의학박사 학위논문

A retrospective study on the clinicopathologic features and Akt/mTOR, STAT3, ERK, cyclin D1 and PD-L1 expression of dermatofibrosarcoma protuberans

융기성피부섬유육종에서 임상병리학적 소견 및 Akt/mTOR, STAT3, ERK, cyclin D1과 PD-L1 발현에 관한 후향적 연구

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박선영

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Abstract

A retrospective study on the clinicopathologic features and Akt/mTOR, STAT3, ERK, cyclin D1 and PD-L1 expression of dermatofibrosarcoma protuberans

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Little is known regarding oncoproteins other than platelet-derived growth factor subunit B in dermatofibrosarcoma protuberans (DFSP). Moreover, the risk factors for worse prognosis are controversial.

We sought to determine the clinicopathologic features and key factors for adverse outcome in DFSP, including the implication of expression of protein kinase B (Akt)/mammalian target of rapamycin (mTOR), signal transducer and activator of transcription 3 (STAT3), extracellular signal regulated kinase (ERK), cyclin D1, and programmed death ligand 1 (PD-L1).

Clinicopathologic and immunohistochemical analyses were performed for 44 DFSPs having wide local excision and 92 dermatofibromas as controls.

Compared with the 35 nonrecurrent DFSPs, the 9 recurrent DFSPs exhibited larger tumor size, deeper invasion beyond the subcutis, and more diverse histologic subtype. The fibrosarcomatous subtype revealed frequent mitotic figures and a high cyclin D1–positive index. The 2 metastatic DFSPs (1 each of the fibrosarcomatous and myxoid subtypes) demonstrated 4 and 11 instances of local recurrence, respectively, as well as larger tumor size, deeper invasion beyond the subcutis, and high expression of cyclin D1. Expression of Akt/mTOR, STAT3, ERK, and PD-L1 ranged from none or low in the primary skin lesions to high in the corresponding metastatic sites. Akt/mTOR and ERK were expressed more frequently in DFSP than in dermatofibroma.

In conclusion, the risk factors for worse prognosis are comprehensive in DFSP. The Akt, mTOR, and ERK proteins may be involved in the development, and Akt, mTOR, STAT3, and PD-L1 may contribute to progression. The elucidation of the status of signaling related oncoproteins may be useful in determining molecularly targeted chemotherapy for intractable, inoperable, or advanced metastatic DFSP

Keyword: cyclin D1; dermatofibrosarcoma protuberans; metastasis; recurrence; fibrosarcomatous; prognosis

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Chapter 1. Introduction

1.1. Study Background

Dermatofibrosarcoma protuberans (DFSP) constitutes a rare skin tumor that exhibits intermediate malignancy characterized by high rates of local recurrence (1–3) but a limited risk of metastasis (4–6). DFSP is generally initially diagnosed in young to middle-aged adults in their 20s to 40s (5, 7, 8), occurring most frequently on the trunk (5, 7–9). Microscopically, DFSP usually localizes within the dermis and subcutaneous tissue and is composed of slender spindle tumor cells with uniform nuclei and rare mitotic figures arranged in a storiform pattern (5, 10). In contrast, the fibrosarcomatous subtype of DFSP is characterized by a long fascicular "herringbone" arrangement, as well as by increased mitotic activity and cellularity with higher nuclear atypia (2, 3, 5). Moreover, the prevailing view has been that the fibrosarcomatous subtype shows a more aggressive clinical course and is associated with a higher risk of distant metastasis than that of the nonfibrosarcomatous variant (2, 3), although some authors have found no such associations (11). With respect to the molecular mechanisms of DFSP, the constitutional production of platelet-derived growth factor subunit B (*PDGFB*), which results from the fusion of the collagen type I alpha 1 (*COL1A1*) and *PDGFB* genes (12), leads to tumor cell proliferation **(Figure 1)** (10, 13–15). Imatinib, selective receptor tyrosine kinase inhibitor is indicated for treatment of unresectable or metastatic DFSP based on

the molecular pathogenesis (5). Although platelet-derived growth factor-induced signaling pathways have been studied (16, 17), little information is available regarding the signaling pathways utilized in DFSP cells. Such signaling pathways play crucial roles in controlling cell proliferation, apoptosis, and immune evasion, thus contributing to the oncogenesis of diverse human malignancies. Treatment with imatinib, a tyrosine kinase inhibitor, deregulates PDGFB/platelet derived growth factor receptor beta (PDGFRB) signaling and alters the biologic effects of various signaling transduction pathways, including the phosphatidylinositol 3 kinase/protein kinase B (Akt), signal transducer and activator of transcription 3 (STAT3), and Ras/mitogen activated protein kinase (MAPK)/extracellular signal regulated kinase (ERK) pathways (18). In 2017, a correlation was demonstrated between PDGFRB and the Akt/mammalian target of rapamycin (mTOR) signaling pathway in DFSP, suggesting that the Akt/mTOR pathway may have a positive effect on DFSP tumor growth (19). In particular, these pathways include activation of cell cycle-related proteins such as cyclin D1 (20, 21). In addition, emerging evidence suggests that programmed death ligand 1 (PD-L1) is central to tumor immune evasion in diverse human malignancies, with PD-L1 expression being promoted by various mechanisms such as activation of epigenetic regulation along with MAPK/ERK, as well as by phosphatidylinositol 3 kinase/Akt pathways (22-26). However, there have been published few reports regarding the function of oncoproteins other than PDGFB in DFSP, with each study being limited to the analysis of only a small number

of proteins (1, 19, 20, 27, 28).

