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Immunohistochemical Analysis of CXCR4 Expression in Fibrohistiocytic Tumors

Seiko Toyozawa[1], Yuki Yamamoto[1], Yuko Ishida[2], Toshikazu Kondo[2], Yasushi Nakamura[3] and Fukumi Furukawa[1]

[1]Department of Dermatology, Wakayama Medical University, Wakayama, Japan, [2]Department of Forensic Medicine, Wakayama Medical University, Wakayama, Japan and [3]Department of Clinical Laboratory Medicine, Wakayama Medical University, Wakayama, Japan

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Functional chemokine receptors are expressed in many malignant tumors. These receptors promote tumor growth and metastasis in response to endogenous chemokines. We analyzed the expression of CXCR4, CCR6 and CCR7 in fibrohistiocytic tumors, including dermatofibrosarcoma protuberance (DFSP), malignant fibrous histiocytoma (MFH), dermatofibroma (DF) using immunohistochemistry. We also investigated the relationship between CXCR4 and CD34, the latter of which is an immunohistochemical marker for DFSP. We observed a higher expression of CXCR4 in DFSP and MFH as compared with DF. Interestingly, a significantly higher expression of CXCR4 was detected in relapsed DFSP than in non-relapsed DFSP, but no significant differences were detected between non-relapsed DFSP and DFSP with CD34 immunostaining. Moreover, MFH had strong immunoreactivity for CXCR4, CCR6 and CCR7. These findings suggest that the assessment of CXCR4 immunoreactivity in fibrohistiocytic tumors is a useful tool for predicting tumor aggressiveness.

Key words: CXCR4, DFSP, immunohistochemistry, fibrohistiocytic tumors

I. Introduction

Recently, functional chemokine receptors have been shown to be expressed by a large number of human malignancies, leading to the hypothesis that these chemokines may stimulate the proliferation, chemotaxis, and site-directed metastasis of tumor cells [14].

Chemokines are molecules that are structurally and functionally similar to growth factors. They bind to G-protein-coupled receptors on leukocytes and stem cells; these receptors are termed this way because they work through guanine-nucleotide-binding (G) proteins to initiate the intracellular signaling cascades which prompt migration towards the chemokine source [4].

High CXCR4 expression was associated with metastasis in breast cancer, malignant melanoma and papillary thyroid carcinoma [4, 8, 12]. Furthermore, recent studies have shown that CXCR4 and CCR7 are consistently expressed in breast cancer cells [4]. Interestingly, these matched chemokines are known to be expressed in sites frequently involved with breast cancer metastasis [4]. However, the role of chemokines in the development of fibrohistiocytic tumors has yet to be clarified.

Dermatofibrosarcoma protuberans (DFSP) is a highly recurrent infiltrative skin tumor of intermediate malignancy. The histogenesis of DFSP is still controversial. DFSP rarely metastasizes (fewer than 10% of cases); however, extensive resection is necessary because of its high tendency to recur. On the other hand, malignant fibrohistiocytoma (MFH) is the most frequently occurring soft-tissue sarcoma. MFH is highly malignant, and the prognosis is very poor [2].

In this study, we investigated the immunohistochemical expression of CXCR4, CCR6 and CCR7 in paraffin-embedded DFSP, MFH and dermatofibroma (DF) tissues to assess the usefulness of assaying the altered expression of these chemokine receptors for diagnosis and prognosis. We also immunohistochemically investigated the relationship between CXCR4 and CD34 which is an immunohistochemical marker for DFSP [3].

Correspondence to: Seiko Toyozawa, M.D., Department of Dermatology, School of Medicine, Wakayama Medical University, 811–1, Kimiidera, Wakayama 641–0012, Japan.
E-mail: seikotoy@wakayama-med.ac.jp

II. Materials and Methods

Tissue samples

This study was performed on tumor tissues from 28 patients with fibrohistiocytic tumors, including 8 DFSP, 6 MFH, and 14 DF patients. These tissues were obtained from the first surgery. The tissue specimens were fixed in neutral buffered formalin, and then paraffin-embedded sections were stained with hematoxylin-eosin (HE) [10]. All tumors were histopathologically diagnosed by at least 3 dermatologists.

