Alteration of PDGFRβ-Akt-mTOR pathway signaling in fibrosarcomatous transformation of dermatofibrosarcoma protuberans

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https://doi.org/10.15017/2348723

出版情報：Kyushu University, 2019, 博士（医学）, 論文博士
バージョン：
権利関係：

Human Pathology (2017) 67, 60–68

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Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Alteration of PDGFRβ-Akt-mTOR pathway signaling in fibrosarcomatous transformation of dermatofibrosarcoma protuberans☆,☆☆

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Received 4 February 2017; revised 23 June 2017; accepted 5 July 2017

Keywords:
Dermatofibrosarcoma protuberans;
Fibrosarcoma;
Akt-mTOR;
PDGFR;
Imatinib

Summary Dermatofibrosarcoma protuberans (DFSP) is a cutaneous mesenchymal tumor of intermediate malignancy and fibroblastic/myofibroblastic differentiation. Fibrosarcomatous (FS) component is a high-grade component of DFSP. The detailed oncogenic difference between DFSP and FS components is not clear. We thus investigated the Akt-mTOR pathway in both components. We used 65 tumor samples obtained from 65 patients. The phosphorylation of Akt-mTOR pathway proteins (Akt, mTOR, 4EBP1, and S6RP) and PDGFRα/β was assessed by immunohistochemical staining, the results of which were confirmed by Western blotting. The immunohistochemical results were as follows: in ordinary DFSP components, p-PDGFRα–positive tumors were 41.9% (18/43 cases), p-PDGFRβ 55.8% (24/43 cases), p-Akt 51.2% (22/43 cases), p-mTOR 39.5% (17/43 cases), p-4EBP1 46.5% (20/43 cases), and p-S6RP 41.8% (18/43 cases); in DFSP components of FS-DFSP, 52.6% (10/19 cases), 47.4% (9/19 cases), 52.6% (10/19 cases), 36.8% (7/19 cases), 52.6% (10/19 cases), and 52.6% (10/19 cases); and in FS components, 45.5% (10/22 cases), 36.4% (8/22 cases), 72.7% (16/22 cases), 54.5% (12/22 cases), 72.7% (16/22 cases), and 68.2% (15/22 cases), respectively. There were significant positive correlations of the phosphorylation of most of the Akt-mTOR pathway proteins (p-Akt, p-mTOR, p-4EBP1, and p-S6RP) with each other ($P < .05$). Phospho-PDGFRβ was well correlated with the phosphorylation of Akt-mTOR pathway proteins in DFSP components of ordinary and FS-DFSPs, but these correlations were weaker in FS components. This study suggested the association of activation of Akt-mTOR pathway proteins and PDGFR with the progression of DFSP to FS. The Akt-mTOR pathway is thus a potential therapeutic target in imatinib-resistant DFSP/FS.

☆ Competing interests: The authors declare that there are no conflicts of interest.

☆☆ Funding/Support: This study was supported by a JSPS KAKEN grant (25293088) and by funds from the Scholarship Program of the Takeda Science Foundation.

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http://dx.doi.org/10.1016/j.humpath.2017.07.001
0046-8177/

1. Introduction

Dermatofibrosarcoma protuberans (DFSP) is a cutaneous soft tissue tumor classified as a fibroblastic/myofibroblastic tumor of intermediate malignancy (rarely metastasizing) according to the World Health Organization classification, characterized by the infiltrative proliferation of mildly atypical spindle-shaped cells and their storiform arrangement [1]. A high-grade spindle cell component, called the *fibrosarcomatous* (FS) *component*, is known to appear in 10%-20% of DFSPs, which is characterized by higher mitotic activity and a tumor cell arrangement involving herringbone architecture [2]. Distant metastasis occurs in 13% of DFSPs carrying an FS component (FS-DFSPs) [1] and may result in an unfavorable prognosis [2-5]. The FS component of DFSP is loosely distinguished from the ordinary DFSP area by immunohistochemical negative conversion of CD34 and elevated Ki-67 index, but the essential oncogenic difference between the 2 components is still unknown.