1.2. Purpose of Research

The goals of the present study were to determine the clinicopathologic features of nonrecurrent, recurrent, and metastatic DFSPs along with the risk factors for aggressive tumor behavior and to simultaneously assess the implication of expression of multiple oncoproteins, including Akt, mTOR, STAT3, ERK, cyclin D1, and PD-L1.

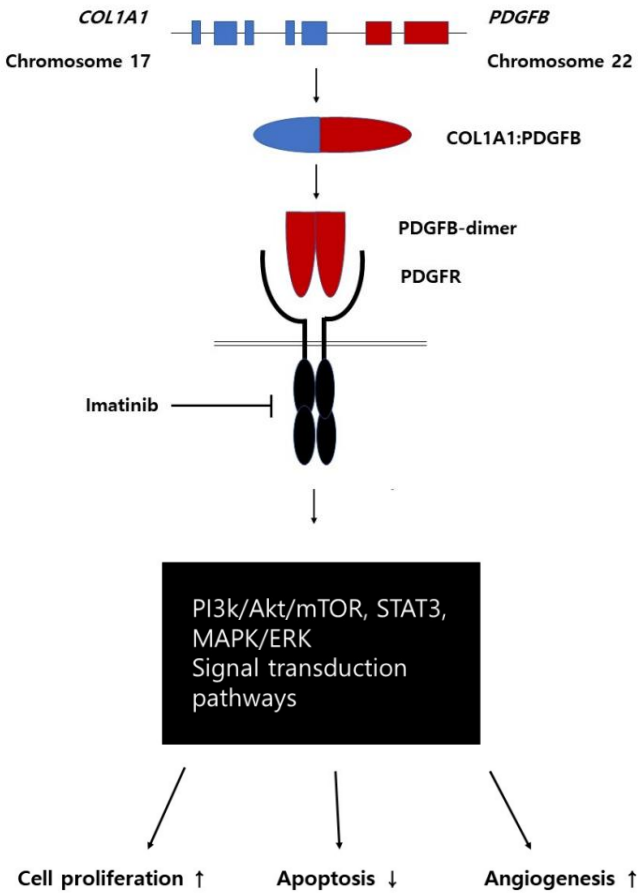

Figure 1. Schematic illustration of molecular biology in dermatofibrosarcoma protuberans. Chromosomal translocation t (17;22) and followed fusion of the *COL1A1* and *PDGFB* cause constitutive expression of COL1A1−PDGFB protein. Continuous activation of PDGF receptor (PDGFR) alters biologic effects which causes tumor growth. Imatinib is selective inhibitor of receptor tyrosine kinase.

Chapter 2. Material and Methods

2.1. Patients

We retrieved database information on DFSP cases that were treated by wide local excision (i.e., surgically excised with at least 1 cm of tumor-free surgical margin) between 2000 and 2015 at Seoul National University Hospital and Boramae Medical Center in Seoul, Korea. Of the patients comprising the 44 collected cases, 35 received their original diagnosis and treatment at our institutions and 9 were referred to our institutions to have surgery for recurrent DFSP. Clinical information, including age, sex, tumor location, clinical skin manifestation, treatment, recurrence, and metastasis, was obtained from medical records.

2.2. Ethics statement

All human tissue specimens were obtained by surgical resection for diagnostic or therapeutic purposes. This retrospective study was performed by using the paraffin blocks containing tissue samples of patients after pathologic diagnosis, and all samples were anonymized before the study. This study was approved by the institutional review board of Seoul National University Boramae Medical Center under the condition of the anonymization (institutional review board No. 06-2011-15).

2.3. Immunohistochemistry

Immunohistochemistry was conducted for Akt, mTOR, STAT3, ERK, cyclin D1, and PD-L1 on paraffin block-embedded tissues by using an automated immunostainer (Ventana BenchMark XT, Ventana Medical Systems Inc., Tucson, AZ) according to the manufacturer's protocol. The antibodies against phospho-Akt (Ser473 and 587F11), phospho-mTOR (Ser2448 and 49F9), phospho-STAT3 (Tyr705 and D3A7), and ERK (phospho-p44/42 MAPK, Thr202/Tyr204, and D13.14.4E) were purchased from Cell Signaling Technology (Danvers, MA) and used at a 1:50 dilution. Anti-PD-L1 (SP263) and anti-cyclin D1 (SP4) antibodies were provided by Ventana Medical Systems in ready-to-use form.