This study was approved by the ethics committee of the Wakayama Medical University and informed consent was obtained from each of the patients.

Immunohistochemistry

Immunohistochemistry was performed on deparaffinized, 4 μm-thick sections. The tissue sections were incubated for 20 min in 3% H_2O_2 at room temperature (RT) to block endogenous peroxidase activity, and then incubated in a blocking solution (1% normal rabbit serum and 1% BSA) to eliminate non-specific binding. The specimens were then incubated for 2 hr at RT with a polyclonal goat anti-CXCR4 antibody (1:500 dilution; GeneTex, Inc., San Antonio, TX, USA), anti-CCR6 antibody (1:300 dilution; GeneTex) or anti-CCR7 antibody (1:300 dilution; GeneTex). Thereafter, the specimens were incubated for 1 hr at RT with a biotinylated secondary antibody (1:400 dilution; DAKO, Japan). After extensive rinsing, they were developed using the streptavidin-biotin-peroxidase complex technique (LSAB2 kit/HRP, DAKO).

CD34 immunostaining was performed with an anti-CD34 antibody (1:200 dilution; DAKO). The sections were incubated for 2 hr at RT with this antibody. The bound primary antibodies were then detected using an Envision labeled polymer (DAKO). The peroxidase reaction was visualized with 0.2 mg/ml 3,3'-diaminobenzidine tetrahydrochloride. The sections were counter-stained with hematoxylin. The control sections were not exposed to the primary antibody.

Evaluation of immunolabeling

The immunohistochemical scoring was performed independently by 3 dermatologists or a pathologist who had no clinical knowledge of the patients and who were blinded to the procedure. The immunostained sections were scanned by light-microscopy. Necrotic areas and the edges of the tissue sections were not included in the counting. The immunohistochemical staining was scored on a 0 to 4 scale on one field as follows: 0 (membranous or cytoplasmic labeling either absent or less than 5% of the tumor cells), 1 (in 5–50% of the tumor cells), 2 (in 50–70% of the tumor cells), 3 (in 70–90% of the tumor cells), or 4 (in 90–100% of the tumor cells). In each specimen, 5 fields of ×400 high power view were assessed randomly in all specimens. The immunostaining score of each specimen is presented by total scores in all 5 fields. We marked on a maximum scale of 20 points (=0~4 ranks×5 fields).

Statistical analysis

Statistical significance was evaluated using a one-way analysis of variance with posthoc testing with Scheffe's *F* multiple comparison tests. $P<0.05$ was regarded as statistically significant.

III. Results

Tumor characteristics and patient profiles

The characteristics of the patients are shown in Table 1. Three of the 8 DFSP patients relapsed within 3 years after the first operation, but none of the DFSP cases had lymph node metastasis or distant metastasis. Two of the 6 MFH patients relapsed within 3 years, and only 1 case of MFH had a lung metastasis within 2 years after the first operation.

High expression of CXCR4 in relapsed DFSP and MFH

Representative results of the immunohistochemical staining for CXCR4 are shown in Figure 1. CXCR4 immunoexpression was detected in 8/8 (100%) cases of DFSP, 6/6 cases (100%) of MFH, 12/14 (86%) cases of DF. As shown in Figure 2, the average scores of the CXCR4

Table 1. *Patients and tumor characteristics*

		DFSP	MFH	DF
Total number of patients		8	6	14
Median age		47.9	61.5	31.1
Gender	Female	6 (75)	2 (33)	11 (79)
	Male	2 (25)	4 (67)	3 (21)
Tumor size	<2 cm	0 (0)	1 (17)	14 (100)
	2~5 cm	2 (25)	4 (67)	0 (0)
	>5 cm	6 (75)	1 (17)	0 (0)
Relapse or metastasis within 3 years	Yes	3 (38)	3 (50) (one of 3; lung metastasis)	0 (0)
	No	5 (62)	0 (0)	14 (100)
	Resected within 3 years	0 (0)	3 (50)	0 (0)

NOTE. The numbers in parentheses are percentages.

