship is also not clear. PUVA or UVB therapy for psoriasis may trigger the development of BP but our patient had not received any PUVA or UVB therapy.[10]

We have described the first case of psoriasis vulgaris with BP autoantibodies against the C-terminal of Col17 protein which was successfully treated. We hope that any future cases may shed more light on the pathomechanisms of this disease.

Department of Dermatology,
Hokkaido University Graduate School of Medicine,
N 15 W 7, Kita-ku, Sapporo 060-8638, Japan
**Sapporo Railway Hospital, Sapporo, Japan*
†Department of Dermatology,
Kurume University School of Medicine, Kurume, Japan
E-mail: *inokuma@med.hokudai.ac.jp*

D. Inokuma
K. Kodama*
K. Natsuga
M. Kasai*
M. Abe*
W. Nishie
R. Abe
T. Hashimoto†
H. Shimizu

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Key words: bullous pemphigoid, Col17 C-terminal domain, psoriasis vulgaris

Conflicts of interest: none declared.

Folliculosebaceous cystic hamartoma differentiates toward the infundibulum, sebaceous duct and sebaceous cells: immunohistochemical study of keratins and filaggrin

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Sir, Folliculosebaceous cystic hamartoma (FSCH) is a rare cutaneous hamartoma with varying proportions of epithelial components and mesenchymal overgrowth.[1] The epithelial components consist of an infundibular cystic structure to which mature sebaceous lobules are attached via sebaceous ducts. The histogenesis of FSCH remains unclear.

We report a case of FSCH occurring on the nasolabial fold in an elderly Japanese man. To determine the differentiation of FSCH, we performed an immunohistochemical study of keratins[2,3] and filaggrin[4] (filament aggregating protein). To our knowledge, this is the first report of FSCH with an immunohistochemical study of keratins and filaggrin. Since this tumour was first described by Kimura et al. in 1991,[1] about 30 cases have been reported.[5]

A 78-year-old man presented with a 3-year history of a slow-growing, pink-yellow, elastic hard, pedunculated asymptomatic nodule 17 × 13 mm in size on the right side of his nose. Specimens were fixed in neutral formalin, embedded in paraffin and stained with haematoxylin and eosin. Serial sections were used for the immunohistochemical study. We used 10 antikeratin antibodies: 34βB4 [keratin 1 (K1)], LP5K (K7), LP3K (K8), LHP1 (K10), LL022 (K14), LHK15 (K15), LL025

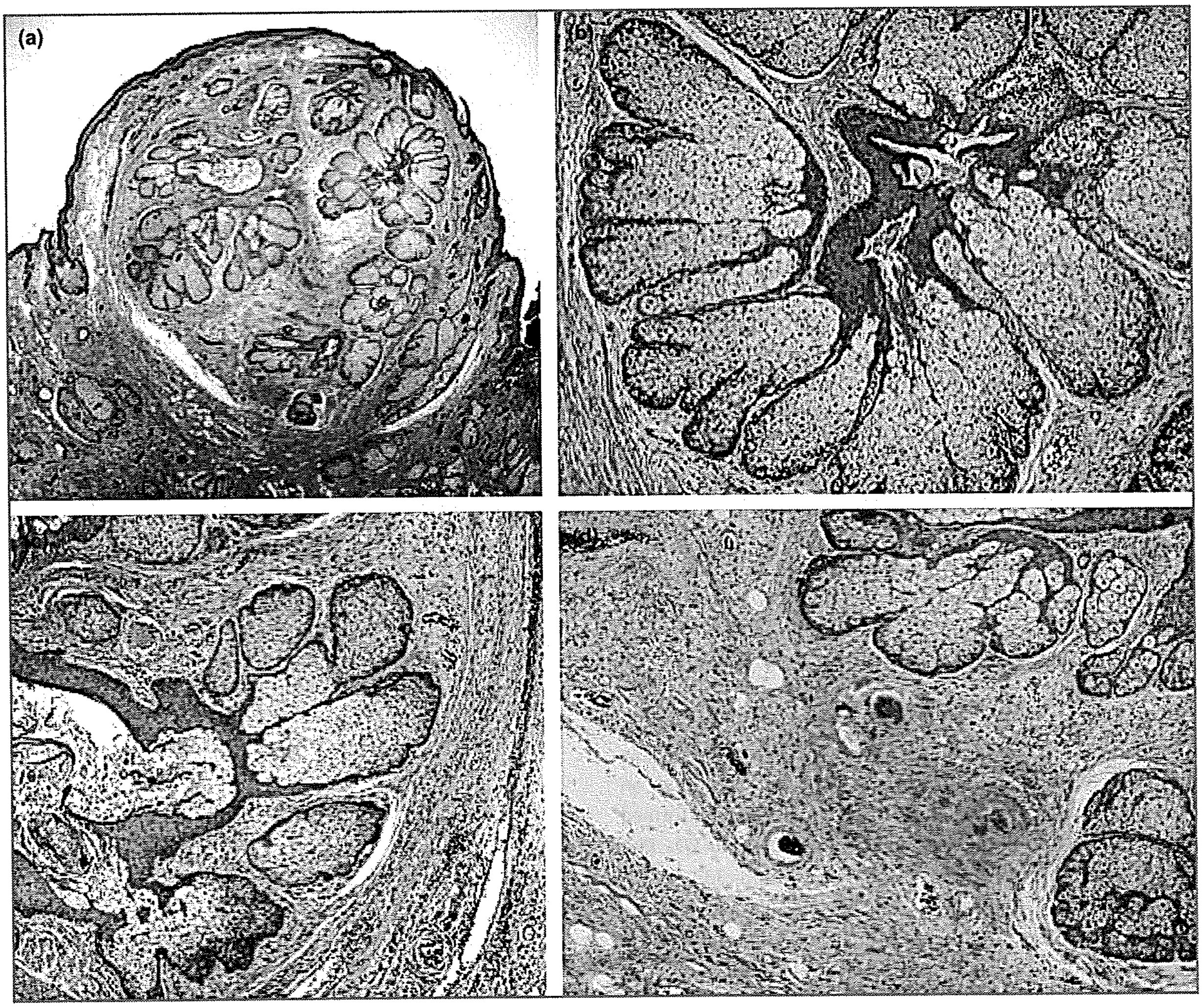

Fig 1. Haematoxylin and eosin staining. (a) The tumour in the dermis and was sharply demarcated from adjacent compressed fibrous tissue of the surrounding dermis by prominent clefts. (b) 'Folliculosebaceous units', which consist of infundibular cystic structures, sebaceous duct-like structures and hypertrophic sebaceous lobules, surrounded by lamellar fibroplasia. (c) Sebaceous duct-like structure. (d) Fibroepithelial unit.

Bowen's Disease of the Nail Matrix Presenting as Melanonychia: Detection of Human Papillomavirus Type 56

Daisuke Inokuma[1], Satoru Aoyagi[1], Nao Saito[1], Maria Maroto Iitani[1], Erina Homma[1], Kokichi Hamasaka[2] and Hiroshi Shimizu[1]
[1]Department of Dermatology, Hokkaido University Graduate School of Medicine, N15 W7, Sapporo 060-8638, and [2]Hamasaka Skin Clinic, Sapporo, Japan. E-mail: inokuma@med.hokudai.ac.jp

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Sir,

Bowen's disease (BD) is one of the most common premalignant conditions of the skin. It can occur at any location on the body, presenting as a reddish plaque with scales. Rare cases of presentation as lesions on the nail unit with longitudinal melanonychia have also been described (2). Trauma, exposure to radiation, and intake of arsenic have been cited as causative factors of BD (1, 2). Recently human papillomavirus (HPV), in most cases HPV type 16, has been implicated as another causal agent of BD (1). We report here a case of BD presenting as melanonychia on the nail matrix detected as HPV type 56 (HPV-56) (2, 3).

CASE REPORT

A previously healthy 41-year-old Japanese man presented with a 2-mm-wide black streak on the nail and a 2-mm-wide, hyperkeratotic black macule on the lateral nail fold of the right index finger, which had been present for more than 2 years (Fig. 1). The Hutchinson sign was not present. There was no evidence of viral warts anywhere, including on the hands, feet and genital region, and no dark streaks in the nails of his other digits. Dermoscopic examination of the nail plate revealed longitudinal black pigmentation with parallel regular lines. Dermoscopic findings around the nail revealed homogenous black pigmentation and hyperkeratosis without vessels. A longitudinal excisional biopsy of the nail bed and the proximal and lateral nail fold was performed. Histopathological findings revealed acanthotic epidermis with atypical dyskeratotic keratinocytes (Fig. 2a). The nuclei of the atypical cells were large, pleomorphic, hyperchromatic, and bizarre mitotic. Many granules of melanin were seen in the nail plate and the epidermis of the nail bed without increases in the number of melanocytes (Fig. 2b). The papillary dermis was not involved in the malignant process. A diagnosis of BD was made. Polymerase chain reaction (PCR) amplification for DNA of HPV-6, 11, 16, 18, 30, 31, 33, 35, 39, 45, 51, 52, 56, 59, and 66 from the tissue of the lesion was positive only for HPV-56.