We evaluated nuclear staining for STAT3, ERK, and cyclin D1; cytoplasmic and/or nuclear staining for Akt and mTOR; and membranous staining for PD-L1. With regard to PD-L1, the percentage of tumor cells at any intensity was evaluated according to the manufacturer's instruction. Cyclin D1 nuclear staining pattern was found with homogeneously strong intensity; thus, the percentage of tumor cells with positive staining was called the positive index. For the remaining proteins, staining intensity was estimated in a 3-tier system: weak, moderate, and strong. Consequently, a sample was defined as positive when staining was observed in more than 20% of tumor cells at weak intensity, more than 10% at moderate intensity, or more than 5% at strong intensity. To our knowledge, the

immunohistochemical evaluation of these proteins in DFSP tissue has rarely been reported; specifically, one group of authors has suggested the positive cutoff for Akt or mTOR as representing at least 10% of the tumor cells without consideration of staining intensity (19), whereas another group has interpreted cases as positive if any tumor cells (even a single cell) are stained for STAT3 or ERK at even weak intensity (20). To better understand protein expression patterns, 92 cases of dermatofibroma tissues were used as a control, as DFSP and dermatofibroma share some similarities with regard to histomorphologic features (5, 29).

2.4. Statistical analysis

We analyzed correlations of categoric features by using the Pearson X^2 test and Fisher's exact test. Correlations of continuous variables were analyzed by using the Mann–Whitney U test and Kruskal–Wallis test. p values less than 0.05 were considered statistically significant. All statistical analysis was performed with SPSS Statistics software (version 20.0, IBM Inc, Armonk, NY).

Chapter 3. Results

3.1. Clinicopathologic features and patient outcome

At the time of assessment at our hospital, the DFSP of 35 patients was diagnosed for the first time and that of 9 patients was recurrent. In particular, the latter had surgical excisions for the first diagnosed tumor and each recurrence at other hospitals, and eventually they were referred to our institutions and underwent wide local excision at our hospital.

The baseline features of patients with DFSP are summarized in Table I. The median age at the time of first diagnosis was 38 years (range, 19–79 years) (Figure 2). There were slightly more female than male patients (57% vs 43%). Tumor location had a predilection for the trunk (55%), followed by a lower extremity (22%), the head and neck (14%), and an upper extremity (9%) (Figure 3). Clinical skin lesions were mainly manifested as a mass/nodule (in 84% of cases), with less frequent appearance of keloid scar–like lesion, patch, and plaque (Figure 4). Mitotic figures ranged from 1 to 25 per 10 high–power fields (median, 2; mean, 4). Histologically, the classic subtype was predominant (64%), with pigmented, myoid, myxoid, or fibrosarcomatous subtypes being uncommon (Figure 5).

Table I. Baseline clinicopathological features and protein expression

of dermatofibrosarcoma protuberans

	Total (N = 44)	Nonrecurrent group (n = 35)	Recurrent group (n = 9)	*p* value
Sex				0.932
Male	19 (43%)	15 (43%)	4 (44%)	
Female	25 (57%)	20 (57%)	5 (56%)	
Age at 1st diagnosis, y				0.886
median (mean±SD) (range)	38 (40±15.2) (19–79)	34 (40±16.1) (19–79)	37 (38±10.8) (26–59)	
Location				0.157
Trunk	24 (55%)	17 (49%)	7 (78%)	
lower extremity	10 (22%)	10 (27%)	0 (0%)	
head and neck	6 (14%)	4 (12%)	2 (22%)	
upper extremity	4 (9%)	4 (12%)	0 (%)	
Clinical skin lesion				0.57
mass/nodule	37 (84%)	28 (80%)	9 (100%)	
keloid scar-like	4 (9%)	4 (11%)	0	
Patch	2 (5%)	2 (6%)	0	
Plaque	1 (2%)	1 (3%)	0	
*Tumor size, mm				0.021
median (mean±SD) (range)	25 (26±14.2) (5–55)	22 (24±13.1) (5–51)	40 (36±14.3) (13–55)	
*Histologic subtype				0.02
Classic	28 (64%)	25 (71%)	3 (33%)	
Pigmented	3 (7%)	3 (9%)	0	
Myoid	4 (9%)	2 (6%)	2 (22%)	
Myxoid	4 (9%)	1 (3%)	3 (33%)	
Fibrosarcomatous	5 (11%)	4 (11%)	1 (11%)	
Mitosis (/10HPF)				0.184
median (mean±SD) (range)	2 (4±5.3) (1–25)	2 (3±3.3) (1–11)	4 (8±9.1) (1–25)	
*Invasion beyond subcutis	2 (5%)	0	2 (22%)	0.038
*Metastasis, present	2 (5%)	0	2 (22%)	0.038
Protein expression				
Positive for Akt	37 (84%)	31 (89%)	6 (67%)	0.138

Positive for mTOR	11 (25%)	9 (26%)	2 (22%)	0.829
Positive for STAT3	5 (11%)	5 (14%)	0	0.566
Positive for ERK	28 (64%)	21 (60%)	7 (78%)	0.45
Cyclin D1-positive index				
median (mean±SD) (range)	10 (15±17.8) (0-80)	10 (15±19.0) (0-80)	10 (12±12.7) (0-40)	0.775

*p value < 0.05 between non-recurrent and recurrent groups.

CyclinD1-positive index: percent of tumor cells showing nuclear staining for cyclin D1.

