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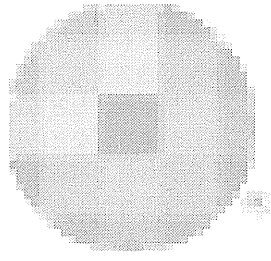

陸沿岸部震災被災地域との皮膚科遠隔診療の試み
ー陸前高田診療所（岩手県医師会）と岩手医科大学皮膚科との遠隔皮膚科診療ー

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The trial of dermatological tele-medicine with Rikuzen-Takata clinic (Iwate medical association) in the Sanriku shore area where is the earthquake disaster stricken area and the dermatology of Iwate Medical University

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要旨

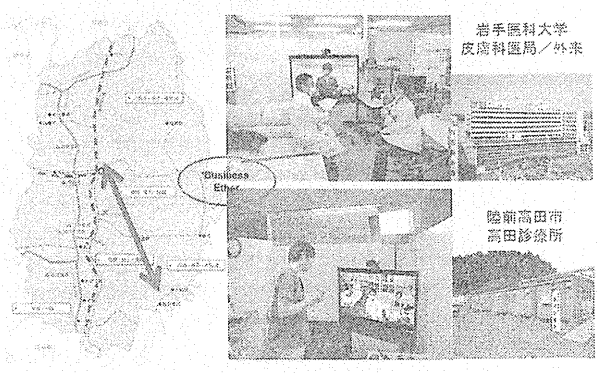
東日本大震災津波により壊滅的な被害を受けた陸前高田地域の皮膚科医は皆無となった。そこで、陸前高田診療所（岩手県医師会）と岩手医科大学皮膚科との遠隔皮膚科診療を試み、遠隔診療が医療過疎の解消の一助となるか検討した。通信方法は専用回線を用い、診療現場に検査機器、ムービーカメラ、照明器具を用い、岩手医科大学には皮膚科専門医が待機するシステムを構築して行った。その結果、1）遠隔医療機器システム立ち上げまでの時間は平均40分であった。2）1人の患者に要する遠隔医療の時間は平均34分であった。患者への説明と同意取得、診断機器や映像機器の切り替えに時間を要した。3）診断一致率は22例中21例が一致（95%）していた。診断確定に苦慮した例の多くは、①頭皮の毛髪間や指間、口腔内、陰部・殿裂部などの皮疹の焦点が合わない、②蕁麻疹など淡い紅斑の色調あるいは常色の軽い扁平な盛り上がりが画像で認識しがたい、③アナフィラキシー紫斑病など微小点状出血は映像では不明瞭である、④悪性黒色腫の初期病変や軽症の太田母斑の淡い黒色斑や青色斑は映像で不明瞭である、⑤真菌検査の菌糸の画像が不鮮明である、などであった。これらの問題は診断を補助する機器の充実で改善すると考えられた。4）患者からの遠隔診療に対する評価はVASで66%であった。①大きなモニター画像に映し出され、おどろいた、②診察のスキンシップが感じられない、⑤診療時間が長すぎる、⑥カメラに追い回されている感じがする、などの意見があった。しかし、意見の多くは専門医の診療・判断を仰ぐことができ、安心感を示すものが多くみられた。本研究によって、他科の医師と機器操作に熟練した技術員の存在のもとに皮膚科遠隔医療が可能であることが示唆された。しかし、緊急に改善するべき、①遠隔医療に関する受診者の理解、②運用性に優れたムービーカメラの精度向上、などの問題点が提起された。

キーワード：東日本大震災津波、陸前高田地域、皮膚科遠隔診療、高性能ムービーカメラ、NTT専用回線

1. はじめに

岩手県三陸沿岸地域は以前から医療過疎地域であった。皮膚科診療を有する総合病院が5カ所（うち皮膚科常勤医は1人）、皮膚科開業医診療所が3カ所と皮膚科領域についても医療過疎地域であった。東日本大震災津波により壊滅的な被害を受けた三陸沿岸地域、特に陸前高田地域では開業医1人によって皮膚科診療が行われていたが、震災に被災し大都市に避難したため同地域には皮膚科医は皆無となった。一方、岩手医科大学附属病院は、「岩手県東日本大震災津波復興計画」のなかで、被災した医療過疎地域に対して皮膚科領域も含め医療情報機器等を活用した遠隔医療によって高度な専門医療を提供する役割を求められている。

一方、従来の皮膚科遠隔医療は個別的な支援や簡単な疾患の診断に留まっており、検査や診断・治療など総合的医療の提供はできていない。本研究では三陸沿岸部震災被災地域である陸前高田診療所（岩手県医師会）と岩手医科大学皮膚科との遠隔皮膚科診療を試み、遠隔診療が医療過疎の解消の一助となるかを検討した。



【図1】遠隔医療実証実験プロジェクト概要

2. 目的

本研究では、①被災した医療過疎地域において災害拠点病院である大学病院が皮膚科遠隔医療によって高度医療を安定的に提供するための施設・設備・人員体制・コスト等についての検討と②対面診療と比較した遠隔医療の質についての検討を行う。①においては、専用回線を用い、診療現場に検査機器、ムービーカメラ、照明器具を用い、これらの器材の使用法に熟練した人材がいることでることで、また、岩手医科大学には皮膚科専門医が2名待機するシステムを構築する。②においては、皮膚疾患患者を対象として、陸前高田診療所における皮膚科専門医による対面診療と遠隔診療とを比較検討する【図1】。

3. 方法

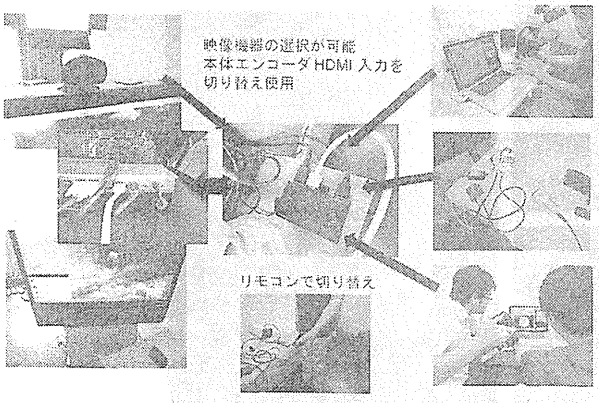
1. 研究倫理および記録保存

本研究は岩手医科大学倫理委員会の許可を得た。実験は患者のインフォームドコンセントを得て行う。患者情報や画像は匿名化し、個人を特定できないようにする。また、各患者の対面診療の動画は岩手医科大学情報センターにサーバー室を設けて保管した。医療情報は高田診療所の診療録に同診療所医師が記載し、同診療所に保管した。診療録の一部は患者および高田診療所の許可のもと、研究材料として用いた。

2. 利用回線および診療現場の器材

画像および医療情報の更新はNTT専用回線（NTT Business Ether）を使用した。実験に先駆けて、対面診療による問診のためにテレビ電話付き大型モニターを含むテレビ会議交信システム（フルHD（1080P/30fps））【図2】、患部の撮影のため2機の高性能ムービーカメラ、1機の接写カメラ、真菌検査および病理組織検査標本確認のためにオリンパス顕微鏡、患者情報記録のためノートパソコン、FAX機を設置した【図3】。それぞれを接続し、必要に応じてこれらの機器を切り替えて使用した。また、画像の色調を統一化、一定化するためにLED照明システムを使用した。これらのシステムで遠隔診断と医療提供が可能かを評価すると共にシステム設定にかかる時間も計測した。

【図2】高田診療所のシステム機器

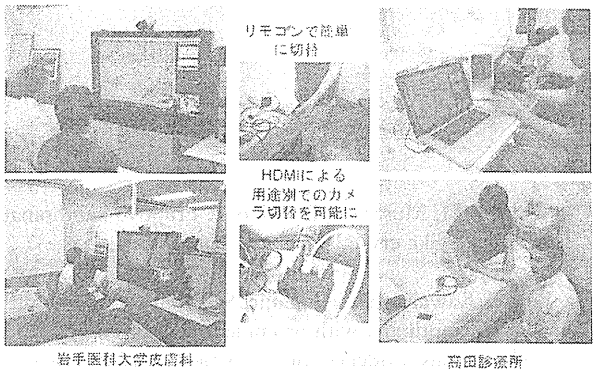


【図3】高田診療所カメラの切り替え状況

3. 遠隔対面診療の評価方法

研究の大半は高田診療所に皮膚科専門医が出向き、インフォームドコンセントの取得、患者の問診、診療録記載、処方箋発行、皮膚検査、機器の設定、皮膚病変の撮影、岩手医科大学皮膚科専門医との交信を行い、以下について評価した。平成25年1月末まで計22人の皮膚科患者の診療を行った【図4】。