The Akt (also known as *protein kinase B*)–mammalian target of rapamycin (mTOR) pathway plays essential roles in controlling cellular functions and activating molecules such as ribosomal protein S6 kinase (S6RP) and the eukaryotic translation initiation factor 4E–binding protein (4EBP1), which contribute to the regulation of cell size, proliferation, and survival [6,7]. Therefore, the Akt/mTOR pathway is regarded as an oncogenic signaling system and has the potential to be a therapeutic target in many sarcomas. However, whether this pathway is activated in DFSP remains to be clarified.

In this study, we investigated the phosphorylation status of Akt/mTOR proteins (Akt, mTOR, 4EBP1, and S6RP) and the receptor tyrosine kinases (RTKs) PDGFRα and β in a large series of DFSP, FS-DFSP, and pure FS and evaluated the relationships of their activation with clinicopathological and histopathological features.

2. Materials and methods

2.1. Materials

This study was conducted in accordance with the principles embodied in the Declaration of Helsinki. This study was also approved by the Ethics Committee of Kyushu University (No. 26-49) and conducted in accordance with the Ethical Guidelines for Epidemiological Research enacted by the Japanese Government. Informed consent was obtained from the subjects or guardians.

We used 65 tumor samples obtained from 65 patients who were registered in the database of the Department of Anatomic Pathology, Kyushu University, Fukuoka, Japan. All 65 tumors were diagnosed by histopathological features, immunohistochemical CD34 positivity, and/or detection of the *COL1A1-PDGFB* fusion gene in accordance with the World Health Organization classification [1]. Formalin-fixed, paraffin-embedded samples of 43 primary DFSPs, 19 FS-DFSPs, and 3 primary FSs were prepared. All 3 primary FSs were purely composed of an FS component and contained the *COL1A1-PDGFB* fusion gene. Neither imatinib nor irradiation was used in any case.

In total, 43 ordinary DFSP components, 19 DFSP components of FS-DFSPs, and 22 FS components of FS-DFSPs were histopathologically reviewed by hematoxylin and eosin staining (Supplementary Table 1).

2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue was sliced at a thickness of 3 μm. Antigen retrieval was performed by boiling the slides in Target Retrieval Solution (Dako, Carpinteria, CA). The rabbit polyclonal antibodies for phosphorylated (p)-PDGFRα (Tyr754; 1:200 dilution) (Cell Signaling Technology, Danvers, MA), p-PDGFRβ (Tyr857; 1:200 dilution) (Santa Cruz Biotechnology, Dallas, TX), p-Akt (Ser473; 1:50 dilution) (Cell Signaling Technology), p-mTOR (Ser2448; 1:50 dilution) (Cell Signaling Technology), p-4EBP1 (Thr37/46; 1:400 dilution) (Cell Signaling Technology), and p-S6RP (Ser235/236; 1:75 dilution) (Cell Signaling Technology) were used as primary antibodies. The immune complex of anti–p-Akt was detected with EnVision FLEX Rabbit Linker (Dako) and EnVision FLEX/horseradish peroxidase (Dako), whereas the others were detected with the EnVision Detection System (Dako).

Coexisting endothelial cells (ECs) were evaluated as a positive internal control of anti–p-Akt, anti–p-mTOR, anti–p-4EBP1, and anti–p-S6RP. The proportion of immunoreactive cells and staining intensity compared with those of the ECs adjacent to the tumor cells were evaluated in the most representative areas, with reference to the evaluation method of Dobashi et al [9]. Nuclear or cytoplasmic staining of tumor cells was considered to represent positive staining for p-Akt, p-mTOR, p-4EBP1, and p-S6RP antibodies. Nuclear, perinuclear, and cytoplasmic staining was judged as positive staining for p-PDGFRα and p-PDGFRβ antibodies. The expression of these molecules was judged to be positive when at least 10%

Table 1 Histological findings

		P-DFSP	F-DFSF	FS component
Case number		43	19	22
Myxoid change	+	6	4	3
	−	37	15	19
Pigmentation	+	2	1	1
	−	41	18	21
Giant cells	+	1	0	1
	−	42	19	22
Myoid differentiation	+	3	1	2
	−	40	18	20
Mitosis	+	4	6	11
	−	39	13	11

Table 2 Histological findings

Component	Status	Ordinary DFSP	DFSP of FS-DFSP	FS
Case number		43	19	22
Myxoid differentiation	+	6 (14%)	4 (21%)	3 (14%)
Pigmentation	+	2 (5%)	1 (5%)	1 (5%)
Giant cells	+	1 (2%)	0 (0%)	1 (5%)
Myoid differentiation	+	3 (7%)	1 (5%)	2 (9%)
Mitosis	Any mitotic activity	4 (9%)	6 (32%)	11 (50%)

of the cells were positively stained, specifically to Akt-mTOR pathway proteins, more strongly than adjacent ECs; this threshold was established with reference to a previous report [9].