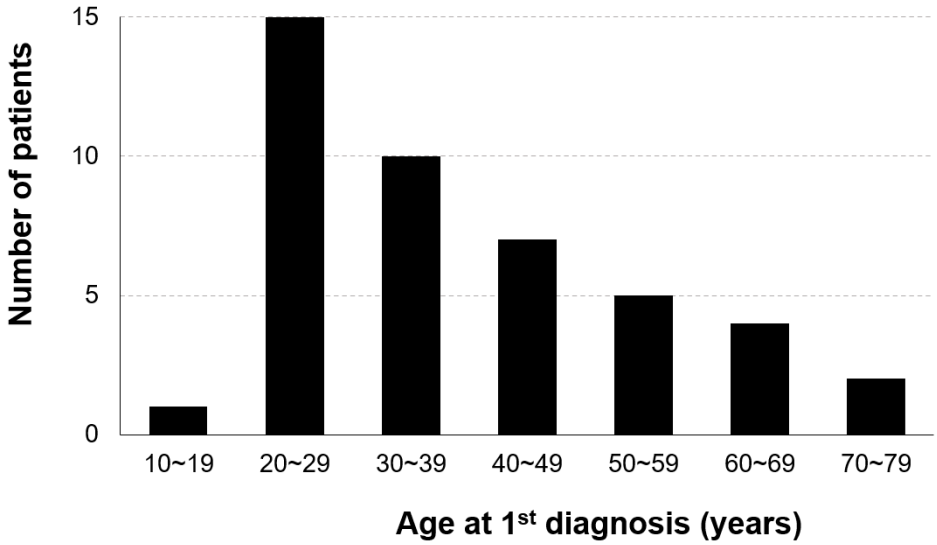

Figure 2. Age distribution of the first diagnosis in patients with dermatofibrosarcoma protuberans. Peak incidence is observed in patients in their 20s to 40s.

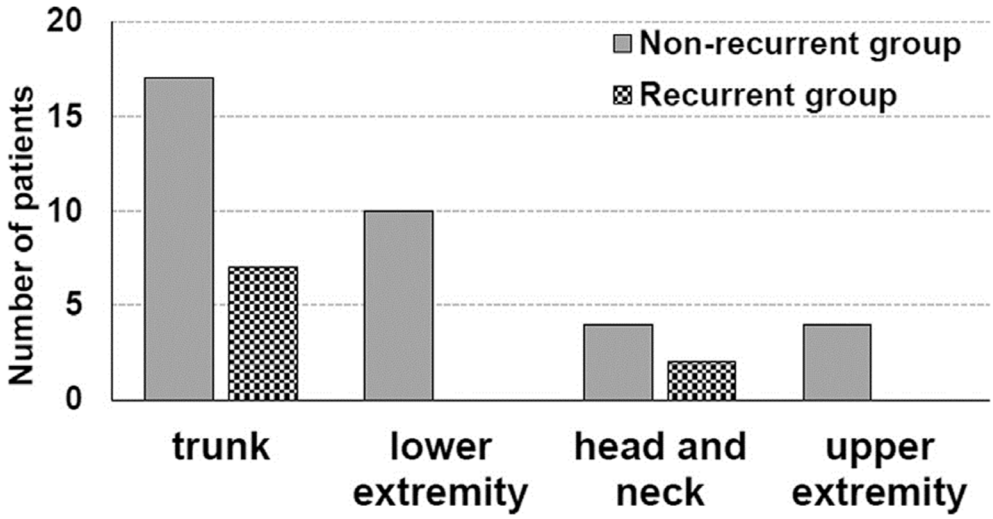

Figure 3. Anatomic location of dermatofibrosarcoma protuberans. Tumors are located predominantly on the trunk.

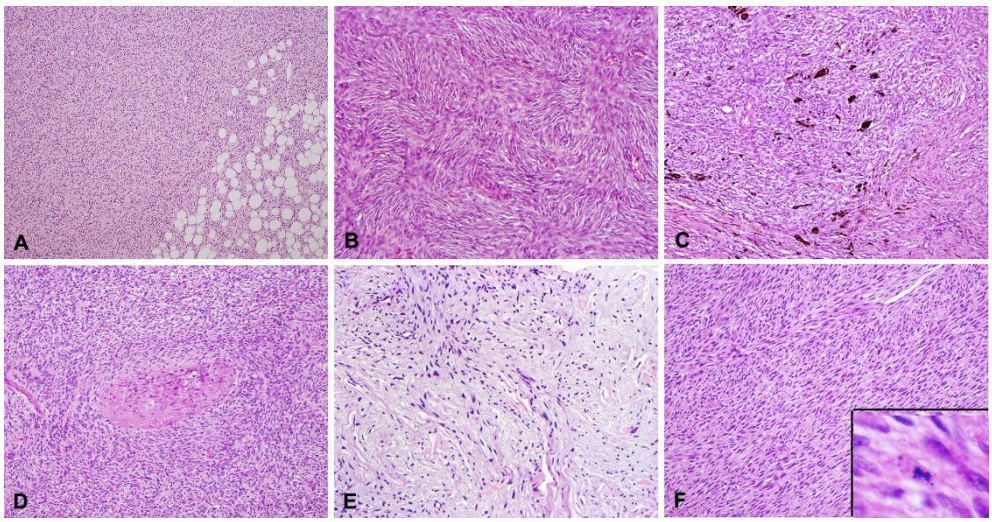

Figure 4. Histologic features of dermatofibrosarcoma protuberans upon hematoxylin and eosin staining. (A), Classic subtype in which tumor cells infiltrate the subcutaneous layer in a honeycomb pattern. (B), Classic subtype in which slender spindle tumor cells are arranged in storiform fashion. (C), Pigmented subtype in which melanin pigments are noted. (D), Myoid subtype in which myoid ball is observed. (E), Myxoid subtype in which myxoid change is conspicuous. (F), Fibrosarcomatous subtype in which spindle tumor cells are arranged in a long fascicular fashion with brisk mitotic activity (inset).