1) 患者1人の診察時間
2) 診断名：高田診療所と岩手医科大学の皮膚科専門医の診断の一致率
3) 皮疹の部位で診断しにくい部位
4) 皮疹の形態で診断しにくい皮疹
5) 患者満足度（通常の対面診療と比較したVASで表示：100%が通常対面診療と同等、0%が全く対面診療に値しない）



【図4】皮膚科患者画像の送受信状況

4. 結果

1) 診療前の遠隔医療機器システム立ち上げまでにかかる時間は平均40分であった。熟練すると短縮可能と考えられた。
2) 1人の患者に要する遠隔医療の時間は最短26分、最長52分、平均34分であった。患者への説明と同意取得、診断機器や映像機器の切り替えに時間を要した。
3) 診断一致率は22例中21例が一致（95%）していた。診断確定に苦慮した例の多くは、①頭皮の毛髪間や指間、口腔内、陰部・殿裂部などの皮疹の映像の焦点が合わない、②蕁麻疹など淡い紅斑の色調あるいは常色の軽い扁平な盛り上がりが画像で認識しがたい、③アナフィラキシー紫斑病など微小点状出血は映像では不明瞭である、④悪性黒色腫の初期病変や軽症の太田母斑の淡い黒色斑や青色斑は映像で不明瞭である、⑤真菌検査の菌糸の画像が不鮮明である、などによるものであった。これらの問題は診断を補助する機器の充実で改善すると考えられた。
4) 患者からの遠隔診療に対する評価はVisual Analog Scale（VAS）で66%であった。①大きなモニター画像に映し出され、おどろいた、②診察のスキンシップが感じられない、⑤診療時間が長すぎる、⑥カメラに追い回されている感じがする、などの意見があった。しかし、意見の多くは専門医の診療・判断を仰ぐことができ、安心感を示すものが多くみられた。

5. 考察

本研究の最終目標は遠隔地に皮膚科専門医がいない状況での遠隔診療である。他科の医師と機器操作に熟練した技術員の存在のもとに皮膚科遠隔医療が可能であることが示唆された。しかし、緊急に改善するべき以下の問題点が提起された。①遠隔医療に関する受診者の理解、②他科の医師の皮膚科遠隔医療に対する理解、③カメラ、検査機器、コンピュータの操作に熟練した技術員の存在、④患者誘導や発疹の選択に熟練した看護師の存在、⑤運用性に優れたムービーカメラの精度向上、⑥診断精度向上のための機器（皮膚温検査機、エコー機器など）の必要性、⑦画像および遠隔診療カルテの保存方法の改善、⑧診療費用の配分。

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Annular elastolytic giant cell granuloma developing on lesions of vitiligo

Dear Editor,

A 74-year-old woman presented with a two-year history of reddish annular nodules on the neck, trunk, and forearms. She had a 10-year history of generalized vitiligo that had been untreated. The nodules with elevated borders and central atrophy and hypopigmentation, 0.5–1 cm in diameter, were located on the neck, trunk, and forearms (Fig. 1). These nodules had developed mainly on the preexisting vitiligo lesions. The patient had been taking benidipine hydrochloride for hypertension for the last 14 years. She had no history of diabetes mellitus or sarcoidosis.

A skin biopsy was taken from a nodule on the forearm. Hematoxylin and eosin staining of the specimen showed a granulomatous infiltrate of lymphocytes, histiocytes, and multinucleated giant cells in the upper and middle dermis without palisading (Fig. 2a). Immunohistochemistry showed that the lymphocytes in the inflammatory infiltrate were positive for CD3. $CD4^+$ T-cells predominated over $CD8^+$ T-cells, and the histiocytes and multinucleated giant cells were positive for CD68. Almost all of the epidermal cells were negative for Melan-A (Fig. 2b). Slight mucin deposition was evident. Elastica van Gieson staining showed that elastic fibers were absent from the reticular dermis in the area surrounded by the granulomatous infiltrate (Fig. 2c). Fragmented elastic fibers were present within some of the histiocytes and multinucleated giant cells (Fig. 2d). Ziehl-Neelsen and PAS staining revealed no acid-fast bacilli or fungal organisms. Laboratory examinations, including a full blood cell count, routine biochemistry, fasting blood sugar level, and urinalysis gave normal results. These findings were consistent with

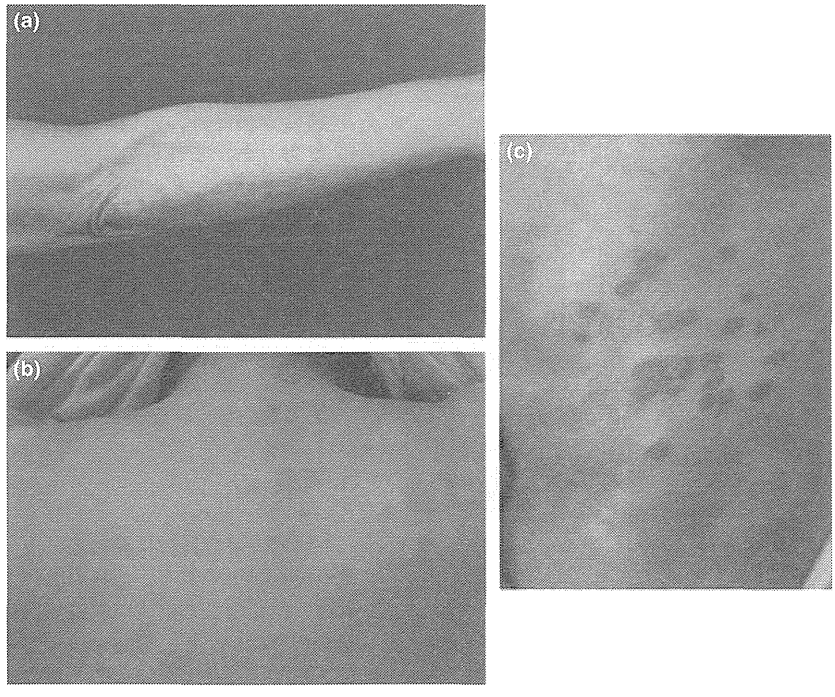

Figure 1 Multiple, erythematous, marginally elevated, annular nodules and round papules, developed mainly on lesions of preexisting vitiligo, distributed on the back (a), forearms (b) and abdomen (c)

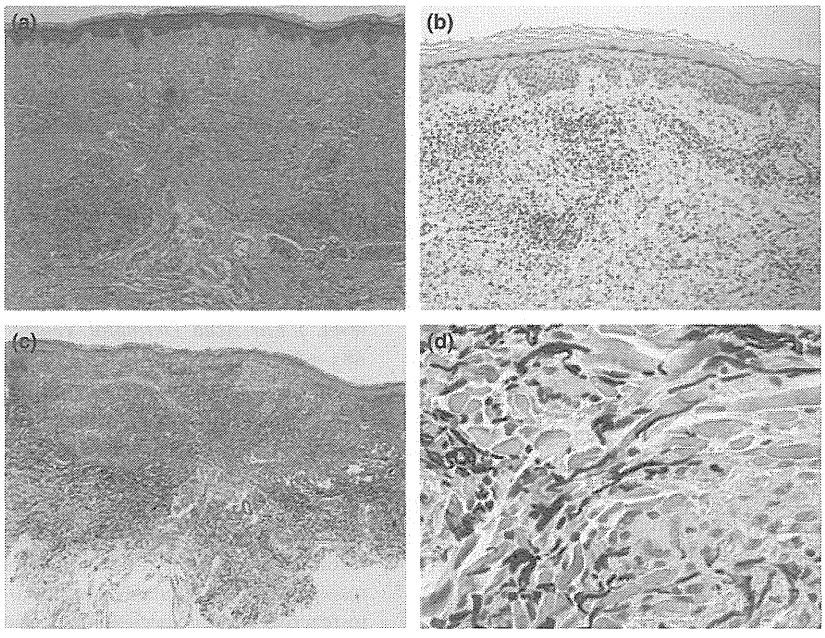

Figure 2 (a) Granulomatous infiltrates of lymphocytes, histiocytes and multinucleated giant cells in the upper and middle dermis without palisading (hematoxylin and eosin, original magnification ×40). (b) Almost all of the epidermal cells were negative for Melan-A (original magnification ×100). (c) Elastica van Gieson staining demonstrates the absence of elastic fibers in the reticular dermis in the area surrounded by the granulomatous infiltrate (original magnification ×40). (d) Fragmented elastic fibers are present within some of the histiocytes and multinucleated giant cells (original magnification ×400)

annular elastolytic giant cell granuloma (AEGCG). Thereafter, the patient was treated with topical corticosteroid (clobetasol propionate). This resulted in gradual flattening of the nodules, some of which finally disappeared.