The tumors with co-phosphorylation of Akt and mTOR were judged as ones in which the Akt-mTOR pathway was activated. The phosphorylation of PDGFRα/β was judged as activation of the proteins in this investigation.

2.3. Western blotting

Western blot analysis was performed as reported previously [10] using frozen samples from 9 tumors (4 ordinary DFSP components, 3 DFSP components of FS-DFSP, and 2 FS components). Normal tissue samples were available in 5 cases (subcutaneous adipose tissue). Rabbit monoclonal antibodies

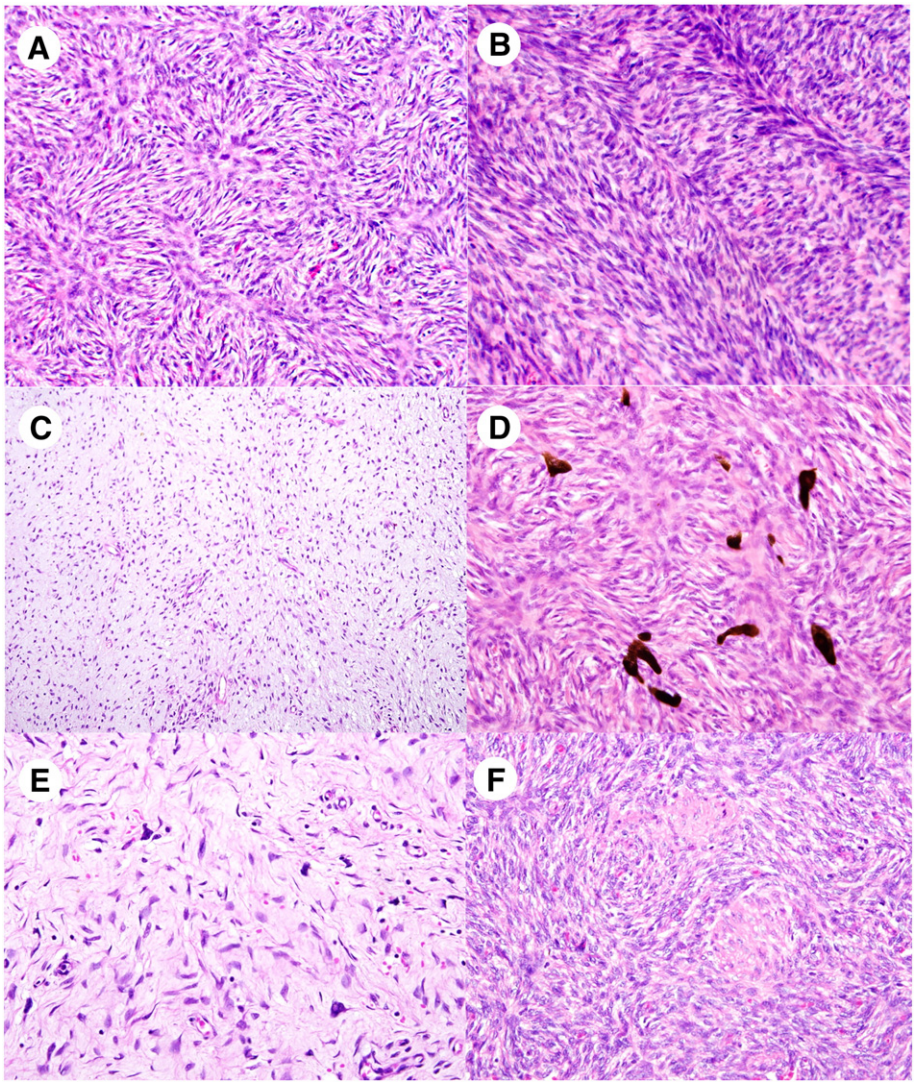

Fig. 1 Histopathological findings: A, Ordinary DFSP component shows a proliferation of spindle cells in storiform pattern (original magnification ×200). B, Fibrosarcomatous component shows a proliferation of spindle cells in herringbone pattern (×200). C, Myxoid change in DFSP (×40). D, Pigmentation in DFSP (×400). E, Giant cells in DFSP, so-called giant cell fibroblastoma (×400). F, myoid differentiation in DFSP (×200).