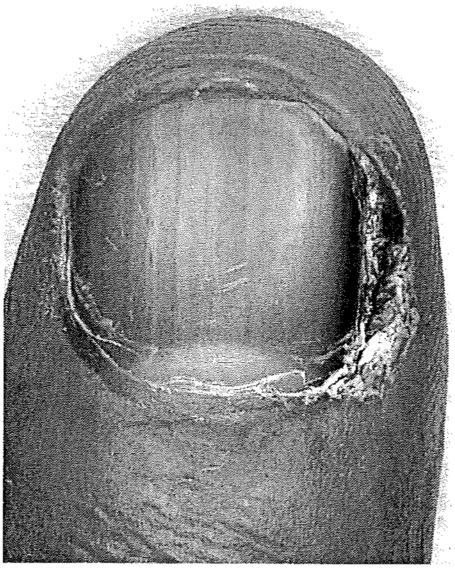

Fig. 1. A black streak on the nail and hyperkeratotic black macule on the lateral nail fold of the right index finger.

DISCUSSION

In most cases of BD with HPV, the lesions present as black papules in the genital region or as melanonychia, and the HPV detected from the lesion is typically HPV-16 (1). However, there is no information on the skin phototypes of people involved in these cases. In recent studies, however, HPV-56 has rarely been identified from BD lesions. HPV-56 was first detected from cervical intraepithelial neoplasia in 1989 (4); since then, it has frequently been detected in cervical neoplasia, in contrast to the low incidence of detection in normal cervical tissues and condylomas. At present, HPV-56 is included in the high-risk group or the mucous membrane group. In 1999, the first case of BD in an extra-genital area in which HPV-56 was detected was reported (5), and in 2003 the first case of detection of HPV-56 in the nail matrix with BD was reported (2). Since then, only two other similar cases have been reported (3). In all of these cases, the clinical features showed regular pigmented streaks and histopathological findings revealed many granules of melanin and atypical keratinocytes with large, hyperchromatic and bizarre mitotic nuclei.

In our case there were no control samples from matched locations in the same individual and, to the best of our knowledge, in others where HPV-56 has been found, there have been no control samples. In other patients with BD we examined who had no pigmentation, we did not detect HPV-56.

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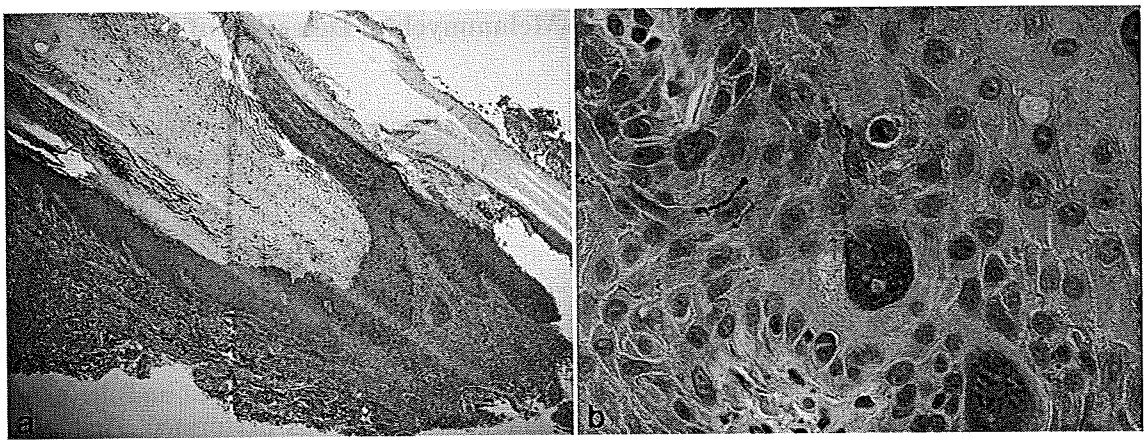

Fig. 2. (a) Acanthotic epidermis with dyskeratotic and atypical keratinocytes. (b) Large, pleomorphic, hyperchromatic, bizarre mitotic nuclei of atypical keratinocytes. Many melanin granules are seen in the nail plate and the epidermis of the nail bed. Haematoxylin and eosin (H&E) stain (a: ×40, b: ×400).

We hypothesize that HPV-56 is not only associated with the pathomechanism of carcinogenesis in BD, but that it is also a causative factor of pigmentation in this disease.

The authors declare no conflict of interest.

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suggest that the SNP had no influence on the C5 synthesis. Similar analyses in RA patients are needed to confirm this observation.

In this study, the lack of association of the SNP G/A (rs10818488) with pemphigus emphasizes the dilemma concerning the contribution of complement in pemphigus pathogenesis.

In fact, complement was described as an effective mediator in many studies based on cell culture detachment in the presence or absence of complement.[2] However, extensive erosions were obtained in C5-deficient mice suggesting that acantholysis could be complement independent.[9]

Further studies are needed to clarify if this polymorphism is restricted to RA or associated with other autoimmune diseases, where complement has a crucial role in disease pathogenesis, such as systemic lupus erythematosus.[10] Evaluating the association of other SNPs in the *TRAF1/C5* region will be needed to clarify their effect on the signalling cascade and to elucidate their involvement in autoimmune diseases.

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K. MEJRI
H. MBAREK*
M. KALLEL-SELLAMI
E. PETIT-TEIXEIRA*
Y. ZERZERI
O. ABIDA†
M. ZITOUNI
M. BEN AYED†
M. MOKNI‡
B. FEZZA§
H. TURKI¶
F. TRON**
D. GILBERT**
H. MASMOUDI†
B. PRUM††
F. CORNELIS*
S. MAKNI

Laboratoire d'Immunologie, Hôpital la Rabta, Tunis, Tunisia
**Genhotel-EA3886, Evry & Paris 7 Universities, AP-HP and CHSF Hospitals, AutoCure European Consortium member, 91057 Evry-Genopole, France*
†Laboratoire d'Immunologie, Hôpital Habib Bourguiba, Sfax, Tunisia
‡Service de Dermatologie, Hôpital la Rabta, Tunis, Tunisia
§Service de Dermatologie, Hôpital Charles Nicolle, Tunis, Tunisia
¶Service de Dermatologie, Hôpital Hédi Chaker, Sfax, Tunisia
***INSERM U519, Institut Fédératif de Recherche Multidisciplinaire sur les Peptides (IFR23), Faculté Mixte de Médecine et de Pharmacie, Rouen, France*
††Laboratoire Statistique et Génome, Evry, France
Correspondence: Sondes Makni.
E-mail: sondes.makni@rns.tn

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Key words: *complement C5, desmoglein, pemphigus, single-nucleotide polymorphism (SNP)*

Conflicts of interest: none declared.

Birt–Hogg–Dubé syndrome with clear-cell and oncocytic renal tumour and trichoblastoma associated with a novel *FLCN* mutation

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SIR, Birt–Hogg–Dubé (BHD) syndrome is a rare autosomal dominant syndrome characterized by skin hamartomas and multiple renal tumours.[1,2] FLCN (previously known as BHD) is the responsible gene, encoding a folliculin, which is suspected to be a tumour suppressor.[3] We report here a case of a Japanese patient with BHD syndrome with a new mutation in exon 12 of the FLCN gene, manifesting a trichoblastoma on the scalp and a renal tumour with atypical histological features.

A 68-year-old Japanese man was transferred to our hospital for further treatment of a cerebral infarction. From his early years he had had copious papules all over his face and neck. His mother and sister died from unknown renal disease. The patient's son also had similar papules since his early years. The patient's daughter had no such lesions. There was an 18 × 19 × 9 mm, dome-shaped, smooth-surfaced, greyish, arranged in a cribriform pattern with peripheral palisading, partly with follicular differentiation (Fig. 1b). We finally diagnosed this tumour as a trichoblastoma.

Computed tomography scans showed no pulmonary lesion, but identified two enhanced heterogeneous masses in the bilateral kidneys, the larger one (50 mm) was in the middle portion of the left kidney, and the smaller one (5 mm) was in the upper pole of the right kidney. The tumours were enhanced in dynamic early phase and washed out in dynamic parenchymal phase. These findings were consistent with renal cell carcinoma. Further imaging studies detected no lymphadenopathy or metastasis, and the clinical diagnosis was left renal cell carcinoma, T1bN0M0 and right renal cell carcinoma, T1aN0M0 (Union Internationale Contre Cancrum) (Fig. 1c). Initially, a laparoscopic left radical nephrectomy was performed, because the tumour was very close to the renal pelvis and partial nephrectomy was technically difficult. Macroscopically, the tumour in the left kidney was 50 mm in diameter with a fibrous capsule and a mahogany-brown colour of its cut surface with no haemorrhage or necrosis. A histopathological examination revealed that the tumour was mainly composed of cells with clear to acidophilic cytoplasm arranged in alveolar, tubular and focal papillary fashion, indicating clear-cell carcinoma of the kidney (Fig. 1d). Focal oncocytic areas were also observed in which round to oval tumour cells with granular acidophilic cytoplasm were organized into various-sized nests within a loose fibrous stroma. The nuclei in the oncocytic area were uniform and round, with or without prominent nucleoli (Fig. 1e). Immunohistochemically, the tumour cells in the clear-cell carcinoma area were positive for vimentin, but the tumour cells in the oncocytic area were negative for vimentin (not shown). This feature is compatible with oncocytoma. Neither capsular nor vascular invasion was seen. The right-side tumour has been followed by imaging without therapy.

Total genomic DNA was extracted from the patient's peripheral blood leucocytes, and the segments of the FLCN gene including all exons (4–14) were amplified and directly sequenced. This mutation analysis finally detected a duplication of 7 bp (GTTCCAC) at codon 448 (c.1792–1798) in exon 12 (Fig. 2). There was no similar mutation reported in the Human Gene Mutational Database; therefore we suggest that this is a novel mutation in the FLCN gene. No mutation was detected in exon 11, a hot spot in previous mutation analyses in Caucasian and Asian patients.[4,5] This insertion led to a premature termination codon 33 bp downstream from the site of insertion. The patient's daughter did not have this mutation (not shown). Consent for gene analysis of his other family members, including his son was not obtained.