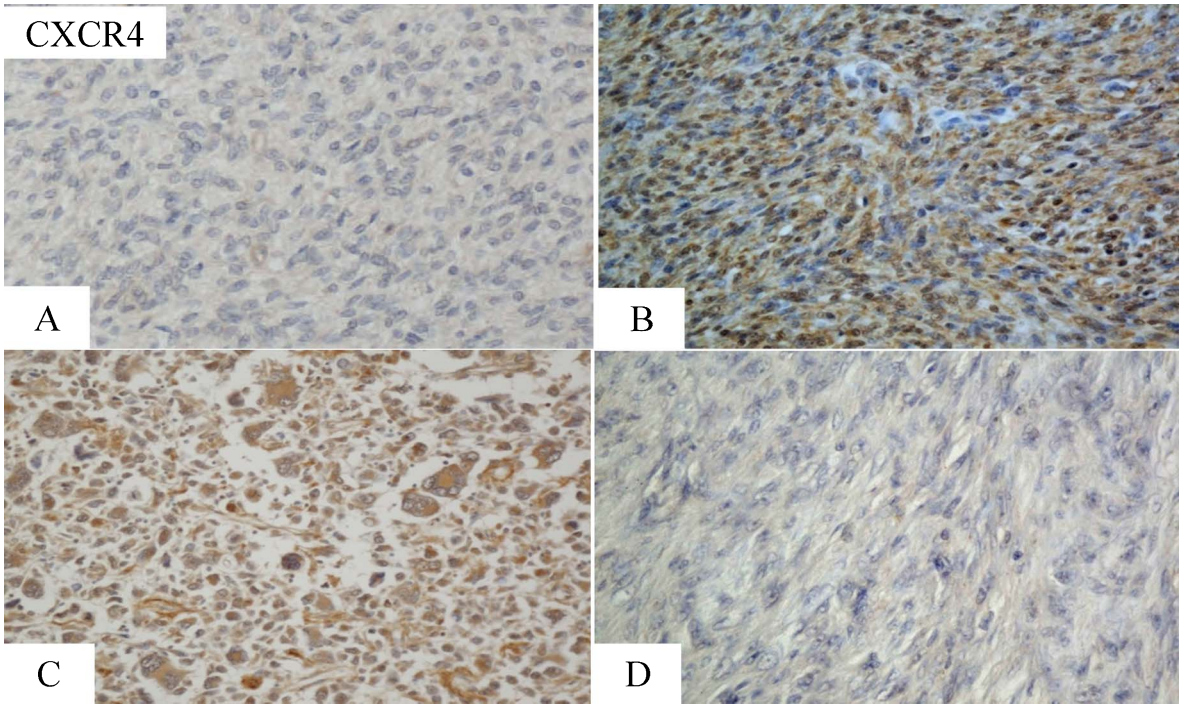

Fig. 1. CXCR4 immunoexpression in fibrohistiocytic tumors (×200). **A.** No expression of CXCR4 was observed in non-relapsed DFSP. **B.** Diffuse CXCR4 expression was observed in relapsed DFSP. **C.** Diffuse CXCR4 expression was observed in MFH. **D.** No expression of CXCR4 was observed in DF.