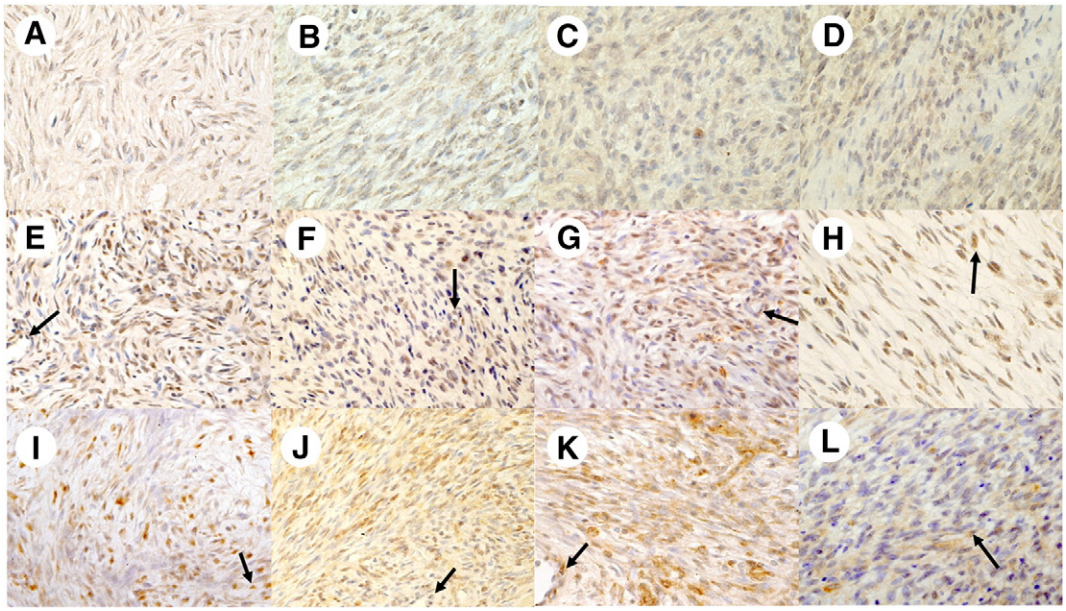

Fig. 2 Immunohistochemical results: A, p-PDGFRα (perinuclear and cytoplasmic staining) in FS-DFSP component (×200). B, p-PDGFRα (perinuclear and cytoplasmic staining) in FS component (×200). C, p-PDGFRβ (perinuclear and cytoplasmic staining) in DFSP components of FS-DFSP (×200). D, Immunohistochemistry of p-PDGFRβ (perinuclear staining) in FS component (×200). E, p-Akt (nuclear and cytoplasmic staining) in P-DFSP component (×200). F, p-Akt (nuclear and cytoplasmic staining) in FS component (×200). G, p-mTOR (nuclear and cytoplasmic staining) in P-DFSP component (×200). H, p-mTOR (nuclear and cytoplasmic staining) in FS component (×200). I, p-4EBP1 (nuclear and cytoplasmic staining) in P-DFSP component (×200). J, p-4EBP1 (nuclear and cytoplasmic staining) in FS component (×200). K, p-S6RP (nuclear and cytoplasmic staining) in F-DFSP component (×200). L, Immunohistochemistry of p-S6RP (nuclear and cytoplasmic staining) in FS component (×200). Arrows, endothelial cells.

for p-Akt (Ser473), p-mTOR (Ser2448), p-4EBP1 (Thr37/46), and p-S6RP (Ser235/236) and goat polyclonal antibodies for p-PDGFRβ (Tyr857) were used as primary antibodies. Images were analyzed using an LAS-4000 Image Reader with Multi-Gauge software (version 3.0; Fujifilm, Tokyo, Japan). Densitometric analysis was performed in the cases that included normal tissue (N). The degree of phosphorylation was calculated as follows, compared with N in each case: (p-protein [tumor]/actin [tumor])/(p-protein [normal]/actin [normal]).