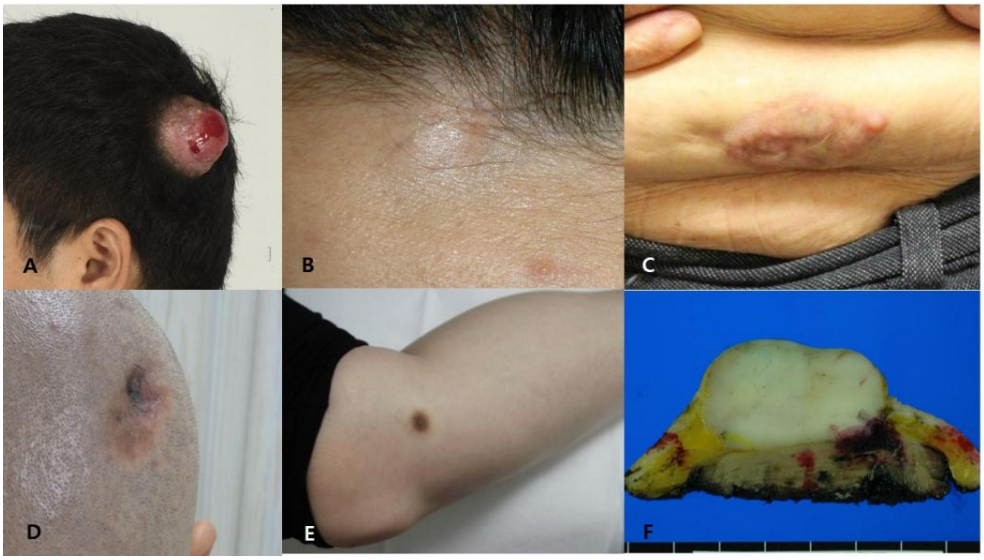

Figure 5. Representative features of clinical skin lesion. (A), mass (B), nodule (C), keloid scar-like (D), patch (E), plaque (F), cross-section of surgical resection specimen.

Fibrosarcomatous DFSP showed more frequent mitotic figures than did the "conventional" group (including classic, pigmented, myoid, and myxoid subtypes) ($p < 0.05$) (Table II), although no statistically significant difference in recurrence or metastasis rate was observed between the fibrosarcomatous type and nonfibrosarcomatous group. The follow-up period in patients with nonrecurrent DFSP ranged from 24 to 155 months (median, 59 months; mean, 64 ± 44.7 months). In particular, 5 cases of the fibrosarcomatous subtype were followed up for 38, 41, 60, 60, and 100 months, respectively.

Recurrent DFSPs showed larger tumor size, deeper invasion beyond the subcutis, and a different spectrum of histologic subtypes compared with nonrecurrent DFSPs ($p < 0.05$, respectively) (Table I). In recurrent DFSPs, the classic subtype was not the only major type, with 3 cases each of classic and myxoid subtypes, 2 cases of the myoid subtype, and 1 case of fibrosarcomatous DFSP also observed. According to patient self-reports, the frequency of recurrence was from 1 to 11 times, and the median time to local recurrence was 2 years (mean, 4; range, 1-9); in particular, the time to recurrence was 1 year for the case of the fibrosarcomatous subtype and 2 and 4 years for each of the 2 cases of the myxoid subtype.

Recurrent DFSP demonstrated more aggressive behavior than did nonrecurrent DFSP, as evidenced by distant metastasis in 2 patients. Patients with recurrent DFSP were followed up from 24 to 109 months after wide local excision at our institution. Metastatic cases

showed frequent recurrence, larger tumor size, and deeper invasion beyond the subcutis compared with cases of nonmetastatic recurrent DFSPs ($p < 0.05$, respectively) (Table III). In particular, 1 patient had 11 instances of local recurrence before visiting our institutions. She had a fibrosarcomatous DFSP that was located on the forehead with bone invasion and metastasis to the maxillary sinus at 17 months after wide local excision of the skin lesion at our institutions. In the other metastatic case, there were 4 instances of local recurrence before transfer to our institutions. Her myxoid DFSP was seated on the lower abdominal wall and extended into the skeletal muscle, and it metastasized to the pleura at 29 months after wide local excision of the skin lesion at our institutions. Both patients were subsequently treated with imatinib and radiotherapy however, they developed progressive disease.