This is the first reported case of a patient with BHD syndrome with trichoblastoma, not basal cell carcinoma.[6] The kidney tumour consisted mainly of clear-cell carcinoma, with oncocytoma and papillary renal cell carcinoma. The histological features were distinctly unusual and were different from those of a hybrid oncocytic tumour, the most common renal cell carcinoma in BHD syndrome, although clear-cell carcinoma is part of the tumour spectrum.[7] A few cases of other mutations in exon 12 have been reported previously. However, these cases do not seem to share apparent common clinical features.[6,8–10] There have been no reports of sporadic trichoblastoma or basal cell carcinoma with mutations in the FLCN gene. Further research is required to define the protein structure and reveal its functionally active sites.

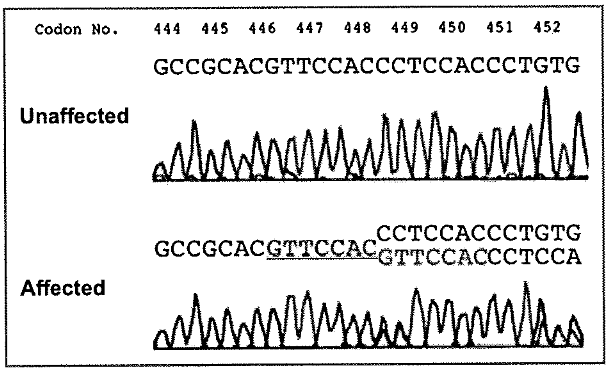

Fig 2. Detection of a mutation in codon 448 (c.1792–1798dup-GTTCCAC) in exon 12, a repeat of the preceding 7 bp in one allele, resulting in unclear multiple chromatograms after the tract.

This case suggests that BHD syndrome is associated with a variety of cutaneous hamartomas including trichoblastoma. A new mutation in the present case of BHD syndrome may be a crucial event in follicular differentiation and renal carcinogenesis.

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Department of Urology,
Graduate School of Medical Sciences,
Kyushu University, Fukuoka, Japan
**Department of Dermatology,*
Kurume University School of Medicine,
67 Asahimachi, Kurume,
Fukuoka 830-0011, Japan
†Department of Anatomic Pathology,
Graduate School of Medical Sciences,
Kyushu University, Fukuoka, Japan
‡Department of Dermatology,
Hokkaido University Graduate School of Medicine,
Sapporo, Japan
§Department of Dermatology,
Hirosaki University School of Medicine,
Hirosaki, Japan
¶Department of Dermatology,
Graduate School of Medical Sciences,
Kyushu University, Fukuoka, Japan
Correspondence: Teruki Dainichi.
E-mail: dainichi@med.kurume-u.ac.jp

K. IMADA
T. DAINICHI*
A. YOKOMIZO
T. TSUNODA
Y.H. SONG†
A. NAGASAKI‡
D. SAWAMURA§
W. NISHIE‡
H. SHIMIZU‡
S. FUKAGAWA¶
K. URABE¶
M. FURUE¶
T. HASHIMOTO*
S. NAITO

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Key words: *Birt–Hogg–Dubé syndrome, mutation, oncocytoma, renal cell carcinoma, trichoblastoma*

Conflicts of interest: none declared.

LEF-1 expression in basal cell carcinomas

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Sir, Basal cell carcinomas (BCCs) are the most frequent tumours of the skin, and are thought to arise from stem

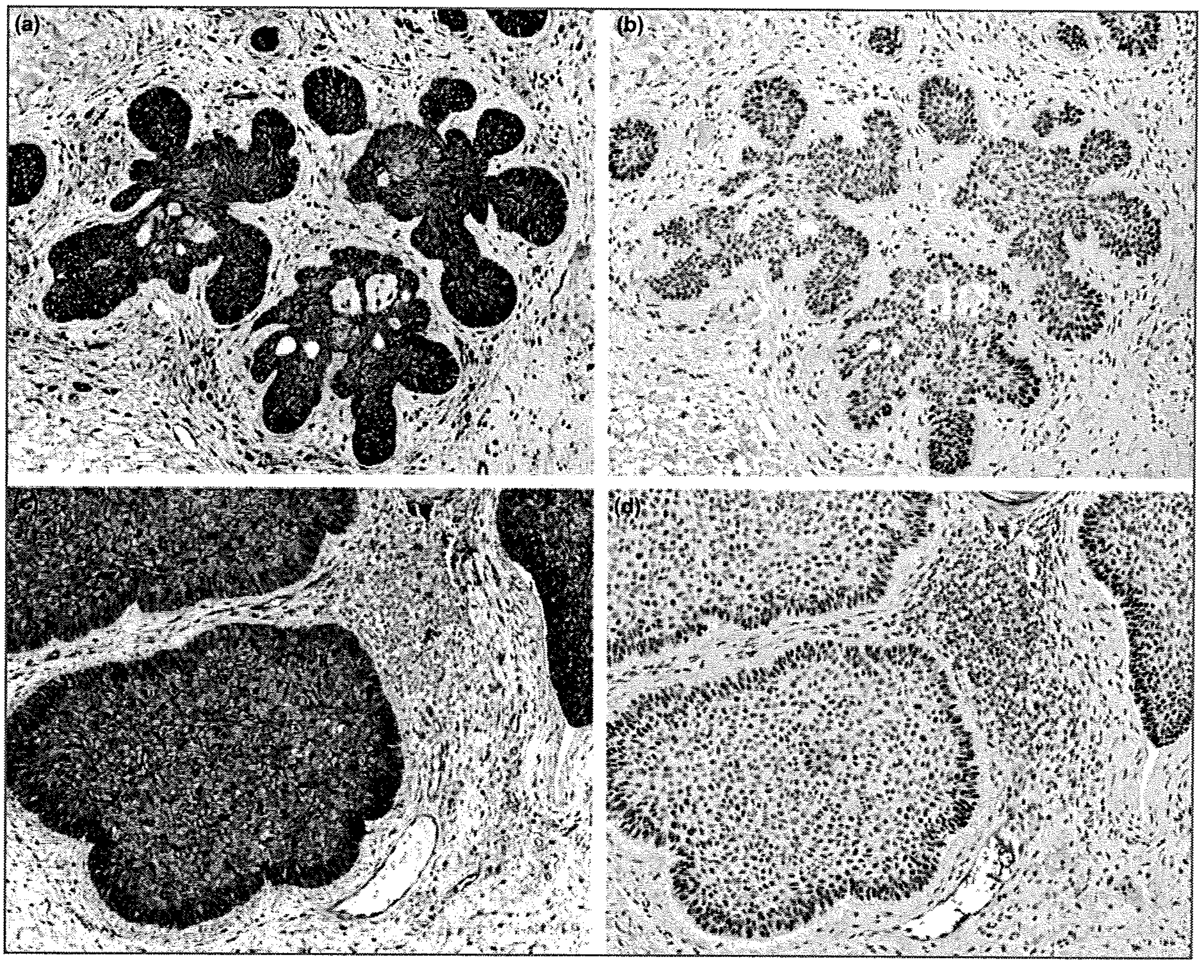

Fig 1. β-catenin and LEF-1 expression patterns. Two patterns of β-catenin and LEF-1 expression were distinguishable in basal cell carcinoma. (a, b) The invasive pattern of β-catenin (a) and LEF-1 (b) displayed an intense nuclear staining of the cells at the invasive margins and an absent nuclear staining of the cells in the tumour centre. (c, d) The diffuse pattern exhibited positive nuclear β-catenin (c) and LEF-1 (d) tumour cells on the periphery and positive cells in the tumour centre. Original magnification: (a–d) × 200.

BJD British Journal of Dermatology

Analysis of Taiwanese ichthyosis vulgaris families further demonstrates differences in *FLG* mutations between European and Asian populations

C-K. Hsu,*†‡ M. Akiyama,* I. Nemoto-Hasebe,* T. Nomura,*§ A. Sandilands,§ S-C. Chao,† J.Y-Y. Lee,† H-M. Sheu,† W.H.I. McLean§ and H. Shimizu*

*Department of Dermatology, Hokkaido University Graduate School of Medicine, North 15 West 7, Kita-ku, Sapporo 060-8638, Japan

†Department of Dermatology and ‡Institute of Clinical Medicine, College of Medicine, National Cheng Kung University Hospital, Tainan, Taiwan

§Epithelial Genetics Group, Division of Molecular Medicine, University of Dundee, Colleges of Life Sciences and Medicine, Dentistry and Nursing, Dundee, U.K.

Summary

Correspondence
Masashi Akiyama.
E-mail: akiyama@med.hokudai.ac.jp

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Key words
atopic eczema, filaggrin, FLG, ichthyosis, mutation

Conflicts of interest
W.H.I.M. has filed patents relating to genetic testing and therapy development aimed at the filaggrin gene.

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Background Mutations in the gene encoding filaggrin (FLG) were identified to underlie ichthyosis vulgaris (IV) and also shown to predispose to atopic eczema. Until now, no FLG mutations have been described in the Taiwanese population.

Objectives To elucidate filaggrin mutations in the Taiwanese population and further to clarify the population genetics of filaggrin gene mutations in the Asian populations.

Methods In the present study, 12 individuals from four unrelated Taiwanese IV families were examined for FLG mutations. We carried out comprehensive sequencing of the entire FLG coding region using an overlapping polymerase chain reaction strategy.