2.4. Statistical analysis

Fisher exact test was used to evaluate differences between pairs of populations established according to each parameter. Thus, all of the clinicopathological, histopathological, and genetic parameters (age: >39 or ≤39, size: >4 cm or ≤4 cm, location: trunk or others, with or without recurrence/metastasis, histology, myxoid degeneration, pigmentation, giant cells, myoid differentiation, and mitosis: >1/10 high-powered fields or ≤1/10 high-powered fields), as well as the immunohistochemical parameters (p-Akt, p-mTOR, p-4EBP1, p-S6RP, p-PDGFRα, and p-PDGFRβ), were analyzed for their correlations with one another using Fisher exact test. The immunohistochemical parameters were also analyzed to examine the difference among 3 groups—ordinary DFSP components, DFSP components of FS-DFSP, and FS components—using Fisher exact test. A $P < .05$ was considered statistically significant. Odds ratio (OR) and risk ratio (RR) were also calculated. RR was calculated as the ratio of p-PDGFRβ–positive cases to –negative ones. Survival analysis was omitted because of the data on the clinical outcome being inadequate for a statistical analysis.

3. Results

3.1. Clinicopathological and histopathological results

The clinicopathological findings are summarized in Table 1. The age at tumor onset ranged from 1 to 73 years (mean, 40.3): 1 to 73 years (mean, 39.2) in DFSP cases, 26 to 65 years (mean, 40.8) in FS-DFSP cases, and 47 to 64 years (mean, 53.3) in FS cases. The median tumor size was 3.75 cm (mean, 4.74 cm [range, 1-12 cm]; SD, 3.16): 3 cm (mean, 3.95 cm [range, 1-11 cm]; SD, 2.81) in DFSPs, 6 cm (mean, 6.34 cm [range, 2-12 cm]; SD, 3.56) in FS-DFSPs, and 5 cm (mean, 4.93 cm [range, 3.5-6.3 cm]; SD, 1.40) in FSs. There was no bias in the distribution of the different disease types between the sexes (20 males and 23 females in DFSPs, 11 males and 8 females in FS-DFSPs, 2 males and 1 female in FS). These tumors were located on the trunk (34 DFSPs, 16 FS-DFSPs, and 3 FSs), extremity (5 DFSPs and 2 FS-DFSPs), cervicofacial area (4 DFSPs), and scrotum (1 FS-DFSP). There were 3 recurrences in DFSP cases and no cases of metastasis/tumor death.

The histopathological findings are summarized in Table 2, and the representative histology is presented in Fig. 1. Histopathologically, all 65 samples showed a proliferation of