Table Ⅱ. Comparison of clinicopathological features and protein expression between conventional and fibrosarcomatous subtypes in dermatofibrosarcoma protuberans

	Conventional (n = 39)	Fibrosarcomatous (n = 5)
Sex		
male	19 (49%)	0
female	20 (51%)	5 (100%)
Age at 1st diagnosis, y		
median (mean ± SD) (range)	37 (40 ± 15.7) (19–79)	43 (39 ± 10.8) (27–52)
Location		
trunk	22 (56%)	2 (40%)
lower extremity	8 (21%)	2 (40%)
head and neck	5 (13%)	1 (20%)
upper extremity	4 (10%)	0
Clinical skin lesion		
mass/nodule	32 (82%)	5 (100%)
keloid scar-like	4 (10%)	0
patch	2 (5%)	0
plaque	1 (3%)	0
Tumor size, mm		
median (mean ± SD) (range)	22 (24 ± 13.4) (5–55)	36 (38 ± 15.6) (15–55)
*Mitosis (/10HPF)		
median (mean ± SD) (range)	2 (4 ± 5.5) (1–25)	5 (7 ± 2.7) (5–10)
Invasion beyond subcutis	1 (3%)	1 (20%)
Recurrence, present	8 (21%)	1 (20%)
Metastasis, present	1 (3%)	1 (20%)
Protein expression		
Positive for Akt	33 (85%)	4 (80%)
Positive for mTOR	10 (26%)	1 (20%)
Positive for STAT3	5 (13%)	0
Positive for ERK	26 (67%)	2 (40%)
†Cyclin D1-positive index		
median (mean ± SD) (range)	10 (13 ± 18.0) (0–80)	20 (26 ± 13.4) (10–40)

p value = 0.014*, 0.021†.

Table III. Clinicopathological features and protein expression in subgroups stratified by metastatic status in recurrent dermatofibrosarcoma protuberans

	Nonmetastasis (n = 42)	Metastasis (n= 2)
Sex		
male	19 (45%)	0
female	23 (55%)	2 (100%)
Age at 1st diagnosis, y		
median (mean ± SD) (range)	36 (39 ± 15.4) (19–79)	45 (45 ± 9.9) (38–52)
*Frequency of recurrence		
once or twice	7 (17%)	0
more than 3 times	0	2 (100%)
Location of skin lesion		
trunk	23 (55%)	1 (50%)
head and neck	5 (12%)	1 (50%)
lower extremity	10 (24%)	0
upper extremity	4 (9%)	0
Clinical skin lesion		
mass/nodule	35 (83%)	2 (100%)
keloid scar-like	4 (9%)	0
patch	2 (5%)	0
plaque	1 (3%)	0
†Tumor size of skin lesion, mm		
median (mean ± SD) (range)	24 (25 ± 13.6) (5–55)	47 (48± 10.6) (40–55)
Mitosis (/10HPF) of skin lesion		
median (mean ± SD) (range)	2 (4 ±4.3) (1–19)	15 (15 ± 14.1) (5–25)
‡Invasion beyond subcutis	0	2 (100%)
Histologic subtype		
classic	28 (67%)	0
pigmented	3 (7%)	
myoid	4 (9%)	0
myxoid	3 (7%)	1 (50%)

fibrosarcomatous	4 (9%)	1 (50%)
Protein expression		
Positive for Akt	35 (83%)	2 (100%)
Positive for mTOR	11 (26%)	0
Positive for ERK	26 (62%)	1 (50%)
§Cyclin D1, high expression	12 (29%)	2 (100%)
Cyclin D1-positive index		
median (mean ± SD) (range)	14 (10 ± 17.9) (0-80)	27 (28 ± 17.7) (15-40)

p value = 0.028*, 0.042†, 0.001‡, 0.034§.

Cyclin D1, high expression: the number of cases with a higher index than the mean value of the cyclin D1-positive index in recurrent DFSP.

3.2. Protein expression in DFSP

The fibrosarcomatous subtype of DFSP showed a higher cyclin D1-positive index than the conventional group did ($p < 0.05$) (Table II). The metastatic cases revealed higher expression of cyclin D1 than did the cases of nonmetastatic recurrent DFSP (Table III). PD-L1 expression was found in only 1 case of metastasis to the maxillary sinus (membranous staining in 2% of tumor cells), whereas no PD-L1 staining was observed in the remaining cases, including a metastatic site to the pleura, or in skin lesions of the 44 DFSP cases. Representative immunohistochemical features for each protein are displayed in Figure 6.

Notably, an alteration of protein expression patterns was observed: from no or low expression in primary skin lesions to higher expression in corresponding metastatic sites (Table IV). The only exception to this trend was for cyclin D1 expression, which revealed high index similarly in both skin lesions and metastatic sites. Among the examined proteins, Akt, mTOR, and ERK were expressed in DFSP more frequently than in dermatofibroma ($p < 0.05$ for each) (Table V).