Results We identified three FLG mutations in the Taiwanese IV families. One mutation E1795X was a previously unidentified FLG mutation, which might be specific to the Taiwanese. Interestingly, another FLG mutation 3321delA is prevalent in the Japanese population and the other mutation Q2417X was found in the Singaporean Chinese population. No FLG mutation identified in the white European population was found in the Taiwanese population.

Conclusions The present findings suggest that the Taiwanese population, as an East Asian group, share FLG mutations with both the Japanese and the Singaporean Chinese population. In addition, these results exemplify differences in the population genetics of filaggrin between Europe and Asia.

Ichthyosis vulgaris (IV; OMIM 146700) is a common inherited skin disorder exhibiting scaling and dry skin that is particularly prominent on the extensor surfaces of limbs and the lower abdomen, and is associated with palmoplantar hyperlinearity.[1–3] Histologically, IV is characterized by a decrease in the size and number or complete absence of keratohyaline granules in the upper epidermis.[1] Loss-of-function mutations in the profilaggrin/filaggrin gene (FLG) which resides within the epidermal differentiation complex on chromosome 1q21 have been identified as the cause of IV.[2] After establishment of sequencing methods for the entire FLG coding region,[4] 22 nonsense or frameshift mutations in FLG have been reported to date.[5] Most FLG mutations are specific to particular ancestral groups, such as the white European,[4] Japanese[6,7] and Singaporean Chinese populations.[8]

In this study, we identified three FLG mutations in Taiwanese IV families. One mutation E1795X was a previously unidentified FLG mutation which might be specific to the Taiwanese population. The other two mutations were recurrent mutations previously identified in the Japanese or the Singaporean Chinese population, but not in the white European population. These results exemplify differences in the population genetics of filaggrin between Europe and Asia.

Materials and methods

Patients

In total, 12 individuals from four unrelated Taiwanese IV families, designated families 1–4, were examined for FLG mutations (Fig. 1). The diagnosis of IV was established from clinical features of variable scaling on the extremities, dry skin, palmoplantar hyperlinearity, early onset and a positive family history. These Taiwanese families had no traceable Japanese or

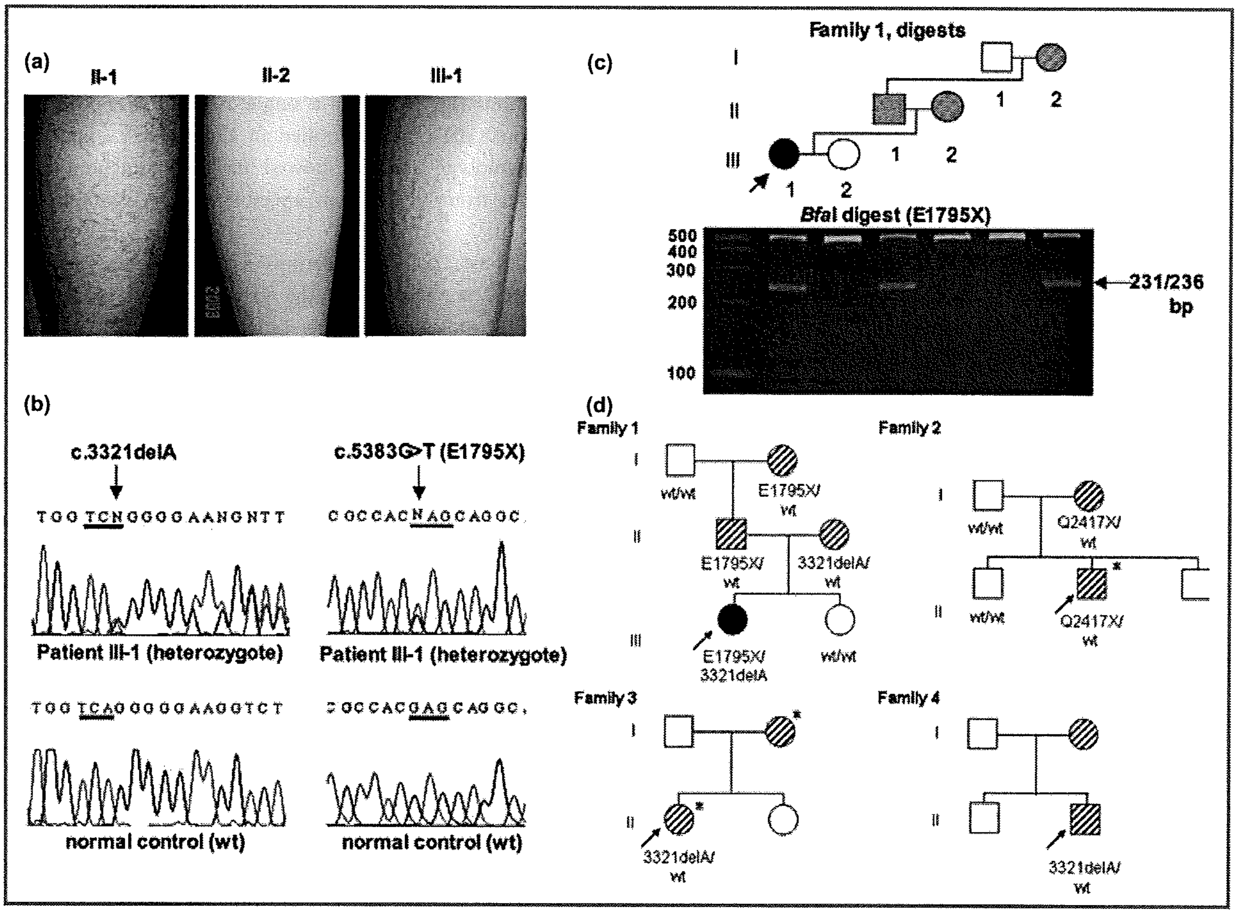

Fig 1. *FLG* mutations in Taiwanese ichthyosis vulgaris (IV) families. (a) Fine scaling on the patients with IV in family 1. The proband (III-1) showed a more severe IV phenotype than her father (II-1) and mother (II-2). (b) The proband of family 1 was a compound heterozygote for a deletion mutation c.3321delA and a previously unidentified nonsense mutation c.5383G>T (E1795X). (c) Verification of the mutation E1795X by *BfaI* restriction enzyme digestion: 467-bp fragments amplified from the mutant alleles are digested into 236-bp and 231-bp fragments, whereas those from wild-type alleles are uncut. The polymerase chain reaction products from I-2, II-1 and III-1 (heterozygotes for E1795X) revealed two bands, 467-bp and 236-bp/231-bp bands, because 236-bp and 231-bp products were detected as one overlapping band in this gel electrophoresis. I-1, II-2 and III-2 had only wild-type alleles. (d) Family trees and *FLG* mutations in the IV families studied. Solid symbols refer to the marked IV presentation; cross-hatched symbols refer to the milder IV presentation. In addition, three of 10 patients with IV had concomitant dermatologist-diagnosed atopic eczema (*). wt, wild-type for *FLG* mutations.

European ancestry, and they were all from the southeast coast of China. We performed a skin biopsy from the upper left arm of the proband in family 4. Light microscopy and immunohistochemical staining were performed as previously described[6] and showed mild hyperkeratosis with a marked reduction in epidermal filaggrin expression compared with healthy individuals.

Mutation detection

Genomic DNA samples from peripheral blood cells of the patients and their family members were analysed for filaggrin mutations. The medical ethical committee at Hokkaido University Graduate School of Medicine and the Human Experiment and Ethics Committee of National Cheng Kung University Hospital approved all the studies. The study was conducted according to the Declaration of Helsinki Principles. Participants or their legal guardians gave their written, informed consent.

All samples from the four IV families were screened for the six previously reported Japanese-specific mutations (3321delA, S1695X, Q1701X, S2554X, S2889X and S3296X),[6,7,9] as well as the six mutations found in Singaporean Chinese (441delA, 1249insG, Q2417X, E2422X, 7945delA and R4307X).[8] *FLG* mutations were studied by direct sequencing, fluorescent polymerase chain reaction (PCR) and/or enzyme digestion assays as described previously.

To identify further mutations, we carried out comprehensive sequencing of the *FLG* gene using an overlapping PCR strategy that allows routine diagnostic sequencing of the entire filaggrin coding sequence.[4]

Results

We identified two recurrent mutations: 3321delA, previously reported in the Japanese population, in three families (families

1, 3 and 4) and Q2417X, previously reported in the Singaporean Chinese population, in one family (family 2) of the four Taiwanese IV families studied here (Fig. 1).

Sequencing of the entire coding region of *FLG* revealed a previously unidentified *FLG* mutation, E1795X, in the proband, her father and paternal grandmother of family 1 (Fig. 1). Presence of the mutation E1795X was confirmed by enzyme digestion assay using restriction enzyme *BfaI* (Fig. 1). This mutation was not detected in 50 unrelated control alleles. The proband in family 1 was compound heterozygous for E1795X and 3321delA and showed a much more severe phenotype than that of her parents, consistent with the reported semidominant pattern of inheritance.[2]

Discussion

Previously reported *FLG* mutations seem to be population specific. Several prevalent *FLG* mutations were reported in the European populations.[4] However, these mutations were rarely found in the Japanese[6] or in the Singaporean Chinese populations.[8] The Japanese and the Singaporean Chinese populations were reported to have *FLG* mutations specific to their own populations (Fig. 2).[6–8] In a single case, the European-specific mutation R501X was identified in a Japanese family;[10] however, haplotype analysis showed that the mutation was not inherited from a European ancestor but recurred *de novo* in Japan.