Annular elastolytic giant cell granuloma is an entity that was proposed originally by Hanke *et al.*[1] for cutaneous annular lesions characterized by elastolysis, elastophagocytosis, and an infiltrate of multinucleated giant cells. The pathogenesis of AEGCG remains unclear. It has been postulated that exposure to the sun or other unknown factors alters the antigenicity of the elastic fibers and induces cell-mediated immune reactions.[2]

Interestingly, in the present case, AEGCG occurred mainly on pre-existing lesions of vitiligo. This anatomical co-localization suggests that the association between the two disorders is not accidental. The pathogenesis of vitiligo is also still unknown, although recently it has been shown that oxidative stress and accumulation of free radicals (FRs) acting as a trigger of melanocyte degeneration in the epidermis of affected skin are involved.[3] Oxidative stress can be induced by an increase in the generation of reactive oxygen species (ROS) and other radicals. Recent studies of vitiligo have shown that FRs are increased and that antioxidant systems are deficient. It has been demonstrated both *in vivo* and *in vitro* that patients with vitiligo accumulate high levels of hydrogen peroxide (H_2O_2), which leads to the destruction of melanocytes in the epidermis.[4] On the other hand, high superoxide dismutase activity in the serum and skin of patients with stable vitiligo has been reported.[5,6] These changes would lead to the accumulation of H_2O_2.

Recent studies of photoaging have revealed that ultraviolet (UV) irradiation induces the formation of ROS in skin tissue. Dermal fibroblasts exposed to ROS show increased expression of mRNA for matrix metalloproteinases (MMP)-1 and MMP-2, which have the ability to degrade collagen and elastic fibers.[7,8] Igawa *et al.*[9] have reported that oral Dapsone, which has an anti-oxidative effect, is effective for treatment of AEGCG. In our present patient, annular nodules were located on covered, as well as sun-exposed areas, and the patient had not been treated with UV irradiation. We postulate that in this case, degradation of dermal elastic fibers in the vitiligo lesions may have triggered the accumulation of lymphocytes and macrophages, elastophagocytosis, and subsequent granuloma formation.

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Conflicts of interest: None declared.

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Spiny keratoderma of the palms in an insulin-treated diabetic patient

Spiny keratoderma is a rare disease characterized by multiple discrete keratotic plugs, resembling a "music box spine", arising from the palms, soles, or both.[1] It was first described in 1971 by Brown[2], who called it punctate keratoderma. These spiny lesions have been described as filiform hyperkeratosis,[3] minute digitate hyperkeratosis,[4] punctate keratoderma,[2] punctate porokeratotic keratoderma[5] and, most recently, spiny keratoderma of the palms and soles.[1] Most of the cases described represent acquired disease, but there are also familial cases. Associations with a systemic disease or malignancy occur in some acquired cases. We present the case of a man with acquired spiny keratoderma who was receiving insulin treatment for type 2 diabetes mellitus.

A 58-year-old Japanese male was referred to us because of multiple asymptomatic, keratotic papules on his fingers and palms. He had a more than 20-year history of diabetes mellitus. He had noted the lesions more than 10 years ago and had started insulin treatment around the same time. Family history was negative for keratoderma, ichthyosis, or other dermatological diseases. He had no history of renal failure or hyperlipidemia and was unaware of any arsenic exposure.

On physical examination, numerous firm keratotic spicules were seen on the volar surface of the palms and fingers (Fig. 1). The lesions were 0.5–1 mm in diameter, 1–2 mm in length, and skin-colored. There were no similar lesions at other sites, including the soles.

Histopathological examination of skin biopsy specimens of the palms showed large columns of keratin arising from the epidermis in an area with a focally decreased granular layer and parakeratosis (Fig. 2). No dyskeratotic or vacuolated keratinocytes were seen in the underlying epidermis. Blood tests and computer tomography scan showed no abnormalities. Results of upper and lower endoscopy did not reveal any malignant neoplasms.

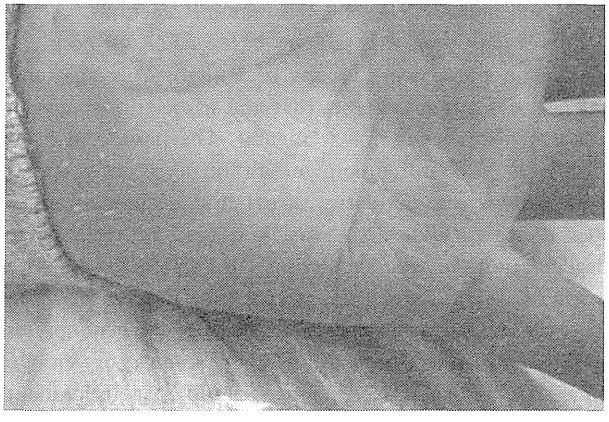

Figure 1 Multiple yellowish filiform keratotic projections on the left palm

in the antimelanogenic action down-regulating the expression of MITF or tyrosinase gene in cAMP-elevated melanocyte cultures and UV-irradiated dorsal skins of guinea pigs (Supplementary Figure S12 online). Finally, this study suggests a potential application of 4H3MC in the treatment of hyperpigmented skin disorders.

Animal experiments were carried out according to the protocols approved by Animal Experimentation Ethics Committee in CBNU institute.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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A Somatic Mutation of the *KEAP1* Gene in Malignant Melanoma Is Involved in Aberrant NRF2 Activation and an Increase in Intrinsic Drug Resistance

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TO THE EDITOR

Among the several characteristics of malignant melanoma, insensitivity to anti-cancer agents is a frequent clinical problem in the treatment of patients (Grossman and Altieri, 2001). In fact, the most commonly used chemotherapy agents cisplatin and dacarbazine for malignant melanoma elicit a response rate of only 10% (Flaherty, 2010).

The small-molecule inhibitor of BRAF, vemurafenib (also known as PLX4032), elicits potent tumor regression in patients with BRAF-positive stage IV melanoma (Huang *et al.*, 2012), and its use is expected in patients harboring the $BRAF^{V600E}$ mutation (Tsai *et al.*, 2008). However, vemurafenib is not effective against melanomas with wild-type BRAF protein (Joseph *et al.*, 2010).

Acral lentiginous melanoma (ALM) is one of the subtypes of cutaneous melanoma most frequent in colored races (Bradford *et al.*, 2009). In fact, only about 10% of ALM cases harbor the $BRAF^{V600E}$ mutation, compared with over 60% of cases of superficial spreading melanoma (SSM), which is most frequent in Caucasian populations (Saldanha *et al.*, 2006). Because these melanomas are insensitive to BRAF inhibitors (Joseph *et al.*, 2010), a search for molecular targets that would enhance sensitivity to standard treatment with cisplatin or dacarbazine would seem justified.