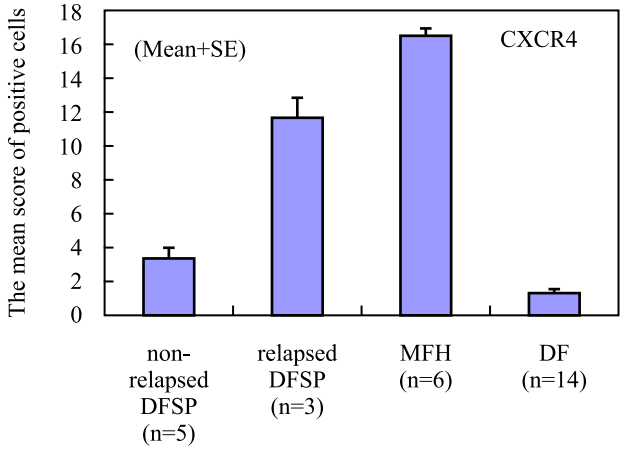

Fig. 2. The mean score of CXCR4-positive tumor cells. The mean scores of the CXCR4 immunostaining were significantly higher in DFSP and MFH as compared with DF (p<0.01). Moreover, CXCR4 immunoexpression was significantly higher in relapsed DFSP as compared with non-relapsed DFSP (p<0.01).
Statistics
Non-relapsed DFSP
vs relapsed DFSP p<0.01; *vs* MFH p<0.01; *vs* DF p<0.05
Relapsed DFSP
vs MFH p<0.01; *vs* DF p<0.01
MFH
vs DF p<0.01

immunostaining were significantly higher in DFSP and MFH as compared with DF (p<0.01). Moreover, CXCR4 immunoexpression was significantly higher in relapsed DFSP as compared with non-relapsed DFSP (p<0.01). However, there was no difference in immunostaining intensity between the two DFSP groups.

High expression of CCR6 and CCR7 in MFH

The immunohistochemical staining results for CCR6 are shown in Figure 3. As shown in Figure 4, the average scores for the CCR6 immunostaining were significantly higher in only MFH as compared with DF (p<0.01). However, no significant difference in immunostaining score was observed between non-relapsed DFSP and relapsed DFSP.

The immunohistochemical staining results for CCR7 are shown in Figure 5. As shown in Figure 6, the average scores for CCR7 immunostaining were significantly higher in non-relapsed DFSP (p<0.05) and MFH (p<0.01) as compared with DF. However, no significant difference in immunostaining score was observed between non-relapsed DFSP and relapsed DFSP.

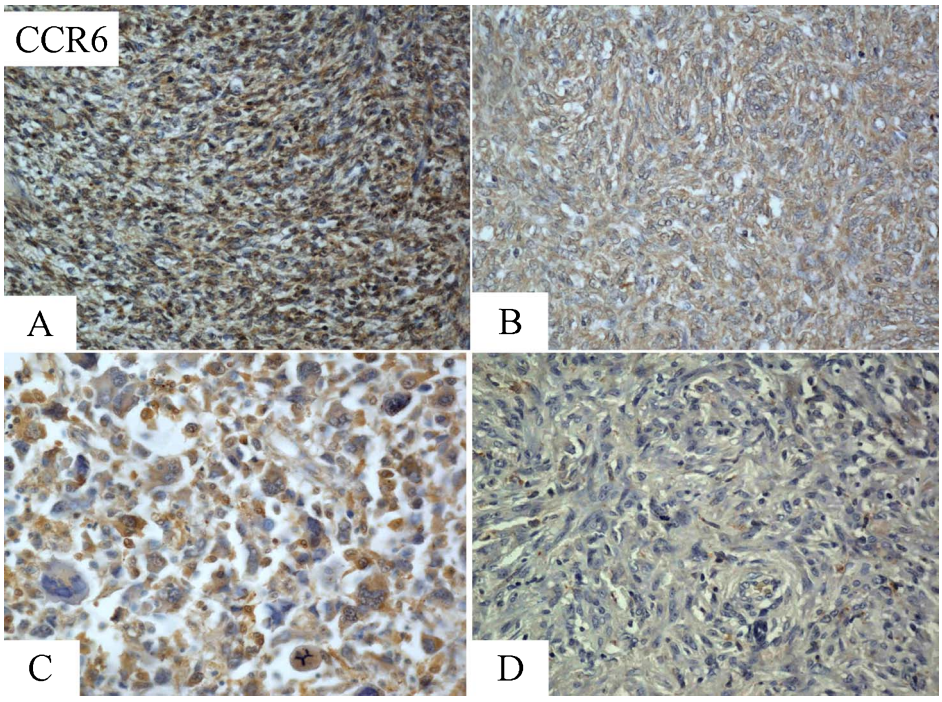

Fig. 3. CCR6 immunoexpression in fibrohistiocytic tumors (×200). **A.** Diffuse CCR6 expression was observed in non-relapsed DFSP. **B.** Diffuse CCR6 expression was observed in relapsed DFSP. **C.** Diffuse CCR6 expression was observed in MFH. **D.** No expression of CCR6 was observed in DF.