Until now, no *FLG* mutations have been described in the Taiwanese population. In the present study, we identified three *FLG* mutations in the Taiwanese population. Interestingly, one *FLG* mutation 3321delA is prevalent in the Japanese population.[6,9] Another mutation Q2417X was found in the Singaporean Chinese population.[8] The remaining mutation, E1795X, is a previously unidentified *FLG* mutation which might be unique in the Taiwanese population. The Taiwanese are a mixture of people originating from both south and north China, and native Taiwanese people. The Japanese population comprises native Japanese and immigrants from the Asian

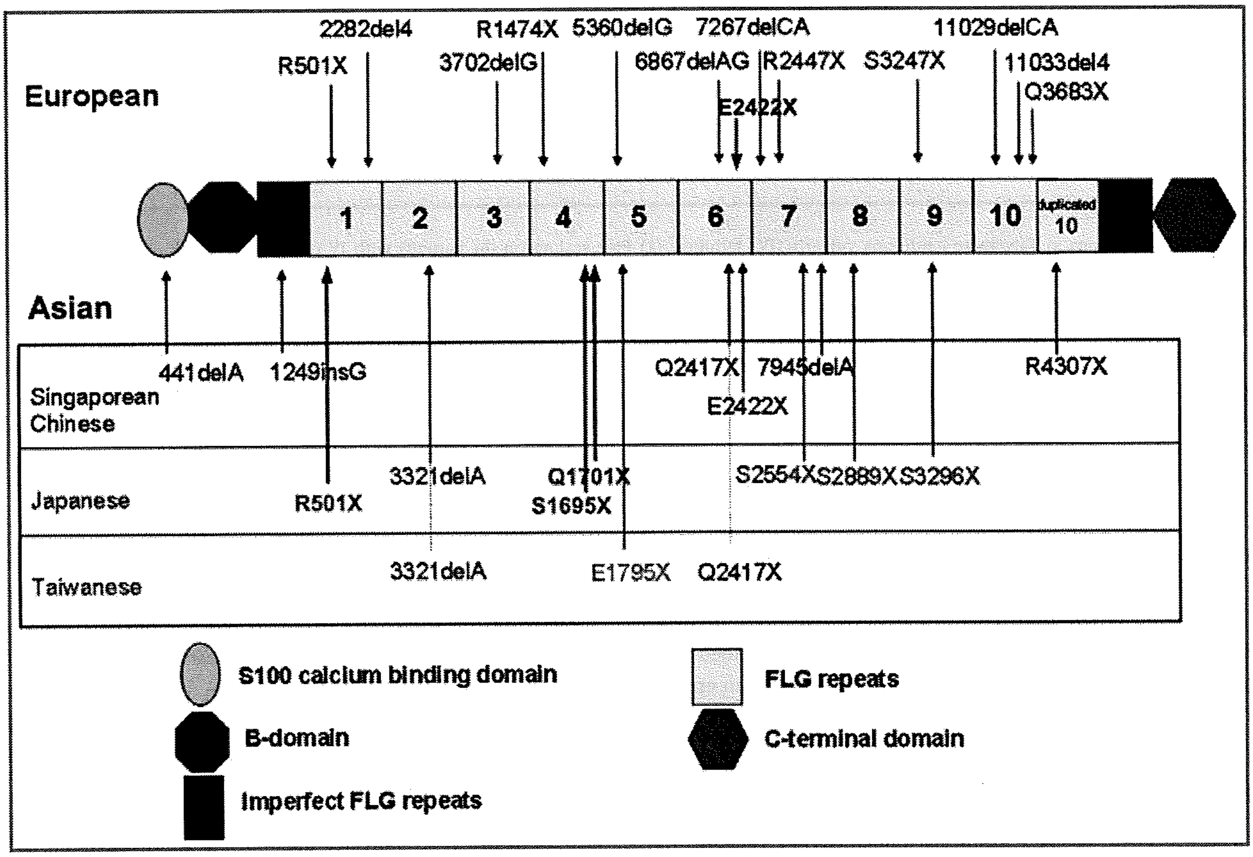

Fig 2. Difference in *FLG* mutations between European and Asian populations. Molecular structure of profilaggrin and *FLG* mutations detected among the European (top) and the Asian (bottom) populations. Profilaggrin contains 10–12 highly homologous filaggrin-repeat domains. *FLG* mutations among the European and the Asian populations appear to be unique. The previously identified *FLG* mutation 3321delA in the Japanese population (red) and Q2417X in the Singaporean Chinese population (blue) are found in the present Taiwanese IV families. E1795X is a previously unidentified mutation. Interestingly, the Taiwanese population shares *FLG* mutations with both the Singaporean Chinese population (Q2417X) and the Japanese population (3321delA), although the Singaporean Chinese population and the Japanese population do not share any of the known *FLG* mutations.

continent via Korea or China. Thus, the present results might be reasonable from the aspect of East Asian population genetics.

None of the previously reported FLG mutations identified in the European population was found in the Taiwanese population. The present results further support the notion that FLG mutation spectra of the white European and the Asian ancestral groups are different, as 25–50% of patients with atopic eczema are expected to harbour FLG mutations.[4,6] In such patients, skin barrier defects due to filaggrin deficiency are thought to play an essential role in the pathogenesis of the disease.[11,12] Thus, it is very important and useful for us to know whether a patient with atopic eczema has an FLG mutation or not, when we see the patient. However, every population is likely to have a unique set of FLG mutations. Specifically, we cannot use the prevalent European FLG mutations to screen Asian patients with atopic eczema. For the proper population-specific mutation screening, we have to obtain information on prevalent FLG mutations in each population. It is therefore important to clarify the worldwide population genetics of FLG mutations.

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Deficient deletion of apoptotic cells by macrophage migration inhibitory factor (MIF) overexpression accelerates photocarcinogenesis

Ayumi Honda[1,2,†], Riichiro Abe[2,†], Yoko Yoshihisa[1], Teruhiko Makino[1], Kenji Matsunaga[1], Jun Nishihira[3], Hiroshi Shimizu[2] and Tadamichi Shimizu[1,*]

[1]Department of Dermatology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Sugitani, Toyama 930-0194, Japan, [2]Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo 060-8638, Japan and [3]Department of Medical Information, Hokkaido Information University, Ebetsu 069-8585, Japan

*To whom correspondence should be addressed. Tel: +81 76 434 7305;
Fax: +81 76 434 5028;
Email: shimizut@med.u-toyama.ac.jp

Chronic ultraviolet (UV) exposure can increase the occurrence of p53 mutations, thus leading to a dysregulation of apoptosis and the initiation of skin cancer. Therefore, it is extremely important that apoptosis is induced quickly after UV irradiation, without any dysregulation. Recent studies have suggested a potentially broader role for macrophage migration inhibitory factor (MIF) in growth regulation via its ability to antagonize p53-mediated gene activation and apoptosis. To further elucidate the possible role of MIF in photocarcinogenesis, the acute and chronic UVB effect in the skin was examined using macrophage migration inhibitory factor transgenic (MIF Tg) and wild-type (WT) mice. The MIF Tg mice exposed to chronic UVB irradiation began to develop skin tumors after ∼14 weeks, whereas the WT mice began to develop tumors after 18 weeks. A higher incidence of tumors was observed in the MIF Tg in comparison with the WT mice after chronic UVB irradiation. Next, we clarified whether the acceleration of photo-induced carcinogenesis in the MIF Tg mice was mediated by the inhibition of apoptosis There were fewer sunburned cells in the epidermis of the MIF Tg mice than the WT mice after acute UVB exposure. The epidermis derived from the MIF Tg mice exhibited substantially decreased levels of p53, bax and p21 after UVB exposure in comparison with the WT mice. Collectively, these findings suggest that chronic UVB exposure enhances MIF production, which may inhibit the p53-dependent apoptotic processes and thereby induce photocarcinogenesis in the skin.

Introduction

Exposure to ultraviolet (UV) radiation leads to various acute deleterious cutaneous effects including sunburn and immunosuppression and also long-term consequences such as premature aging and the potential development of skin cancers (1). UV radiation, particularly UVB, which has a wavelength of between 280 and 320 nm, has been suggested epidemiologically and has been demonstrated experimentally to be the pivotal causal factor for skin cancer in humans and other animals (2). Chronic UVB-induced inflammation and directly damaged DNA can be correlated with skin tumor formation (3,4). Furthermore, the inability to adequately repair DNA after UVB irradiation can result in the formation of skin cancers (5). Chronic UV exposure can increase p53 mutations, thus leading to a dysregulation of apoptosis, an expansion of mutated keratinocytes and the initiation of skin cancer (6).

There is emerging evidence that keratinocytes participate in cutaneous inflammatory reactions and immune responses by producing a variety of cytokines. UV irradiation may trigger cutaneous inflammatory responses by stimulating epidermal keratinocytes to produce biologically potent cytokines such as interleukin (IL)-1 (7,8), IL-6 (9) and tumor necrosis factor (TNF)-α (10). These cytokines are involved not only in the mediation of local inflammatory reactions but also play discrete roles in tumor promotion (11).

The cytokine macrophage migration inhibitory factor (MIF) was first discovered 40 years ago as a T-cell-derived factor that inhibited the random migration of macrophages (12,13). Recently, MIF was reevaluated as a proinflammatory cytokine and pituitary-derived hormone that potentiates endotoxemia (14). Subsequent work has showed that T cells and macrophages secrete MIF in response to glucocorticoids as well as upon activation by various proinflammatory stimuli (15). It has been reported that MIF is expressed primarily in T cells and macrophages; however, recent studies have revealed this protein to be ubiquitously expressed in various cells (16–20). Skin melanoma cells express MIF messenger RNA (mRNA) and produce MIF protein (21). The expression of MIF mRNA and the production of MIF protein have been shown to be much higher in human melanoma cells than in cultured normal melanocytes. Therefore, MIF functions as a novel growth factor that stimulates uncontrolled growth and invasion of tumor cells (16,21,22). In addition, recent studies have suggested a potentially broader role for MIF in growth regulation because of its ability to antagonize p53-mediated gene activation and apoptosis (23,24).