To address the genes responsible for drug resistance in melanoma, whole-exome sequencing was performed. We identified a single-nucleotide deletion in codon 507 from exon 4 of the *KEAP1* gene, common to MM-RU and PM-WK, as a candidate gene for drug resistance

Abbreviations: ALM, acral lentiginous melanoma; CDDP, cis-diamminedichloro-platinum (II); DTIC, 5-(3, 3-dimethyl-1-triazenyl) imidazole-4-carboxamide; FSM, frameshift mutant; ROS, reactive oxygen species; SSM, superficial spreading melanoma

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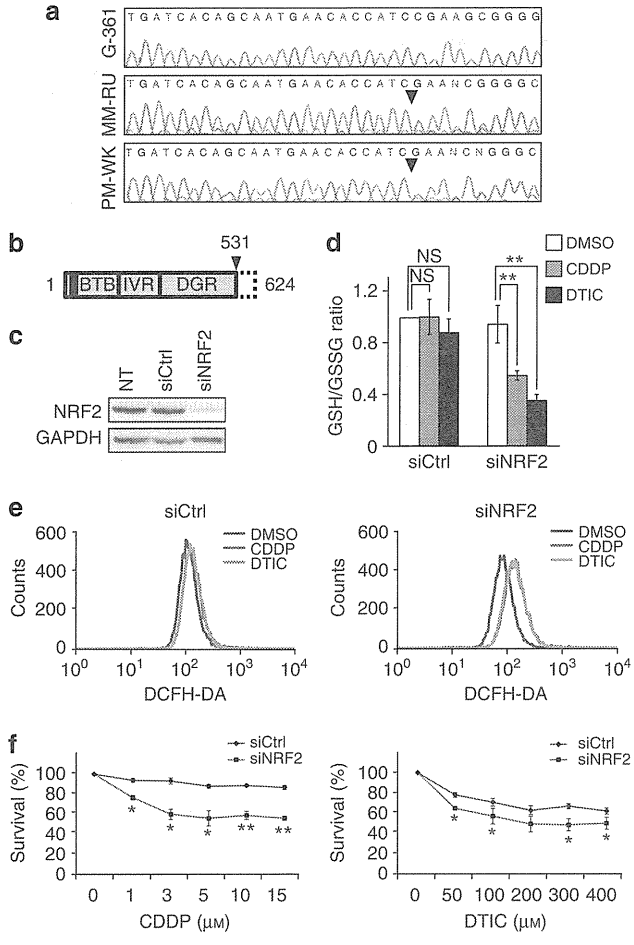

Figure 1. NRF2 contributes to chemoresistance in KEAP1-FSM cells. (**a**) Representative electrophoretograms of *KEAP1* gene exon 4 by capillary sequencing in melanoma cell lines. Arrowheads indicate the single-nucleotide deletion at codon 507 (1518delC). (**b**) Schematic representation of the *KEAP1* protein. Arrowheads indicate the appearance of a stop codon. (**c**) Expression of NRF2 protein in siCtrl- or siNRF2-transfected PM-WK cells. (**d**) Quantification of the ratio of reduced-to-oxidized glutathione (GSH/GSSG) or (**e**) intracellular reactive oxygen species levels after treatment with vehicle, CDDP, or DTIC in siCtrl- or siNRF2-transfected PM-WK cells. (**f**) Cell viability at 24 hours after treatment with vehicle, CDDP, or DTIC in siCtrl- or si*NRF2*-transfected PM-WK cells. NS, no significant difference; $*P<0.05$ and $**P<0.01$ versus control by *t*-test. CDDP, *cis*-diamminedichloro-platinum (II); DTIC, 5-(3, 3-dimethyl-1-triazenyl) imidazole-4-carboxamide.

(Figure 1a). The KEAP1 protein produced by this mutant allele partially lacks the DGR/Kelch domain that is important for interacting with NRF2, a key transcriptional regulator of oxidative stress such as that resulting from reactive oxygen species (ROS; Figure 1b). Under normal conditions, NRF2 is kept transcriptionally inactive through binding to the DGR domains of KEAP1 and constitutively degraded by the ubiquitin proteasome system (Sekhar *et al.*, 2002). In contrast, in the cell lines harboring the *KEAP1* frameshift mutation (KEAP1-FSM), constitutive NRF2 stabilization, nuclear localization (Supplementary Figure S1 online), and target-gene activation were observed (Supplementary Figure S2 online). We also observed strong expression of the NRF2 protein in HMVI cells, which do not harbor *KEAP1* mutation, suggesting that another signaling pathway, such as the PI3K pathway, may regulate the constitutive expression of NRF2 in HMVI cells (Mitsuishi *et al.*, 2012).

Thus far, several somatic mutations of *KEAP1* that affect its NRF2-inhibitory activity have been identified in patients with cancers of the lungs, gallbladder, and liver (Taguchi *et al.*, 2011). Aberrant activation of NRF2 induced by *KEAP1* gene mutation in gallbladder cancer has been reported to lead to 5-fluorouracil (5-FU) resistance (Shibata *et al.*, 2008). However, the relationship between the KEAP1-NRF2 pathway and drug resistance in malignant melanoma still remains to be elucidated.

Cisplatin (*cis*-diamminedichloro-platinum (II), CDDP) and dacarbazine (5-(3, 3-dimethyl-1-triazenyl) imidazole-4-carboxamide, DTIC) have been shown to increase oxidative stress by raising the levels of intracellular ROS such as H_2O_2 (Zhang *et al.*, 2010; Deavall *et al.*, 2012). Therefore, we investigated whether NRF2 is involved in sensitivity to these drugs. The ratio of reduced-to-oxidized glutathione (GSH/GSSG) was decreased by treatment with CDDP or DTIC in si*NRF2*-transfected PM-WK cells (Figure 1c and d). Furthermore, intracellular ROS levels were increased by treatment with CDDP or DTIC in si*NRF2*-transfected cells, suggesting that the H_2O_2 detoxification pathway is active in these cell lines (Figure 1e; Supplementary Figure S3a online). Notably, knockdown of *NRF2* by small interfering RNA in PM-WK and MM-RU cells significantly enhanced their sensitivity to apoptosis by CDDP or DTIC (Figure 1f; Supplementary Figures S3b and S4 online). Conversely, the overexpression of NRF2 or activation of NRF2 by sulforaphane in G-361 cells reduced their sensitivity to CDDP or DTIC (Supplementary Figure S5 online). Moreover, the NRF2-positive cell lines tended to show higher IC_{50} values compared with NRF2-negative cell lines (Supplementary Figure S6 online). Taken together, the data indicate that, in addition to the mechanisms of drug resistance in melanoma, such as drug trapping by melanosomes and elevated expression of ATP-dependent transporters (Gottesman *et al.*, 2002; Chen *et al.*, 2006), aberrantly expressed NRF2 is one of the causes of resistance to CDDP and DTIC.

Next, we examined *KEAP1* gene mutation and NRF2 expression in specimens of primary melanoma. The histological types, patient genders and ages, and the stages of the primary melanomas we examined are shown in Supplementary Table online. Ten (48%), eight (38%), two (10%), and one (4%) of the

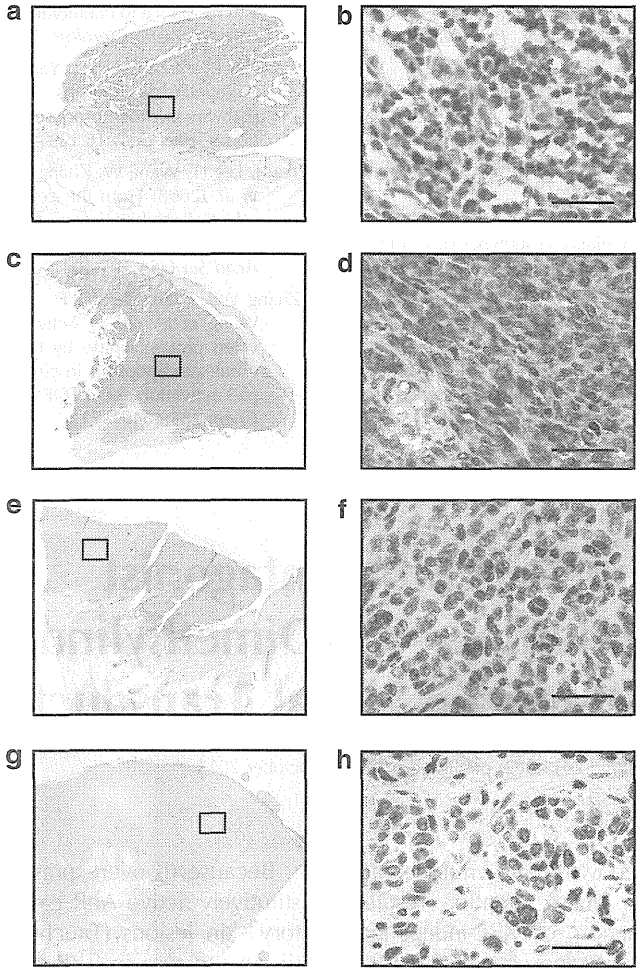

Figure 2. Aberrant NRF2 protein accumulation in primary melanomas harboring *KEAP1* frameshift mutation (FSM). Immunohistochemical analysis of endogenous NRF2 protein expression in melanomas harboring the *KEAP1* FSM (**a** and **b**, #02-2078; **c** and **d**, #03-8008) and with wild-type *KEAP1* (**e** and **f**, #09-7356; **g** and **h**, #07-7252). Cells were stained with NRF2 antibody. High-magnification images correspond to the respective boxed areas in the loupe images. Bar = 100 μm.