Table 3 Relationship between phosphorylated proteins and pathological data

Parameter	Categories	Case	%	*P*					
				p-PDGFRα	p-PDGFRβ	p-Akt	p-mTOR	p-4EBP1	p-S6RP
Ordinary DFSP component				*P* for Fisher exact test					
Age	≥39	20	46.5	1	.5472	.7626	.0677	.3662	.1301
	<39	23	53.5						
Sex	Male	20	46.5	.5375	.5472	1	1	1	.5375
	Female	23	53.5						
Size	≥4 cm	13	30.2	.4813	1	.7221	.7171	.7283	.7171
	<4 cm	20	46.5						
	NA	10	23.3						
Location	Trunk	34	79.1	1	.4766	1	.065	.4674	.7117
	Others	9	20.9						
Myxoid change	+	6	14	.3747	.2048	.1853	.0284	.0064	.0672
	−	37	86						
Pigmentation	+	2	4.7	.5017	.495	1	1	1	1
	−	41	96.3						
Giant cells	+	1	2.3	.4186	1	.4884	1	1	1
	−	42	97.7						
Myoid differentiation	+	3	7	.5624	.5751	.6069	1	1	1
	−	40	93						
Mitosis	≥1/10 HPF	4	9.3	1	.6176	.6069	1	.0393	.2926
	<1/10 HPF	39	90.7						
DFSP component of FS-DFSP									
Age	≥39	11	57.8	.1968	.3698	1	.3765	.1698	1
	<39	8	42.1						
Sex	Male	8	57.8	1	.0968	.6499	.3765	.1698	.1698
	Female	11	42.1						
Size	≥4 cm	11	42.1	.5962	.1058	.2821	.2995	.0256	.5962
	<4 cm	5	26.3						
	NA	3	15.8						
Location	Trunk	16	84.2	1	1	.582	1	.582	.582
	Others	3	15.8						
Myxoid change	+	4	21.1	1	.582	1	1	1	.582
	−	15	78.9						
Pigmentation	+	1	5.3	1	1	.4737	1	.4737	.4737
	−	18	94.7						
Giant cells	+	0	0	0	0	0	0	0	0
	−	19	100						
Myoid differentiation	+	1	5.3	.4737	.4737	1	1	1	1
	−	18	94.7						
Mitosis	≥1/10 HPF	6	31.6	.6285	.3498	.6285	.6169	.1409	1
	<1/10 HPF	13	68.4						
FS component									
Age	≥39	10	45.5	.0743	.0815	.6244	1	1	1
	<39	12	54.5						
Sex	Male	13	59.1	.6656	.187	.655	.6656	.333	.6478
	Female	9	40.9						
Size	≥4 cm	13	59.1	.6285	.1287	1	.1409	1	.6047
	<4 cm	6	27.3						
	NA	3	13.6						
Location	Trunk	10	45.5	1	.5152	1	.1948	.0649	1
	Others	12	54.5						
Myxoid change	+	3	13.6	1	1	.1688	.5714	.1688	.2273
	−	19	86.4						
Pigmentation	+	1	4.5	.4545	1	1	1	1	.3182
	−	21	95.5						
Giant cells	+	0	0	0	0	0	0	0	0

Table 3 (*continued*)

Parameter	Categories	Case	%	*P*					
				p-PDGFRα	p-PDGFRβ	p-Akt	p-mTOR	p-4EBP1	p-S6RP
	−	22	100						
Myoid differentiation	+	2	9.1	1	1	.4805	1	.4805	1
	−	20	90.9						
Mitosis	≥1/10	11	50	.6699	.6594	1	.6699	.6351	1
	<1/10	11	50						

NOTE. *P*: Fisher exact test.

spindle-shaped tumor cells arranged in a storiform pattern in DFSP components (Fig. 1A) and a herringbone pattern in FS components (Fig. 1B). In total, 13 tumor components showed focal myxoid change (6 ordinary DFSP components, 4 DFSP components of FS-DFSPs, 3 FS components) (Fig. 1C), 4 components showed pigmentation (2 ordinary DFSPs, 1 DFSP of FS-DFSP, and 1 FS) (Fig. 1D), 2 components contained giant cells (1 ordinary DFSP and 1 FS) (Fig. 1E), and 6 components showed myoid differentiation (3 ordinary DFSPs, 1 DFSP of FS-DFSP, and 2 FSs) (Fig. 1F). Mitotic figures were observed rarely or occasionally, that is, on average, 1.1/10 high-powered fields (HPFs) in ordinary DFSPs, 0.7/10 HPFs in DFSPs of FS-DFSP, and 3.5/10 HPFs in FSs (Table 2).

3.2. Immunohistochemistry

The results of immunohistochemical staining are presented in Fig. 2 and summarized in Tables 3 and 4.

As for the associations of phosphorylated proteins with the clinicopathological and histological findings, p-mTOR and p-4EBP1 were positively correlated with myxoid change in ordinary DFSP ($P = .0284$ and $P = .0064$), and p-4EBP1 was positively correlated with mitosis in ordinary DFSP ($P = .0393$); p-4EBP1 was negatively correlated with larger tumor size in DFSP component of FS-DFSP ($P = .0256$) (Table 3). No other significant correlation was detected in the relationships between the clinicopathological/histopathological findings and immunohistochemical results.