In the skin, keratinocytes are capable of producing a variety of cytokines and are thought to be a principal source of cytokines from the epidermis after UV irradiation. Previous studies have shown enhanced MIF production in the skin after UVB irradiation (25,26). Solar UV light is a combination of both UVB and UVA wavelengths, each of which stimulate MIF production in both keratinocytes and fibroblasts in the skin. To further elucidate the possible role of MIF in UV-induced carcinogenesis and cell apoptosis, the acute and chronic effect of UVB in skin carcinogenesis was examined using macrophage migration inhibitory factor transgenic (MIF Tg) mice.

Materials and methods

Materials

The following materials were obtained from commercial sources. The Isogen RNA extraction kit was obtained from Nippon Gene (Tokyo, Japan); the DNA random primer labeling kit from Takara (Kyoto, Japan); [^{32}P]dCTP from DuPont-NEN (Boston, MA); anti-CPDs polyclonal antibody from Cosmo Bio Co, Ltd (Tokyo, Japan); anti-p53 polyclonal antibody from Novocastra Lab (Newcastle, UK); anti-p21 polyclonal antibody and anti-BAX polyclonal antibody from Santa Cruz Biotechnology (Santa Cruz, CA) and anti-β-actin antibodies purchased from Sigma–Aldrich Co (St Louis, MO); the western blot detection system was obtained from Cell Signaling Technology (Beverly, MA). The anti-MIF polyclonal antibody was prepared as described previously (27). The Cell Death Detection Kit was provided from Roche Molecular Biochemicals (Indianapolis, IN). Other reagents were of analytical grade.

Mice

The MIF-overexpressed transgenic mice were established following cDNA microinjection and the physical and biochemical characteristics, including body weight, blood pressure, serum levels of cholesterol and blood sugar, were normal as reported previously (28). The expression of the transgene was regulated by a hybrid promoter composed of the cytomegalovirus enhancer and β-actin/β-globin promoter, as reported previously (29). Strain of original MIF-Tg is ICR and backcrossed with C57BL/6 for at least 10 generations. Tg mice were maintained by heterozygous sibling mating. Transgenic and wild-type (WT) mice were maintained under specific-pathogen-free conditions at the Institute for Animal Experiments of Hokkaido University School of Medicine. Experiments using mice were conducted according to the guidelines set out by

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Abbreviations: CPD, cyclobutane pyrimidine dimmer; IL, interleukin; MIF, macrophage migration inhibitory factor; MIF Tg, macrophage migration inhibitory factor transgenic; mRNA, messenger RNA; PBS, phosphate-buffered saline; TUNEL, terminal deoxynucleotidyl transferase nick end labeling; TNF, tumor necrosis factor; UV, ultraviolet; WT, wild-type.

[†]These authors contributed equally to this work.

the Hokkaido University Institutional Animal Care and Use Committee under an approved protocol. All experiments were performed on 8-week-old male adult mice.

UVB irradiation

UVB light source was a FL20SE30 (Clinical Supply Co, Tokyo, Japan) fluorescent lamp that emits 1.0 mW/cm^2 of UV between 280 and 370 nm (peak 305 nm) at a distance of 25 cm, as measured by UV radiometer (Torex Co, Tokyo, Japan). In short-term UVB experiments, MIF Tg and WT mice had their backs shaved with electric clippers and exposed to 200 mJ/cm^2 UVB. After UVB irradiation, the mice were euthanized at the indicated time points. Skin sections were excised from the dorsal surface and used for western blot analyses or immunohistochemical staining. In some experiments for UVB-induced cutaneous inflammation, UVB radiation was administered three times weekly (on days 1, 3 and 5) and skin was obtained on day 7. To examine UVB-induced carcinogenesis, MIF Tg and WT mice had their backs shaved with electric clippers once a week and were UVB irradiated in separate compartments of a modified mouse cage. An incrementally graded UV protocol was used (30): three times weekly a UV dose was delivered of 2.25 kJ/m^2 for 12 treatments (weeks 1–4), 4.05 kJ/m^2 for 24 treatments (weeks 5–12), 5.1 kJ/m^2 for 12 treatments (weeks 13–16) and 6 kJ/m^2 for 33 treatments (week 17 to the end of the experiment at the 27th week).

Skin tumors

Mice were monitored for tumor formation each week. The time to tumor development was taken as the time up to the appearance of a palpable swelling >1 mm subsequently diagnosed as a tumor on histopathological examination after 27 weeks. The tumor size was estimated after 27 weeks using orthogonal linear measurements made with Vernier calipers according to the following formula: volume (mm^3) = [(width, mm)^2 × (length, mm)]/2. The tumors were excised and preserved in 10% formalin, sectioned, stained with hematoxylin and eosin and examined microscopically. The groups each contained 12 MIF Tg and 12 WT mice.

Northern blot analysis

Total cellular RNA was isolated from the epidermis using an Isogen extraction kit according to the manufacturer's protocol. The epidermis was separated from the dermis by incubation in 0.5% dispase in RPMI 1640 at 37°C for 1 h. RNA was quantified by spectrophotometry and equal amounts of RNA (10 μg) from each sample were loaded on a formaldehyde–agarose gel. The gel was stained with ethidium bromide to visualize the RNA standards and the RNA was transferred onto a nylon membrane. Fragments obtained by restriction enzyme treatment for MIF and glyceraldehyde-3-phosphate dehydrogenase were labeled with [α-^{32}P]dCTP using a DNA random primer labeling kit. Hybridization was carried out using the mouse MIF cDNA probe as previously described (28). The membrane was washed twice with 2× saline and sodium citrate (16.7 mM NaCl, 16.7 mM sodium citrate) at 22°C for 5 min, twice with 0.2× saline and sodium citrate containing 0.1% sodium dodecyl sulfate at 65°C for 15 min and twice with 2× saline and sodium citrate at 22°C for 20 min prior to autoradiography. A quantitative densitometric analysis was performed using an MCID Image Analyzer (Fuji Film, Tokyo, Japan). The density of MIF bands was normalized by the intensities of glyceraldehyde-3-phosphate dehydrogenase.

Western blot analysis

The epidermis of each mouse was homogenized with a Polytron homogenizer (Kinematica, Lausanne, Switzerland). The protein concentrations of the cell homogenates were quantified using a Micro BCA protein assay reagent kit. Equal amounts of homogenates were dissolved in a 20 μl solution contained of Tris–HCl, 50 mM (pH 6.8), containing 2-mercaptoethanol (1%), sodium dodecyl sulfate (2%), glycerol (20%) and bromophenol blue (0.04%) and the samples were heated to 100°C for 5 min. The samples were then subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electrophoretically transferred onto a nitrocellulose membrane. The membranes were blocked with 1% non-fat dry milk powder in phosphate-buffered saline (PBS), probed with antibodies against p53, bax and p21 and subsequently reacted with secondary IgG antibodies coupled with horseradish peroxidase. The resultant complexes were processed for the detection system according to the manufacturer's protocol. The relative amounts of proteins associated with specific antibodies were normalized according to the intensities of β-actin.

Immunohistochemical analysis

Five micrometers thick section of dorsal skin were fixed in 10% neutral buffered formalin. After deparaffinization, the sections were treated with target retrieval solution (DAKO, Carpinteria, CA), washed three times with PBS and incubated in H_2O_2/methanol/PBS solution (1:50:50) for 15 min to block endogenous peroxidase activity. After three washes in PBS with 0.5% Tween, the sections were preincubated for 10 min in 10% normal goat serum in PBS and then were incubated with the first antibody overnight at 4°C. After three washes in PBS plus 0.5% Tween, the sections were incubated for 1 h at room temperature with the secondary antibodies. After washing in PBS, staining was performed using the Vectastain Elite ABC kit with diaminobenzidine as the chromagen, according to the manufacturer's instructions (Vector Laboratories, Burlingame, CA). As a negative control, the tissue sections were stained with normal serum and the secondary antibody.

UVB-induced apoptosis in cultured keratinocytes of MF Tg and WT. Mouse keratinocyte (second passage) from MIF Tg or C57BL/6 mice were irradiated with UVB at 50 mJ/cm^2. After 24 h, irradiated cells were analyzed for terminal deoxynucleotidyl transferase nick end labeling (TUNEL) assay or western blot for p53.

TUNEL assay. Cells undergoing apoptosis were detected using TUNEL according to the manufacturer's recommended procedure (R&D Systems, Minneapolis, MN). For statistical analysis, apoptotic cells were counted by light microscopy (×100) and expressed as the mean number (±SD) of apoptotic cells per section. Five random fields per section (one section per mouse, five mice per group) were analyzed.

Cultured apoptotic cells were also detected using TUNEL. Incorporated fluorescein was detected by anti-fluorescein monoclonal antibody Fab fragments from sheep, conjugated with alkaline phosphatase.

Statistics

Values are expressed as the mean ± SEM of the respective test or control group. Statistical significance between the control group and test groups was evaluated by either the Student's *t*-test or one-way analysis of variance.