21 cases were ALM, NM, LMM, and SSM, respectively. We performed direct sequencing of *KEAP1* gene exon 4 using the genomic DNAs extracted from these paraffin-embedded specimens. In two of the 21 cases (~10%), we identified a homozygous single-nucleotide deletion that was the same as that identified in melanoma cell lines (#02-2078, 1518delC) and a heterozygous single-nucleotide deletion in a neighboring position (#03-8008, 1519delG) (Supplementary Table online). The stop codon appeared 23 nucleotides after the 1519delG mutation and also produced the same type of truncated KEAP1 protein as that resulting from 1518delC mutation. Immunohistochemical staining revealed that NRF2 was positive in specimens #02-2078 and #03-8008 (Figure 2b and d, respectively), which were ALM specimens harboring KEAP1-FSM mutation, whereas NRF2 was negative in specimens #09-7356 and #07-7252, which were ALM specimen without KEAP1 mutation (Figure 2, Supplementary Table online). We also performed direct sequencing of *BRAF* gene exon 15 using DNAs from #02-2078, #03-8008, #08-6115, and #09-7356, but the typical $BRAF^{V600E}$ mutation was not identified (Supplementary Table online). Taking these results together, it appears that the loss of function of KEAP1 caused by single-nucleotide deletions is indeed correlated with aberrant NRF2 expression in primary melanoma.

Missense mutations of the *KEAP1* gene have been identified in various cancers. Interestingly, the *KEAP1*-FSMs are most frequently identified in the DGR domain (65%), which is important for interaction with NRF2 (Taguchi *et al.*, 2011). Our results also indicate that the DGR domain of the *KEAP1* gene may be a "hot-spot" for FSM that causes aberrant activation of NRF2 and causes melanomas to become more resistant to CDDP or DTIC.

In conclusion, our present results and available data fully support the possibility that decreased expression or inhibition of NRF2 may provide an avenue for treatment of patients with malignant melanoma, by improving the rate of response to the standard chemotherapy agents, namely, cisplatin and dacarbazine.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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The New Aryl Hydrocarbon Receptor Antagonist E/Z-2-Benzylindene-5,6-Dimethoxy-3,3-Dimethylindan-1-One Protects against UVB-Induced Signal Transduction

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TO THE EDITOR

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates the toxicity of 2,3,7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), polycyclic aromatic hydrocarbons, and related environmental contaminants (Abel and Haarmann-Stemmann, 2010). The unligated AhR is trapped in a cytosolic multiprotein complex, which rapidly dissociates upon ligand binding. Subsequently, the AhR shuttles into the nucleus, dimerizes with ARNT, and binds to xenobiotic-responsive elements (XREs) in the promoter of target genes, e.g., encoding cytochrome P450 (CYP) 1 monooxygenases, to enforce transcription (Abel and Haarmann-Stemmann, 2010). Furthermore, AhR-triggered activation of c-src tyrosine kinase stimulates EGFR and downstream mitogen-activated protein kinase signaling, resulting in the induction of XRE-independent genes, such as cyclooxygenase-2 (COX-2; Abel and Haarmann-Stemmann, 2010). We have previously shown that the AhR in keratinocytes is not only activated by anthropogenic chemicals but also by UVB irradiation, which leads to the intracellular formation of the tryptophan photoproduct and high-affinity AhR ligand 6-formylindolo[3,2-*b*]carbazole (FICZ; Rannug *et al.*, 1995; Fritsche *et al.*, 2007). Indeed, UVB exposure enhances AhR/XRE binding (Supplementary Figure 1 online) and accompanied CYP1A1/1B1 expression (Katiyar *et al.*, 2000), as well as XRE-independent COX-2 expression (Fritsche *et al.*, 2007).

Because (i) overexpression of a constitutively active AhR causes inflammatory skin lesions (Tauchi *et al.*, 2005), (ii) an increase in CYP activity leads to reactive oxygen species formation (Puntarulo and Cederbaum, 1998), (iii) CYP1 enzymes are critical for chemical-induced skin carcinogenesis (Shimizu *et al.*, 2000), and (iv) COX-2 is involved in UV-induced inflammation and carcinogenesis (Elmets *et al.*, 2010), it was postulated that a transient inhibition of AhR may protect human skin against the detrimental effects of UVB irradiation (Agostinis *et al.*, 2007; Haarmann-Stemmann *et al.*, 2012). Moreover, we have shown that the expression of matrix metalloproteinase-1 (MMP-1), which is critically involved in extrinsic skin aging, is upregulated in an AhR-dependent manner in tobacco smoke extract–exposed keratinocytes (Ono *et al.*, 2013). Therefore, we decided to develop an AHR antagonist that is suitable for topical UV-protection. We screened a library of compounds that possess the structural prerequisites to

Abbreviations: AhR, aryl hydrocarbon receptor; BDDI, E/Z-2-benzylidene-5,6-dimethoxy-3,3-dimethylindan-1-one; COX-2, cyclooxygenase-2; CYP, cytochrome P450; EROD, 7-O-ethoxyresorufin-deethylase; FICZ, 6-formylindolo[3,2-b]carbazole; MMP-1, matrix metalloproteinase-1; MNF, 3′-methoxy-4′-nitroflavone; NHEK, normal human epidermal keratinocyte; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; XRE, xenobiotic-responsive element

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Case Study

Subcutaneous Panniculitis-Like T-Cell Lymphoma (SPTCL) with Hemophagocytosis (HPS) : Successful Treatment Using High-Dose Chemotherapy (BFM-NHL & ALL-90) and Autologous Peripheral Blood Stem Cell Transplantation

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Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a rare form of non-Hodgkin lymphoma, in which lymphoma cells infiltrate preferentially into subcutaneous adipose tissue. Although various treatment trials for SPTCL have been attempted, no standardized therapy has been established. Here, we report a case of α/β^+ T-cell-phenotype SPTCL (SPTCL-AB) with hemophagocytosis (HPS) in a 14-year-old girl, who presented with low-grade fever, general fatigue and chest swelling. Laboratory examinations revealed leukocytopenia, and bone marrow aspiration cytology showed HPS. The diagnosis of SPTCL-AB was made by biopsy on the basis of thickened subcutaneous tissue in the chest wall. Following high-dose chemotherapy (HDT) of BFM-NHL & ALL-90, autologous peripheral blood stem cell transplantation (auto-PBSCT) was performed. The patient responded to the treatment and has remained asymptomatic for 2 years. Our results suggest that a combination of HDT of BFM-NHL & ALL-90 and auto-SCT treatment is effective for SPTCL associated with HPS. [*J Clin Exp Hematop 53(2) : 135-140, 2013*]

Keywords: SPTCL, hemophagocytosis, BFM-NHL & ALL-90, auto-PBSCT

INTRODUCTION

In 1991, the distinct clinicopathological features of a T-cell lymphoma in which the lymphoma cells preferentially invade the subcutaneous tissue were described by Gonzalez *et al.*[1] Under the term subcutaneous panniculitis-like T-cell lymphoma (SPTCL), this new condition was established as a distinct disease entity in the World Health Organization (WHO) classification.[2] Because of its peculiar pathological features, SPTCL may be initially misdiagnosed as Weber-Christian disease, a benign inflammatory panniculitis and a granulomatous disease.[3,4] Recent studies have disclosed that cases with an α/β^+ T-cell phenotype (SPTCL-AB) and a γ/δ^+ T-cell phenotype (SPTCL-GD) can be distinguished within the group of SPTCL.[5] SPTCL-ABs have a $CD4^-$, $CD8^+$, $CD56^-$ phenotype, and SPTCL-GDs have a $CD4^-$, $CD8^-$ phenotype with frequent expression of CD56. Compared with SPTCL-ABs, SPTCL-GDs have a poor prognosis.[6,7] On the basis of these observations, the term SPTCL is used only for SPTCL-ABs, and SPTCL-GDs are included within the cutaneous γ/δ^+ T-cell lymphomas.[6,8] Although SPTCL-AB patients without hemophagocytic syndrome (HPS) have a favorable prognosis, the clinical course of cases associated with HPS is generally aggressive, and a delay in diagnosis or treatment may result in a fatal outcome. There have been few reports of successful treatment of SPTCL-AB patients with HPS, and no therapeutic regimen has been established.