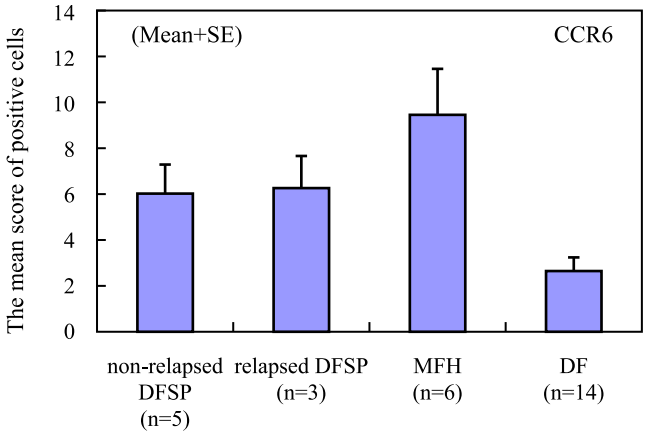

Fig. 4. The mean score of CCR6-positive tumor cells. The mean score of the CCR6 immunostaining was significantly higher in only MFH as compared with DF ($p<0.01$).

Expression of CD34 in DFSP

Anti-CD34 reacted in most of the tumor cells in 2/8 (25%) cases of DFSP, and there was partial immunoreactivity in 5/8 (63%) cases, and no immunoreactivity in 1/8 (13%) case.

In the 3 relapsed DFSP cases, anti-CD34 immunoreactivity was detected in 2 cases (Fig. 7A), but not in 1 case (Fig. 7B).

As shown in Figure 8, no significant difference in the average scores for CD34 immunostaining was observed between non-relapsed DFSP and relapsed DFSP.

IV. Discussion

Recent studies have demonstrated the ability of chemokines to induce cell migration during cancer metastasis [14]. CXCR4 is the physiological receptor for CXCL12, which belongs to a chemokine family that has potent chemotactic activity for lymphocytes. Studies have determined that an upregulation of CXCR4 is observed in breast cancer, squamous cell carcinoma of the head and neck, colorectal cancer, thyroid carcinoma and malignant melanoma [4–6, 9, 13]. However, this chemokine receptor expression pattern has not been previously analyzed in fibrohistiocytic tumors. In this study, immunohistochemical analyses revealed that CXCR4 immunoreactivity was thought to be related to tumor aggressiveness in fibrohistiocytic tumors.

CXCR4, CCR6 and CC7 have a potential to be immunohistochemical indicators of tumor aggressiveness of MFH

MFH is a sarcoma of either fibroblastic or primitive mesenchymal origin, which manifests features of both fibroblastic and histiocytic differentiation. MFH tends to metastasize distantly. In our study, significantly high immunoreactivity for CXCR4, CCR6, and CCR7 was detected in 6/6 cases of MFH, which have the tendency to metastasize.

Recent studies have shown that CXCR4 and CCR7 are consistently expressed in breast cancer cells [4]. Con-