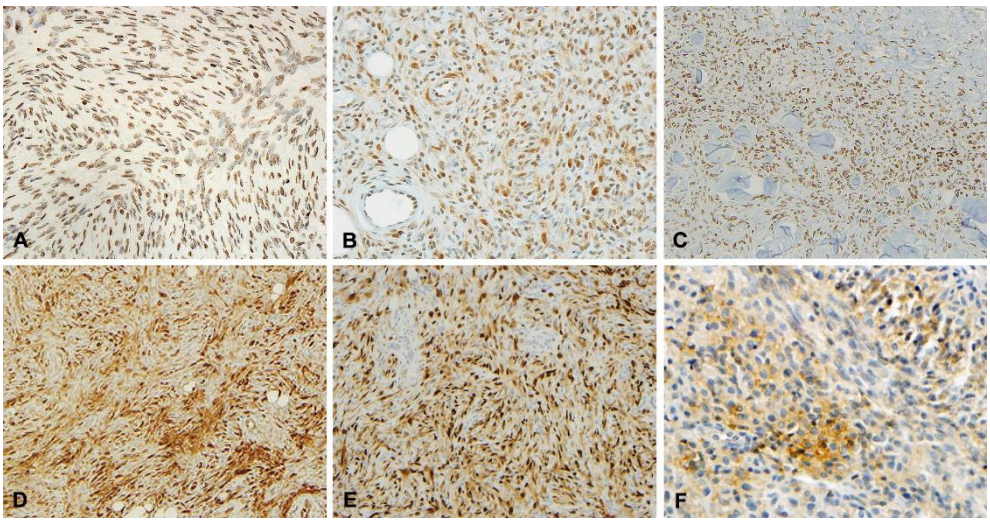

Figure 6. Representative immunohistochemical features in dermatofibrosarcoma protuberans. Protein kinase B positivity (A), mammalian target of rapamycin positivity (B), signal transducer and activator of transcription 3 positivity (C), extracellular signal regulated kinase positivity (D), high cyclin D1-index (E), and programmed death ligand 1 membranous staining (F) are noted in the tumor cells.

Table IV. Protein expression in each skin lesion and its corresponding metastatic site in recurrent dermatofibrosarcoma protuberans with metastasis

	Case 1: Fibrosarcomatous		Case 2: Myxoid	
	Skin lesion	Metastasis	Skin lesion	Metastasis
	Forehead	Maxillary sinus	Abdominal wall	Pleura
Akt	P (2+, 50%)	P (3+, 70%)	P (3+, 60%)	P (3+, 90%)
mTOR	N	P (1+, 20%)	N	P (1+, 30%)
STAT3	N	P (3+, 10%)	N	P (3+, 30%)
ERK	P (2+, 20%)	P (3+, 70%)	P (3+, 20%)	P (3+, 80%)
Cyclin D1-positive index	40%	40%	15%	20%
PD-L1	0	(2+, 2%)	0	0

N, negative; P, positive

Staining intensity (1+ , weak; 2+ moderate; 3+ , strong) and percent of positive staining tumor cells are shown parenthetically for each case.

Table V. Comparison of protein expression between dermato-fibrosarcoma protuberans and dermatofibroma

	Dermatofibrosarcoma protuberans (N = 44)	Dermatofibroma (N = 92)	*p* value
Positive for Akt	37 (84%)	57 (62%)	0.049
Positive for mTOR	11 (25%)	7 (8%)	0.013
Positive for STAT3	5 (11%)	4 (4%)	0.148
Positive for ERK	28 (64%)	18 (20%)	<.001

Chapter 4. Discussion

Our results regarding the clinicopathologic features of DFSP are in line with those in previous reports, such as the peak incidence in patients in their 20s and 40s, the trunk as the most common location, clinical skin presentation mainly as a mass/nodule, a spectrum of subtypes, and high rates of local recurrence with rare metastasis (5, 7–9). Patients with recurrent DFSPs were referred to our institutions for re-excision of locally recurrent tumor after surgical procedures at other hospitals; such patients showed larger tumor size, deeper invasion beyond the subcutaneous layer, and diverse histologic subtypes (beyond the predominant "classic" subtype). Additionally, it is plausible that local recurrence may have derived from an inadequate surgical margin and/or an incorrect first pathologic diagnosis at prior hospitals, considering that there was no local recurrence in 35 patients whose DFSP was first diagnosed at our institutions and who were treated with "wide local excision with adequate surgical margin." It is known that an adequate surgical margin and a correct first pathologic diagnosis are absolutely essential to obtaining local control in patients with DFSP (4, 7, 19, 30, 31).

The results of the present study suggest that determination of the risk factors of metastasis in patients with DFSP may be more complex than just categorization as the fibrosarcomatous subtype. Specifically, frequent recurrences, larger tumor size, deeper invasion beyond the subcutaneous layer, and high expression of cyclin D1

were noted in 2 metastatic cases (1 each of the fibrosarcomatous and myxoid subtypes) compared with in 42 cases of the nonmetastatic group. In comparison, some authors have reported a stronger relationship between DFSP metastasis and tumor size than between DFSP metastasis and frequency of recurrence (32). Several studies have emphasized the fibrosarcomatous subtype as a risk factor for local recurrence and/or metastasis (2, 3, 33-36). Conversely, Goldblum et al. stated that no difference existed between the biologic behavior of fibrosarcomatous subtype and that of the conventional subtypes, suggesting that such conclusions regarding fibrosarcomatous DFSP derived from inadequately excised tumors (11). These findings are similar to our finding that the fibrosarcomatous subtype was not statistically different from the conventional group with regard to local recurrence or metastasis rate. The fibrosarcomatous subtype in the present study revealed more frequent mitotic figures and higher cyclin D1 expression, which may provoke faster tumor growth or an increased potential for metastasis than in the conventional group. However, statistical analysis indicated that our fibrosarcomatous DFSP was not more aggressive clinically than the conventional group. This is consistent with the concept that the recurrence or metastasis rate in fibrosarcomatous DFSP may be only minimally increased over that in the conventional group, provided that the tumor is excised with adequate surgical margin (30).