Here, we report a case of SPTCL-AB with HPS that was treated successfully with a combination of high-dose chemotherapy (HDT) of Berlin-Frankfurt-Münster-non-Hodgkin lymphoma-90 (BFM-NHL-90) and autologous peripheral blood stem cell transplantation (auto-PBSCT).

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CASE REPORT

A previously healthy 14-year-old Japanese girl visited a physician because of a 1-month history of chest swelling.

She also had a 3-month history of general fatigue and low-grade fever. Initial laboratory examinations revealed a white blood cell count of 2.82 × 10^3/μL, hemoglobin of 11.6 g/dL, hematocrit of 33.8 g/dL and platelet count of 16.4 × 10^4/μL. Histopathologic examination of a biopsy specimen of subcutaneous adipose tissue from the chest wall revealed diffuse infiltration of medium-sized lymphocytes. Under a diagnosis of panniculitis due to lupus profundus, administration of low-dose prednisolone (PSL) was started. Several weeks later, the chest swelling expanded gradually. She was therefore referred and admitted to our hospital for further evaluation and treatment.

On physical examination, a massive tumor was evident in her chest subcutaneous tissue (Fig. 1). The findings from laboratory examinations were as follows : aspartate aminotransferase 27 IU/L (normal : 10-40 IU/L), alanine aminotransferase 15 IU/L (normal : 5-35 IU/L), lactic dehydrogenase 235 U/L (normal : 115-359 IU/L), soluble interleukin-2 receptor 641 U/mL (normal : 220-530 U/mL) and ferritin 56 ng/mL. Moreover, previous infection with Epstein-Barr virus was evident. Magnetic resonance imaging revealed noticeable thickening of the subcutaneous tissue, compatible with the region of the massive tumor in the chest (Fig. 2). Histopathology of skin biopsy specimens showed medium-sized lymphocytes diffusely infiltrating into the subcutaneous fat tissue (Fig. 3a). Rimming of the lymphocytes around individual fat cells was observed (Fig. 3b).

Immunohistochemical staining revealed that the infiltrating lymphocytes were $CD3^+$, $CD4^-$, $CD5^+$, $CD8^+$, $CD20^-$, $CD30^-$, $CD45RO^+$, $CD56^-$ and $CD79a^-$ (Fig. 4). They were positive for T-cell receptor-β (Fig. 5a). Cytotoxic molecules such as granzyme B, T-cell intracellular antigen-1 (TIA-1) and perforin were positive (Fig. 5b). Latent membrane protein 1 (LMP-1) and EBER *in situ* hybridization were negative (Fig. 5c & 5d). Bone marrow smears showed hemophagocy-

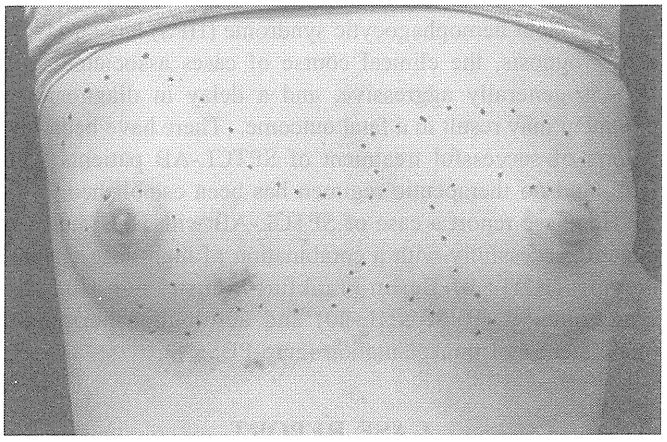

Fig. 1. A massive tumor, indicated by *black dots*, was present in the subcutaneous tissue of the chest.

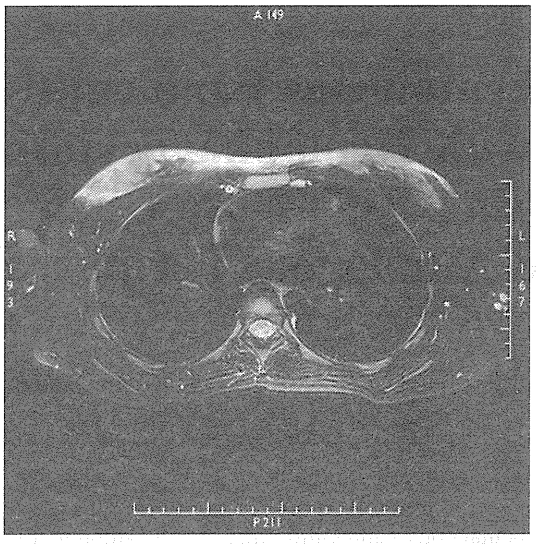

Fig. 2. Magnetic resonance imaging of the chest (T2-weighted). The subcutaneous tumor of the chest wall showed high signal intensity.

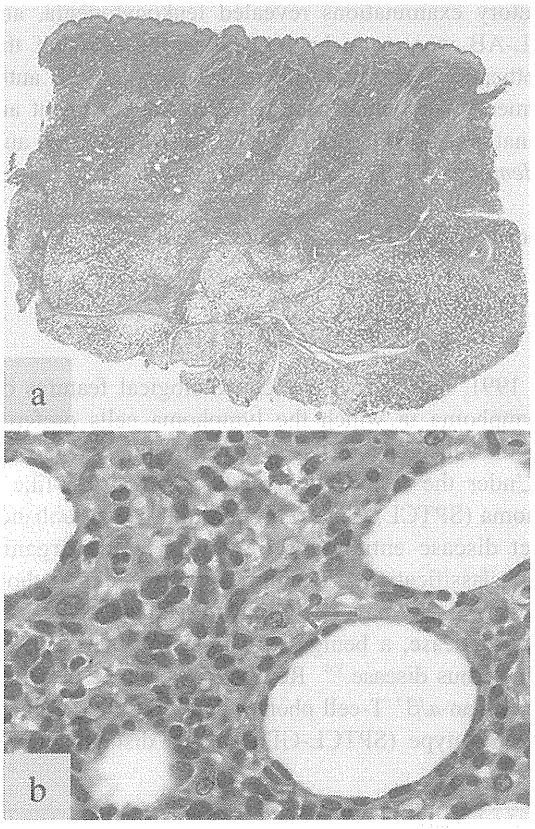

Fig. 3. Histopathology of the skin biopsy specimen (H&E stain). (*3a*) Diffuse cellular infiltration occurred in the subcutaneous fat tissue. (*3b*) Rimming of lymphocytes around fat cells was observed (*arrow*). Bean bag cells, phagocytosing erythrocytes, were found among the lymphocytes. (*3a*) ×10, (*3b*) × 400.

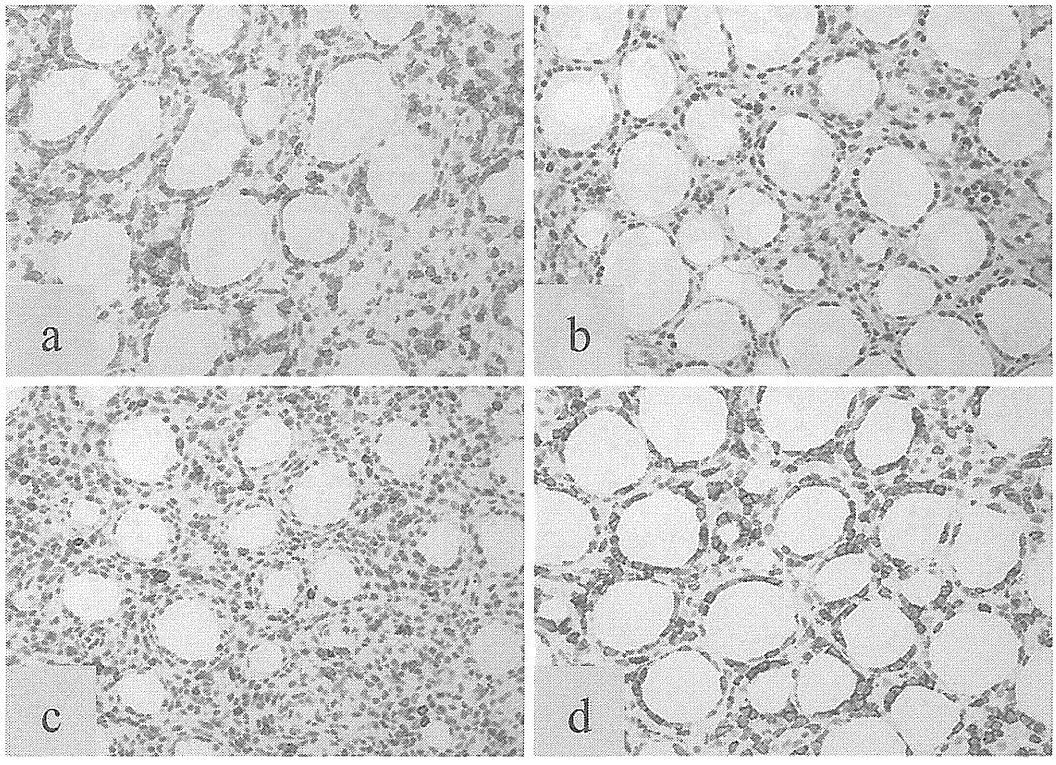

Fig. 4. Immunohistochemistry of CD3 (*4a*), CD4 (*4b*), CD5 (*4c*) and CD8 (*4d*). The infiltrating lymphocytes were positive for CD3, CD5 and CD8, but negative for CD4. ×400.

tosis of red blood cells and neutrophils by histiocytes (Fig. 6).