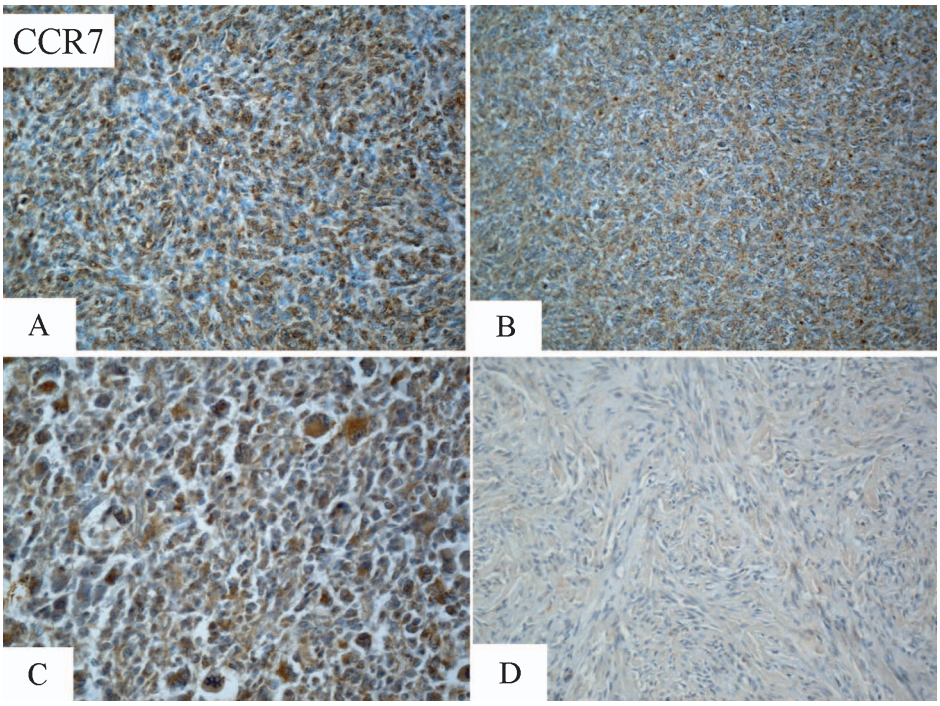

Fig. 5. CCR7 immunoexpression in fibrohistiocytic tumors (×200). **A.** Diffuse CCR7 expression was observed in non-relapsed DFSP. **B.** Diffuse CCR7 expression was observed in relapsed DFSP. **C.** Diffuse CCR7 expression was observed in MFH. **D.** No expression of CCR7 was observed in DF.

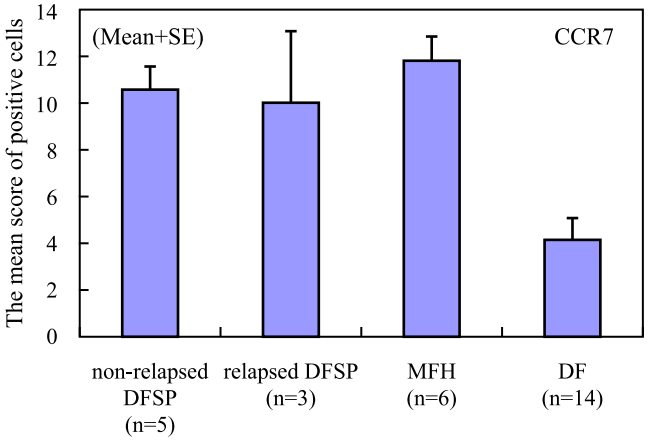

Fig. 6. The mean score of CCR7-positive tumor cells. The mean score of CCR7 immunostaining was significantly higher in non-relapsed DFSP ($p<0.05$) and MFH ($p<0.01$) as compared with DF.

sidering that these matched chemokines are known to be expressed in sites frequently involved with breast cancer metastasis, it is possible that an assessment of the expression of these chemokines in MFH may be a useful tool for predicting tumor aggressiveness.

The assessment of CXCR4 immunoreactivity is a useful tool for predicting tumor aggressiveness of DFSP

DFSP is highly invasive locally, but rarely metastasizes, even after recurrence. It is often difficult to determine the optimal therapy, including the range of excision. In 1990, the first report appeared demonstrating that cells from DFSP express the human progenitor antigen CD34 on their surface [3]. Aiba *et al.* reported that they found strongly positive staining of tumor cells for the CD34 antigen in all seven cases of DFSP [1]. Most studies have documented the prevalence of CD34 staining in DFSP as ranging from 84% to 100% [11].