In contrast, the present study indicates that metastasis may accompany genetic progression accumulated by repeated recurrences. In this study, the Akt, mTOR, STAT3, ERK, and PD-

L1 proteins were expressed at higher levels in the metastatic sites than in each of the primary skin lesions; in addition, cyclin D1 was highly expressed in both skin lesions and metastatic sites. Moreover, Oh et al. tracked a patient with DFSP over a 10-year period, from the first surgical procedure to metastasis (including 3 instances of recurrence), reporting genetically different tumor subclones among the first, recurrent, and metastatic tumors (37) and thus supporting the notion that tumor progression is induced by the sequential selection of more aggressive subclones (38).

The present study may imply that Akt, mTOR, and ERK proteins are involved in the development of DFSP, as these proteins were more frequently expressed in DFSP than in dermatofibroma. Our results are compatible with current data suggesting that PDGFRB and the Akt/mTOR signaling pathway may affect the tumor growth of DFSP (19). Additionally, a recent report has demonstrated a response to everolimus (an mTOR inhibitor) in 1 patient with imatinib-resistant fibrosarcomatous DFSP (39). Moreover, the ERK pathway is involved in increased mitogenic activity and proliferation in DFSP cells (27, 28), and overexpression of ERK protein has been demonstrated in DFSP (20).

Chapter 5. Conclusions

This retrospective study has presented an analysis of nonrecurrent, recurrent, and metastatic DFSPs. In this series, we have suggested that comprehensive clinicopathologic features (rather than simply the fibrosarcomatous subtype) constitute the risk factors for local recurrence and/or metastasis in DFSP. Metastatic DFSP may accompany genetic progression accrued by recurrences. The Akt, mTOR, and ERK proteins may be involved in the development of DFSP, and Akt, mTOR, STAT3, and PD-L1 may contribute to its progression. We believe that elucidation of the status of signaling-related oncoproteins may be useful in determining molecularly targeted chemotherapy, thereby providing additional options to imatinib therapy in the case of intractable, inoperable, or advanced metastatic DFSP.

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국문 초록

융기성피부섬유육종에서 임상병리학적 소견 및 Akt/mTOR, STAT3, ERK, cyclin D1과 PD-L1 발현에 관한 후향적 연구

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융기성피부섬유육종의 발생과 관련된 암 단백질에 대하여 platelet-derived growth factor subunit B 외에는 밝혀진 것이 거의 없으며, 예후에 영향을 미치는 인자에 관하여도 논란이 많다.

본 연구에서는 임상병리학적 소견과 protein kinase B (Akt)/mammalian target of rapamycin (mTOR), signal transducer and activator of transcription 3 (STAT3), extracellular signal regulated kinase (ERK), cyclin D1, and programmed death ligand 1 (PD-L1)와 같은 단백질들의 발현 양상을 분석하여 융기성피부섬유육종의 예후에 영향을 미치는 인자에 대하여 알아보고자 하였다.

수술적 절제한 44례의 육기성피부섬유육종 검체를 대상으로 임상병리학적 분석과 면역조직화학 염색을 시행하였으며, 92례의 피부섬유종 검체를 대조군으로 설정하였다.

9례의 재발한 융기성피부섬유육종 증례들은 35례의 재발하지 않은 증례들에 비하며 종괴의 크기가 크며 침윤 깊이가 깊고 더 다양한 병리적 아형이 관찰되었다. 섬유육종형의 변화를 보이는 아형에서는 더 많은 세포분열과 더 높은 cyclin D1-positive index가 관찰되었다. 2례의 전이성 융기성피부섬유육종 증례 (각각 피부육종형 아형과 점액양 아형)는 각각 5, 11회의 국소 재발이 관찰되었으며, 전이하지 않은 증례에 비하며 큰 종괴 크기, 더 깊은 침윤 깊이, 더 높은 cyclin D1 발현 소견을 보였다. Akt/mTOR, STAT3, ERK, 그리고 PD-L1과 같은 단백질의 발현 양상에서 기존 피부 병변에 비해 전이 부위에서 더 높은 발현을 보이는 것이 관찰되었다. Akt/mTOR 와 ERK의 발현은 융기성피부섬유육종에서 피부섬유종에 비해 더 높게 나타나는 것으로 관찰되었다.

따라서 융기성피부섬유육종에서 나쁜 예후를 시사하는 위험 인자는 여러 임상병리학적 요인이 복합적으로 작용한다는 것을 알 수 있다. 세포 신호 전달 단백질들 중에서 Akt, mTOR, 그리고 ERK는 종양의 발생과 연관이 있으며, Akt, mTOR, STAT3, 그리고 PD-L1은 진행과 연관이 있는 것으로 생각된다. 이러한 세포 신호 전달에 관여하는 암 유발 단백질들을 밝히는 것은 진행성 융기성피부섬유육종에서 새로운 표적항암제를 개발하는 데 도움이 될 것으로 기대되고 있다.

주요어: cyclin D1; 융기성피부섬유육종; 전이; 국소 재발; 피부육종형; 예후

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