Results

Enhanced expression of MIF in MIF Tg mice epidermis

MIF expression in the MIF Tg mouse epidermis was first examined after UVB irradiation. Northern blot analysis revealed that 16 h after 200 mJ/cm^2 UVB irradiation, MIF Tg mice showed higher levels of MIF mRNA expression even before irradiation. After UVB exposure, the MIF mRNA expression dramatically increased in comparison with that of the WT mice (Figure 1).

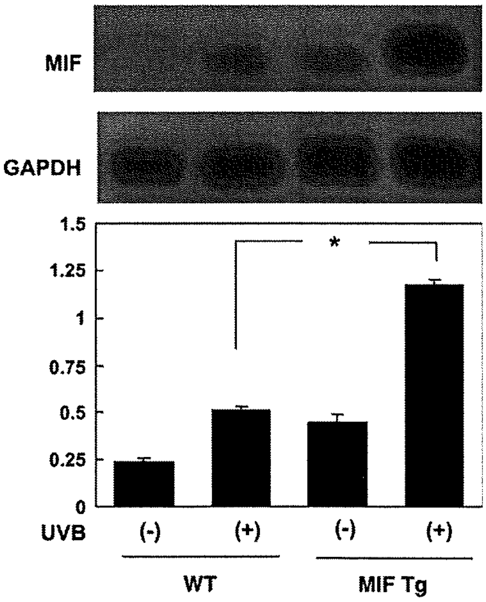

Fig. 1. Enhanced expression of MIF in the MIF Tg mouse epidermis after UV exposure. The expression of MIF mRNA was examined. Total RNA was isolated at 16 h after UVB (200 mJ/cm^2) and analyzed by northern blotting. MIF Tg mice ($n = 5$) showed higher levels of MIF mRNA expression even before irradiation. After UVB exposure, MIF mRNA expression dramatically increased in comparison with that of WT mice ($n = 5$). The experiments were repeated three times with similar results.

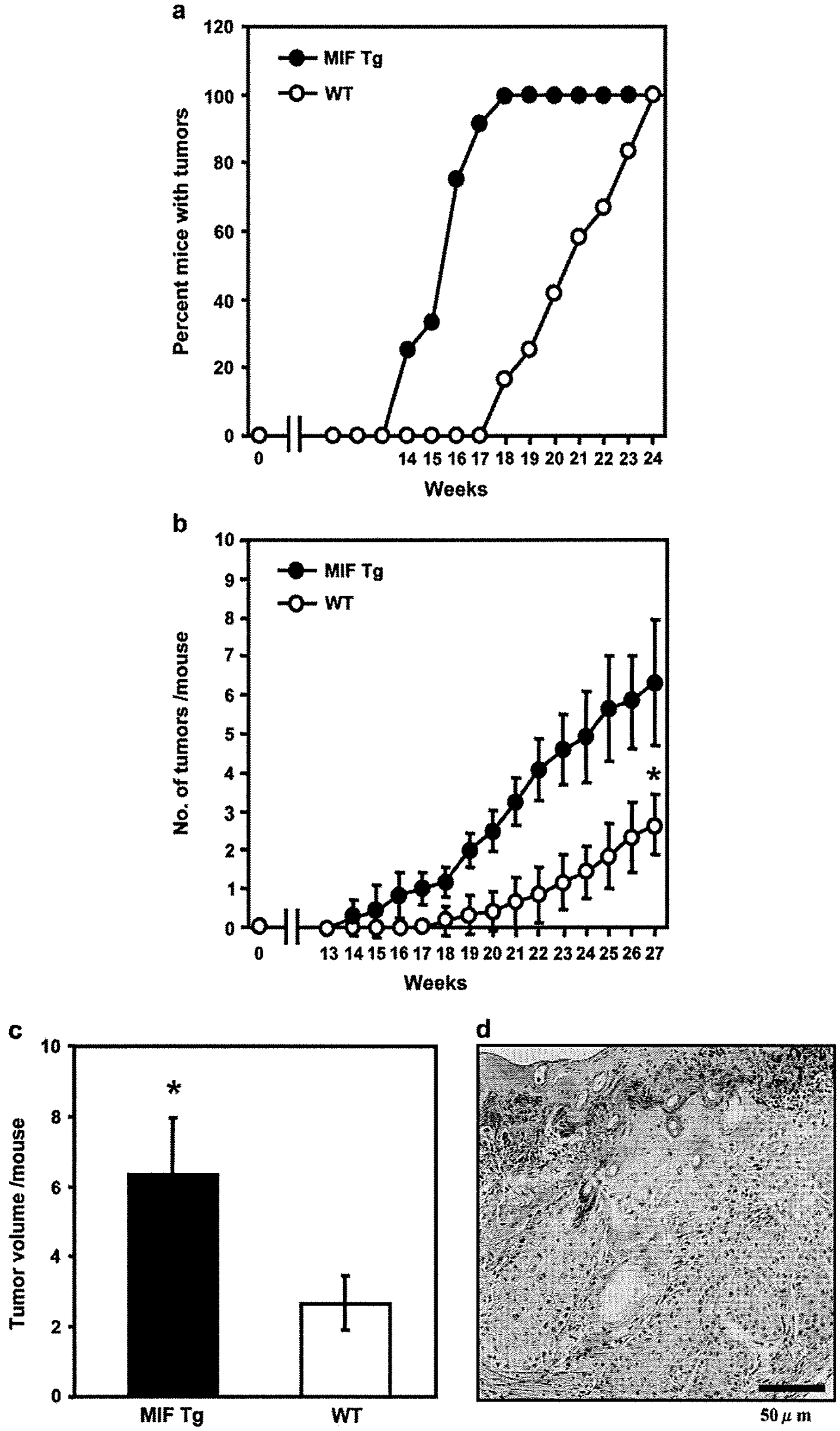

Fig. 2. Accelerated UVB-induced carcinogenesis in the MIF Tg mice. (**a**) MIF Tg and WT mice were subjected to chronic UVB. The details of the protocols are described in Materials and Methods. The formation of skin tumors was determined on a weekly basis. MIF Tg mice exposed to chronic UVB began to develop skin tumors after ~14 weeks, whereas the WT mice began to develop tumors after 18 weeks. (**b**) The incidence of skin tumors was recorded weekly and a tumor was considered to occur when an outgrowth of >1 mm in diameter was observed. MIF Tg mice developed a higher number of tumors in each mouse in comparison with WT mice ($^{*}P < 0.001$). (**c**) The mice were UVB irradiated as in (b). At the end of study at week 27, the volume of all tumors on each mouse was recorded ($^{*}P < 0.001$). (**d**) The histopathology of well-differentiated squamous cell carcinoma from an MIF Tg mouse. Scale bar indicates 100 μm (hematoxylin and eosin staining).

Sensitivity of MIF Tg mice to the development of skin tumors elicited by chronic exposure to UVB

To examine the role of MIF for chronic UV-induced carcinogenesis, MIF Tg and WT mice were subjected to chronic UVB as described in the Materials and Methods and followed up for the formation of skin tumors on a weekly basis. The MIF Tg mice exposed to chronic UVB began to develop skin tumors after ~14 weeks, whereas WT mice began to develop tumors after 18 weeks (Figure 2a). The mean time for tumor development in MIF Tg mice was after 110.3 ± 9.0 days, whereas it was 147.0 ± 15.5 days in WT mice. MIF Tg mice developed a higher number of tumors in each mouse in comparison with WT mice. At the 27th week, the average number of tumors per mouse was 6.33 ± 1.61 in the MIF Tg mice, whereas there were only 2.67 ± 0.78 in the WT mice ($P < 0.001$; Figure 2b). The volume of tumors developed in UVB-irradiated MIF Tg mice was significantly higher in comparison with that of WT mice ($P < 0.001$) (Figure 2c). Tumors measuring <2 mm in diameter proved to be too small for a reliable histological analysis and were assumed to be papillomas. Lesions that were ~2 mm in diameter had multilayered epithelia with irregular cells. These lesions were similar to actinic keratosis, and some large tumors (>3 mm in diameter) were diagnosed as well-differentiated SCC (Figure 2d). Twelve unirradiated MIF Tg mice and 12 unirradiated WT mice developed no tumors during the course of this study.

TUNEL-positive cells in UV-irradiated MIF Tg mouse epidermis

The possible role of MIF in UV-induced cell apoptosis was examined using MIF Tg and WT mice. Twenty-four hours after 200 mJ/cm^2 UVB irradiation, large numbers of sunburned cells and TUNEL-positive cells were detected in the WT mice, whereas, there were fewer sunburned cells and TUNEL-positive cells detected in MIF Tg mice (Figure 3a). Thereafter, the number of TUNEL-positive nuclei in the MIF Tg mice was compared with that in the WT mice. MIF Tg mice showed a significantly smaller number of apoptotic cells than the WT mice ($P < 0.01$; Figure 3b).