On the other hand, several reports suggest that the sarcomatous changes in DFSP, which represents a form of tumor progression, showed weak CD34 immunoreactivity [7]. To date, however, definite markers predicting the aggressiveness and recurrence potential of DFSP have not been established. In our study, 7/8 (87.5%) cases of DFSP had CD34 immunoreactivity. However, there was no significant difference in CD34 immunoreactivity between non-relapsed DFSP and relapsed DFSP. Therefore, it seems difficult to regard CD34 as a prognostic indicator of DFSP.

In contrast, our study showed CXCR4 immunoreactivity was significantly higher (i.e. the number of CXCR4 immunopositive cells) in relapsed DFSP as compared with non-relapsed DFSP. There was no significant difference in the immunoexpression of CCR6 or CCR7.

Our present study revealed that: (i) MFH had a strong expression of CXCR4, CCR6, and CCR7 immunoreactivity as compared with DF. These data suggest that an assessment of the expression of these chemokines in MFH may be a useful tool for predicting tumor aggressiveness. (ii) CXCR4

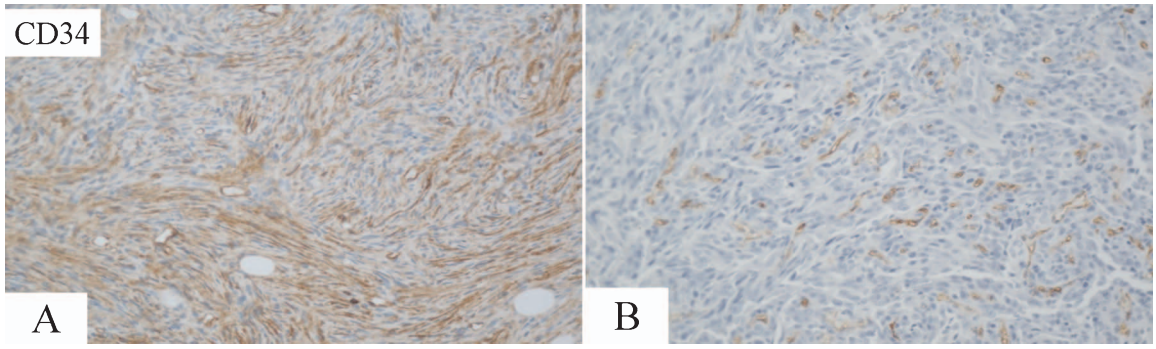

Fig. 7. CD34 immunoexpression in relapsed DFSP (×200). **A.** CD34 positive case of relapsed DFSP. **B.** CD34 negative case of relapsed DFSP.

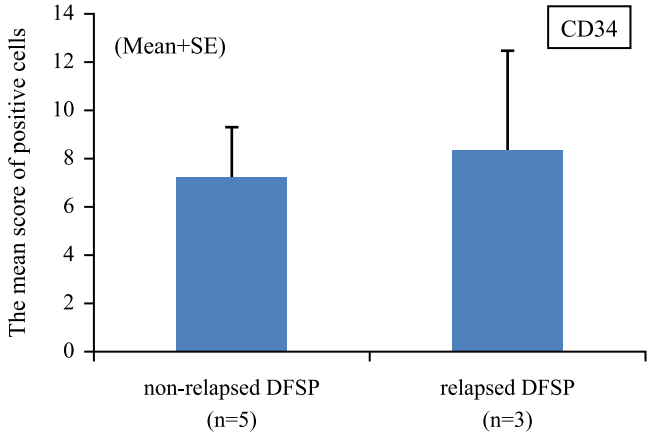

Fig. 8. The mean score of CD34-positive tumor cells. No significant difference in the mean score of CD34 immunostaining was observed between non-relapsed DFSP and relapsed DFSP.

immunoreactivity was significantly higher in relapsed DFSP than in non-relapsed DFSP, even though CD34 immunoreactivity showed no significant differences between the two DFSP groups. These data suggest that the assessment of CXCR4 immunoreactivity in DFSP is a useful tool for predicting tumor aggressiveness.

To confirm these data, additional research with larger study populations will be necessary.