On the basis of these results, the patient was diagnosed as having SPTCL accompanied by HPS in spite of the low levels of lactic dehydrogenase and ferritin, and was treated with high-dose PSL (75 mg/day internally), starting on day 9 of admission.

Nine days later (on day 17 of admission), despite the PSL therapy, the chest tumor was unchanged in size. Additional HDT of BFM-NHL-90 (protocol I) was therefore applied. After starting the first course of the chemotherapy, the tumor in the chest gradually decreased in size. During the phase of recovery from the chemotherapy, peripheral blood stem cells ($CD34^+$ cells) were harvested. Moreover, chemotherapy of BFM-NHL-90 (Protocol M) was applied as a consolidation therapy. In addition, one course of the BFM-acute lymphoblastic leukemia (ALL)-90 regimen (HR3) was applied for further treatment.

Following pretreatment with the MCVAC (ranimustine, cytarabine, etoposide and cyclophosphamide) regimen, the patient was treated with auto-PBSCT. The clinical course thereafter was uneventful. She has been in complete remission for more than 2 years, and there was no evidence of local recurrence in the chest wall and HPS in the bone marrow at the final follow-up examination.

DISCUSSION

SPTCL is a type of skin lymphoma characterized by the infiltration of subcutaneous tissue by pleomorphic T cells and benign macrophages, mimicking lobular panniculitis. This malignancy typically presents in the form of skin nodules that involve the extremities and can become ulcerated.[9] The clinical course associated with HPS is aggressive, and a delay in diagnosis and treatment may result in a fatal outcome.

Although the mechanism of HPS in SPTCL has not been clarified, the phenomenon of HPS results from overproduction of cytokines, including interferon-γ, interleukin-2 (IL-2), IL-6, IL-12, IL-18 and tumor necrosis factor-α, produced by activated T cells (Th1 cells) and macrophages, which leads to a chain reaction of cytokines.[10-13] Control of HPS in SPTCL-AB patients improves the prognosis.

Various therapies such as radiotherapy, PSL, CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine and prednisolone) (-like) chemotherapy and auto/allo-SCT have been applied for SPTCL-AB.[9] For relapsed or refractory disease, various regimens have been attempted as salvage chemotherapy, including cladribine, DHAP (dexamethasone, cytarabine and cisplatin), ESHAP (etoposide, methylprednisolone, cytarabine and cisplatin), FLAG (fludarabine, cytarabine and granulocyte-colony stimulating factor), mini-BEAM (carmus-

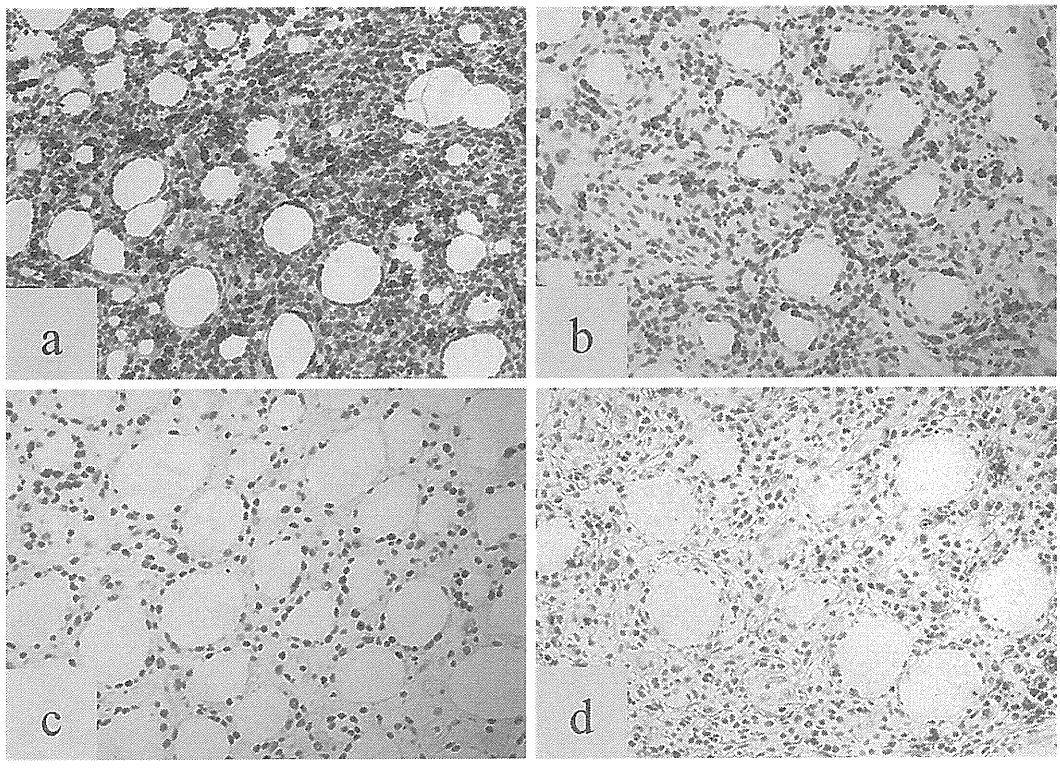

Fig. 5. Immunohistochemistry for T-cell receptor (TCR)-β (*5a*), granzyme B (*5b*), latent membrane protein-1 (LMP-1, *5c*) and Epstein-Barr virus (EVB)-encoded RNA *in situ* hybridization (*5d*). Lymphocytes were positive for TCR-β, and expression of the cytotoxic molecule granzyme B was also found. LMP-1 and EBV were negative. ×200.

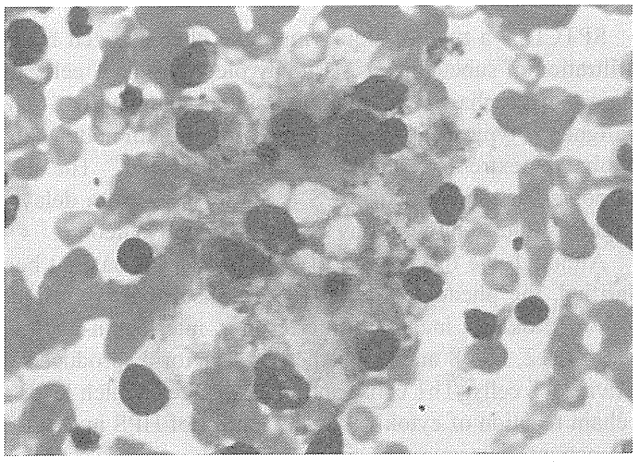

Fig. 6. Hemophagocytosis was seen in a smear of the bone marrow. Giemsa stain, ×1000.

tine, etoposide, cytarabine and melphalan) and VEPPB (vincristine, etoposide, prednisone, pocarbazine and bleomycin).[9] However, no standardized treatment has yet emerged. In SPTCL-AB patients with HPS, a recent report has indicated that CHOP (-like) chemotherapy is not very effective. Auto-SCT or allo-SCT following HDT has been suggested as an important option for patients with refractory or recurrent SPTCL.[4,14]

In the present case, high-dose PSL was initially used, but no response was obtained. Therefore, we treated the patient according to the BFM-NHL & ALL-90 protocol.