Immunohistochemistry: accumulation of DNA damages in the epidermis following UVB. We then investigated cyclobutane pyrimidine dimmers (CPD), as UV-induced DNA damage photoproduct

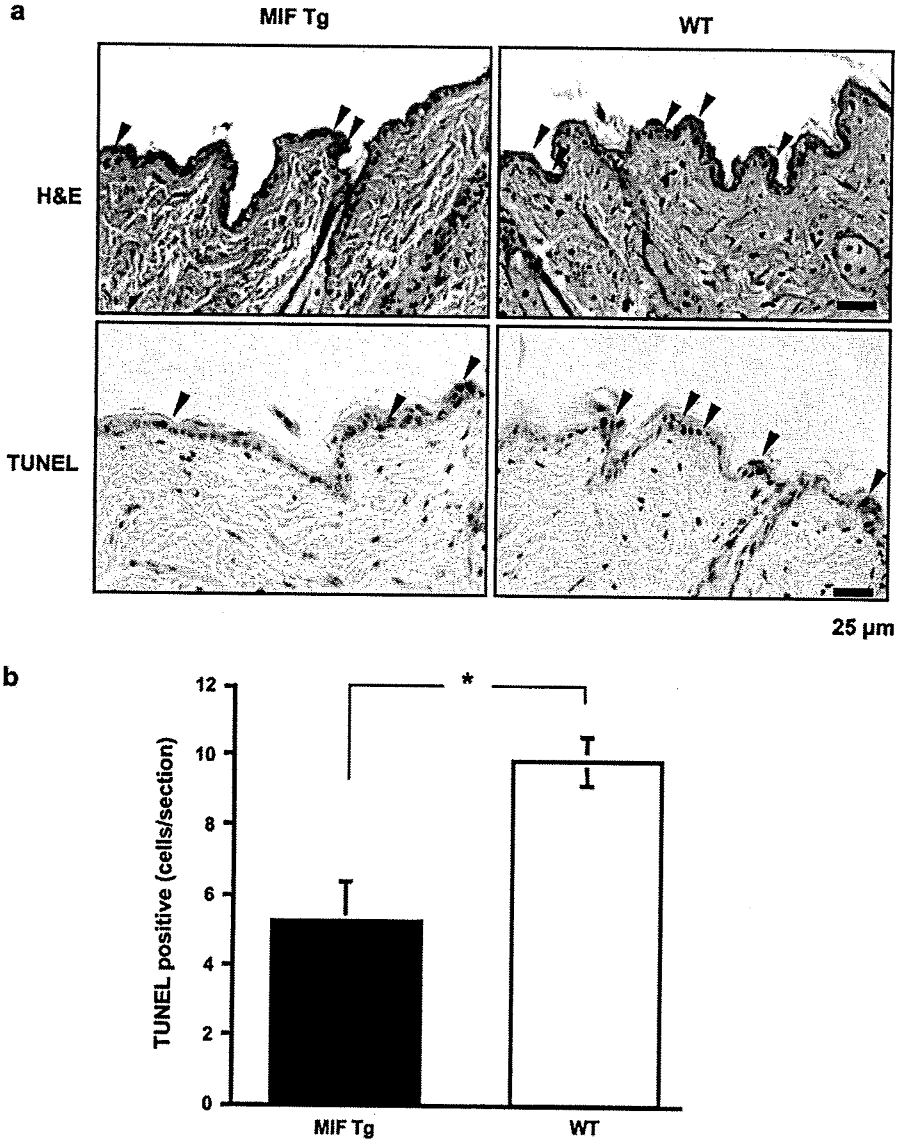

Fig. 3. Sunburn cells in UV-irradiated MIF Tg mouse epidermis. (a) Hematoxylin and eosin (H&E) staining and TUNEL assay for the detection of apoptotic cells in the epidermis of MIF Tg and WT mice skin 24 h after UVB irradiated (200 mJ/cm^2). Sunburn cells and TUNEL-positive cells are indicated by arrowheads. The scale bar indicates 25 µm. (b) The numbers of TUNEL-positive nuclei of MIF Tg mice were compared with the WT mice. Each value represents the mean ± SEM ($n = 5$). Smaller numbers of TUNEL-positive cells were observed in the MIF Tg in comparison with the WT mouse skin (*$P < 0.01$).

(31) in UVB-irradiated skin. Twenty-four or 48 hours after 200 mJ/cm^2 UVB irradiation, a large number of CPD-positive cells was detected in MIF Tg mice. Whereas, there were fewer CPD-positive cells detected in WT mice (Figure 4a). Thereafter, the number of CPD-positive cell in the MIF Tg mice was significantly higher compared with that in the WT mice ($P < 0.001$; Figure 4b).

p53, bax and p21 expression in UV-irradiated MIF Tg mice epidermis

p53 is a key factor in the photoreactive process and bax and p21 are important downstream proteins regulated by p53. To further confirm the role of MIF in influencing p53-mediated gene activation, the time course for the induction of p53, bax and p21 in 200 mJ/cm^2 UVB-irradiated mouse epidermis was investigated by western blot analysis with specific antibodies. The epidermis derived from MIF Tg mice exhibited decreased induction levels of p53 at 12 and 24 h after irradiation in comparison with the WT mice (Figure 5a). Similarly, the induction levels of bax and p21 from the MIF Tg mice substantially decreased in comparison with those of the WT mice at 48 and 72 h after UVB exposure. An immunohistochemical analysis revealed that at 24 h after UVB irradiation, intense nuclear p53 immunostaining was observed in the WT mice epidermis. In contrast, nuclear p53 immunostaining was low in the MIF Tg mice (Figure 5b). Similarly, at 48 h after UVB irradiation, a low level of p21 expression in and around the nuclei was observed in the MIF Tg mice in comparison with the WT mice. Bax immunoreactivity was both perinuclear and cytoplasmic and the MIF Tg mice showed a lower expression level compared with that of the WT mice at 48 h (Figure 5b).

UVB-induced cutaneous inflammation in MIF Tg and WT mice

UVB-induced infiltration of leukocytes is a major source of inflammatory reactions. Therefore, the effect of UVB-induced infiltration was examined in MIF Tg and WT mice after three courses of UVB exposure. UVB exposure in the MIF Tg mice resulted in greater leukocyte infiltration than in the UVB-irradiated WT mice skin ($P < 0.05$; Figure 6).

UVB-induced apoptosis in cultured keratinocytes of MF Tg and WT mice

To confirm that MIF overexpression prevents keratinocyte apoptosis, cultured keratinocyte from the MIF Tg or the WT mice were irradiated with UVB at 50 mJ/cm^2. After 24 h, irradiated cells were analyzed for TUNEL assay or western blot for p53. As shown in Figure 7a and b, apoptotic keratinocytes (TUNEL positive) from MIF Tg mice were significantly reduced compared with that of WT mice ($P < 0.005$). Furthermore p53 expression of MIF Tg keratinocytes was also lower than that of WT mice (Figure 7c).

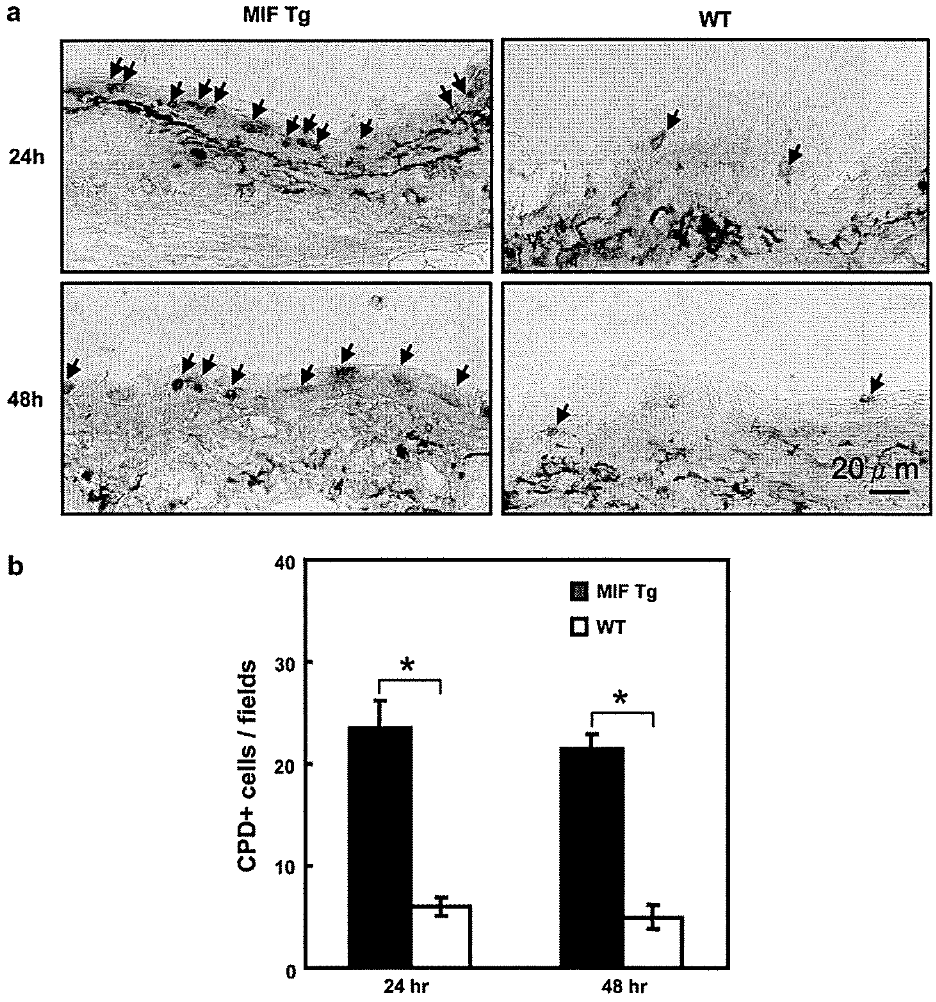

Fig. 4. CPD-positive cells in UV-irradiated MIF Tg mouse epidermis. (**a**) CPD staining in the epidermis of MIF Tg and WT mice skin 24 or 48 h after UVB irradiated (200 mJ/cm^2). CPD-positive cells indicated by arrowheads. Scale bar indicates 20 μm. (**b**) The numbers of CPD-positive cells of MIF Tg mice were compared with WT mice. Each value represents the mean ± SEM ($n = 5$) ($^*P < 0.001$).