V. References

1. Aiba, S., Tabata, N., Ishii, H., Ohtani, H. and Tagami, H. (1992) Dermatofibrosarcoma protuberans in a unique fibrohistiocytic tumor expression CD34. *Br. J. Dermatol.* 127; 79–84.
2. Al-Agha, O. M. and Igbokwe, A. A. (2008) Malignant fibrous histiocytoma. Between the past and the present. *Arch. Pathol. Lab. Med.* 132; 1030–1035.
3. Haycox, C. L., Odland, P. B., Oldricht, S. M. and Piepkorn, M. (1997) Immunohistochemical characterization of dermatofibrosarcoma protuberans with practical applications for diagnosis and treatment. *J. Am. Acad. Dermatol.* 37; 438–444.
4. Muller, A., Homey, B., Soto, H., Ge, N., Carton, D., Buchanan, M. E., McClanahan, T., Murphy, E., Yuan, W., Wagner, S. N., Barrera, J. L., Mohar, A., Verastegui, E. and Zlotnik, A. (2001) Involvement of chemokine receptor in breast cancer metastasis. *Nature* 410; 50–56.
5. Muller, A., Sonkoly, E., Eulert, C., Gerber, P. A., Kubitza, R., Schirlau, K., Franken-Kunkel, P., Poremba, C., Snyderman, C., Klotze, L. O., Ruzicka, T., Bier, H., Zlotnik, A., Whiteside, T. L., Homey, B. and Hoffmann, T. K. (2006) Chemokine receptors in head and neck cancer: association with metastatic spread and regulation during chemotherapy. *Int. J. Cancer* 118; 2147–2157.
6. Murakami, T., Cardones, A. R. and Hwang, S. T. (2004) Chemokine receptors and melanoma metastasis. *J. Dermatol. Sci.* 36; 71–78.
7. Sato, N., Kimura, K. and Tomita, Y. (1995) Recurrent dermatofibrosarcoma protuberans with mixoid and fibrosarcomatous changes paralleled by loss of CD34 expression. *J. Dermatol.* 22; 665–672.
8. Scala, S., Ottaiano, A., Ascierto, P. A., Cavalli, M., Simeone, E., Giuliano, P., Napolitano, M., Franco, R., Botti, G. and Castello, G. (2005) Expression of CXCR4 predicts poor prognosis in patients with malignant melanoma. *Clin. Cancer Res.* 11; 835–841.
9. Schimanski, C. C., Schwald, S., Simiantonaki, N., Jayasinghe, C., Gonner, U., Wilsberg, V., Junginger, T., Berger, M. R., Galle, P. R. and Moehler, M. (2005) Effect of chemokine receptors CXCR4 and CCR7 on the metastatic behavior of human colorectal cancer. *Clin. Cancer. Res.* 11; 1743–1750.
10. Tanaka, A., Kimura, A., Yamamoto, Y., Uede, K. and Furukawa, F. (2008) Expression of histo-blood group A type 1, 2 and 3 antigens in normal skin and extramammary Paget's disease. *Acta Histochem. Cytochem.* 41; 165–171.
11. Tardio, J. C. (2008) CD34-reactive tumors of the skin. An updated review of an ever-growing list of lesions. *J. Cutan. Pathol.* 35; 1079–1092.
12. Wagner, P. L., Moo, T. A., Arora, N., Liu, Y. F., Zarnegar, R., Scognamiglio, T. and Fahey, T. J. 3rd. (2008) The chemokine receptors CXCR4 and CCR7 are associated with tumor size and pathologic indicators of tumor aggressiveness in papillary thyroid carcinoma. *Ann. Surg. Oncol.* 15; 2833–2841.
13. Yasuoka, H., Tsujimoto, M., Yoshidome, K., Nakahara, M., Kodama, R., Sanke, T. and Nakamura, Y. (2008) Cytoplasmic CXCR4 expression in breast cancer: induction by nitric oxide and correlation with lymph node metastasis and poor prognosis. *BMC Cancer* 8; 340.
14. Zlotnik, A. (2006) Chemokines and cancer. *Int. J. Cancer.* 119; 2026–2029.