BFM-NHL-90 is a protocol for pediatric malignant lymphoma and yields a significantly better outcome for non-Hodgkin lymphoma at stage I or II.[15] BFM-NHL-90 is also effective for pediatric anaplastic large-cell lymphoma. BFM-ALL-90 is a superior regimen for high-risk childhood T-cell acute lymphoblastic leukemia.[16]

The present case was treated successfully with a combination of HDT of BFM-NHL & ALL-90 followed by auto-PBSCT, and achieved clinical complete remission (CR). This result suggests that the BFM protocol is applicable and can yield complete remission in cases of SPTCL with HPS. Medhi *et al.* have also reported the value of the BFM-90 protocol for the treatment of patients with SPTCL and HPS.[17]

To date, there have been few reports of effective treatment for SPTCL using HDT following SCT. In almost all of the reported cases, the patients underwent HDT-allo-SCT and its effectiveness was impressive, 92% achieving CR, with a median response duration of ≥ 14 months.[9] In intermediate- and high-grade lymphomas, myeloablative allo-SCT is associated

with a lower relapse rate than auto-SCT for the graft-versus-leukemia effect.[18,19] However, chronic graft-versus-host disease (cGVHD) is a very common complication, with a reported incidence of between 40% and 70%,[20] and is the leading cause of late death in allo-SCT survivors.[20,21]

The median age of patients with SPTCL-AB at diagnosis is 36 years (range : 9-79 years), and about 19% are in the second decade or younger.[8] In children, cGVHD may reduce the quality of life because of the induction of growth irregularity. Mukai *et al.* described a patient with SPTCL and HPS who received HDT of BFM-NHL & ALL-90 and auto-SCT.[4] The present case received a combination of HDT of BFM-NHL & ALL-90 and auto-SCT, and has been in complete remission for more than 2 years. This suggests that auto-SCT might be a feasible option following HDT.

In summary, we have reported a case of SPTCL complicated by HPS, which responded to treatment with HDT of BFM-NHL & ALL-90 and auto-SCT. Although the value of the BFM-NHL & ALL-90 protocol has to be further evaluated in SPTCL cases, our findings suggest that a combination of HDT of BFM-NHL & ALL-90 and auto-SCT is applicable for the treatment of SPTCL with HPS.

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DISCLOSURE/CONFLICT OF INTEREST

The authors state that they have no financial interest in the products mentioned within this article.

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SHORT COMMUNICATION

A Case of Atypical Fibrous Histiocytoma with Positivity for CD163 and CD44

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Atypical fibrous histiocytoma (AFH) is a variant of dermatofibroma (DF) that was first described by Fukamizu et al. (1) in 1983. Histologically AFH is characterized by proliferation of dermal spindle cells composed mainly of atypical histiocytic cells with striking nuclear pleomorphism and atypia, in a background of classic fibrous histiocytoma (2). It is known that many cases of AFH follow a benign course if complete excision is carried out (2, 3). However, because the tumour cells are atypical, AFH must be differentiated from tumours of intermediate malignancy, such as dermatofibrosarcoma protuberans (DFSP) or atypical fibrous xanthoma (AFX), as well as more malignant tumours, such as pleomorphic dermal sarcoma (PDS)/malignant fibrous histiocytoma (MFH).

We report here a case of AFH on the left upper arm of a 63-year-old woman and describe its immunoreactivity in detail. We also discuss the points of histological and immunohistological differentiation between AFH and other cutaneous spindle cell tumours.

CASE REPORT

A 63-year-old woman presented with an 8-month history of a symptomless, slowly growing swelling on the left upper arm. The patient had no unusual medical or family history. Clinical examination revealed an 8-mm black-purplish hard mass with peripheral erythema (Fig. 1A). The tumour had arisen at a site without any known previous history of injury. A haemangioma was clinically suspected, and surgical excision was performed. Microscopic examination revealed a well-defined lesion, located in the dermis and extending to the subcutaneous tissue, with epidermal hyperplasia and a grenz zone (Fig. 1B). The lesion was composed largely of interlacing fascicles of predominant histiocyte-like eosinophilic spindle cells with elongated or plump vesicular nuclei, arranged in a storiform pattern. Abundant pleomorphic giant cells with huge bizarre nuclei (bi-lobed and multi-lobed) and histiocytes with large vesicular nuclei and prominent eosinophilic nucleoli were observed (Fig. 1C). In the peripheral region of the tumour, fibroblast-like spindle cells arranged in a storiform or fascicular pattern with collagen bundles were observed, resembling the classic features of DF. No necrosis was present. Foci of chronic inflammatory cells, including lymphocytes and plasma cells, were also evident. As a typical feature, we noted individual prominent hyalinized collagen bundles surrounded by tumour cells, predominantly in the periphery of the lesion (Fig. 1D). The mitotic count was 3 per 10 high-power fields (HPF).

Immunohistochemical staining revealed diffuse positivity for vimentin, factor XIIIa, CD68, CD163 (Fig. 1E) and CD44 (Fig. 1F). The lesion showed no reactivity for desmin, CD34, AE1/AE3, desmin, S-100 protein, α1-antitrypsin or α1-antichymotrypsin. Ki-67 staining showed less than 5% positive reactivity. Based on these findings, a diagnosis of atypical fibrous histiocytoma was made. As it was suspected that the initial resection may have left some residual tumour cells at depth, expanded excision with a 3-cm margin was performed one month later. At 30 months of follow-up, the patient was asymptomatic with no evidence of tumour recurrence.

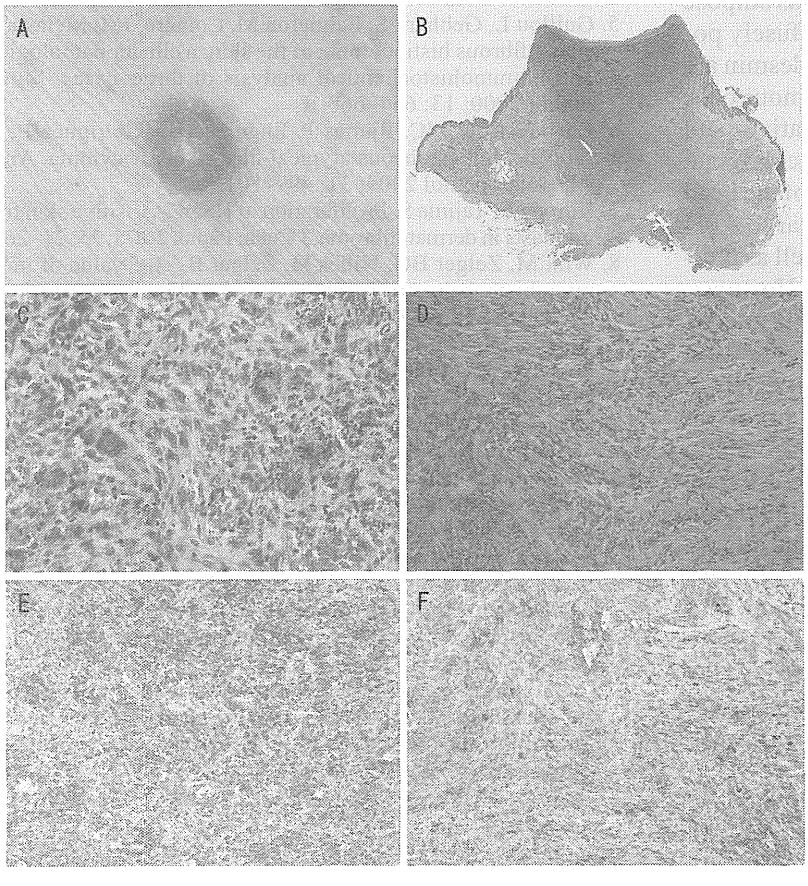

Fig. 1. (A) Macroscopic view of the blackish-purplish skin tumour on the right arm. (B) Dermal-to-subcutaneous tumour with focal extension into the subcutaneous tissue. The epidermis was hyperplastic and a grenz zone was evident (haematoxylin and eosin; HE × 1). (C) The tumour is composed of a proliferation of interlacing fascicles of predominantly histiocyte-like eosinophilic spindle cells with vesicular nuclei. Abundant pleomorphic giant cells with huge bizarre nuclei are present (HE × 100). (D) At the border of the lesion, the cells are interspersed with hyaline collagen bundles (HE × 40). Immunohistochemical studies of the tumour cells. The cells were positive for (E) CD163 and (F) CD44.