The correlations among the immunohistochemical results of Akt-mTOR pathway proteins and p-PDGFRα/β are summarized in Table 4. There were positive correlations of the phosphorylation of all of the Akt-mTOR pathway proteins (p-Akt, p-mTOR, p-4EBP1, and p-S6RP) with each other. Phospho-PDGFRβ was correlated with p-Akt, p-mTOR, p-4EBP1, and p-S6RP in ordinary DFSP components ($P = .0329$, $P = .0058$, $P = .0052$, and $P = .0044$, respectively); p-Akt, p-mTOR,

Table 4 Intercorrelations between Akt-mTOR pathway proteins and RTKs

	Positive cases	[%]	*P*			
			p-Akt	p-mTOR	p-4EBP1	p-S6RP
Ordinary DFSP component (43 cases)						
p-PDGFRα	18	41.9	.358	1	1	1
p-PDGFRβ	24	55.8	.0329	.0058	.0052	.0044
p-Akt	22	51.2	–	–	–	–
p-mTOR	17	39.5	.0001	–	–	–
p-4EBP1	20	46.5	.0007	.0002	–	–
p-S6RP	18	41.8	<.0001	<.0001	<.0001	–
DFSP component of FS-DFSP (19 cases)						
p-PDGFRα	10	52.6	.0055	.1698	.3698	.0698
p-PDGFRβ	9	47.4	.0055	.0198	.0001	.0698
p-Akt	10	52.6	–	–	–	–
p-mTOR	7	36.8	.0031	–	–	–
p-4EBP1	10	52.6	.023	.0573	–	–
p-S6RP	10	52.6	.023	.3498	.023	–
FS component (22 cases)						
p-PDGFRα	10	45.5	.0557	.3913	.3476	.6517
p-PDGFRβ	8	36.4	.3512	.6749	.0511	.0225
p-Akt	16	72.7	–	–	–	–
p-mTOR	12	54.5	.0028	–	–	–
p-4EBP1	16	72.7	.0254	.0028	–	–
p-S6RP	15	68.2	.0536	.1718	.0043	–

NOTE. *P*: Fisher exact test.

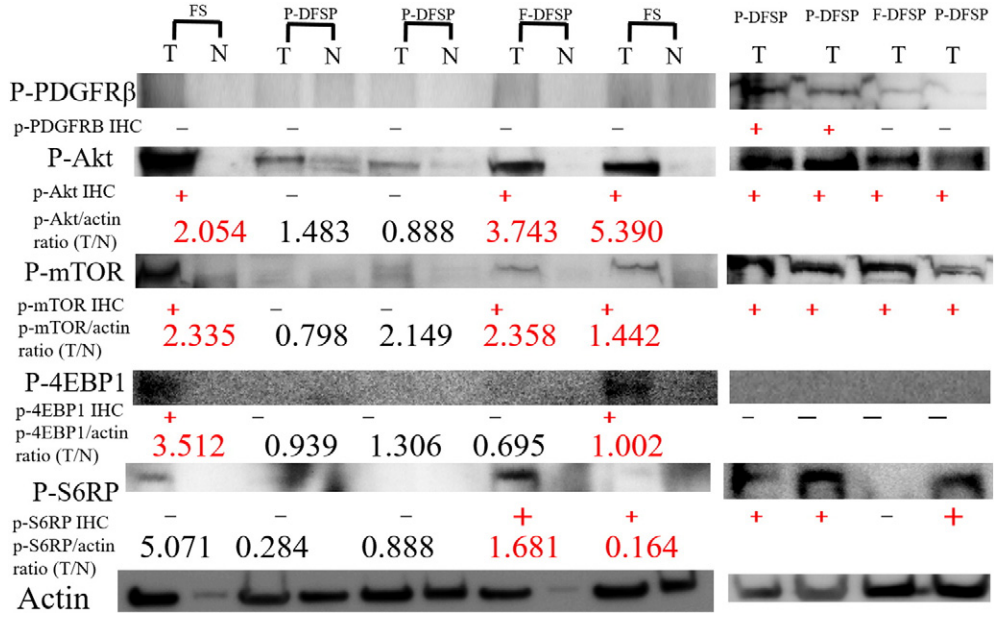

Fig. 3 Results of Western blot analysis. The tumor tissue (T) tended to be phosphorylated more than normal tissue (N). IHC, immunohistochemistry; +, positive; −, negative.

and p-4EBP1 were correlated in DFSP components of FS-DFSPs (P = .0055, P = .0198, and P = .0001, respectively); and only p-S6RP (P = .0225) was correlated in FS components. Phospho-PDGFRα was correlated with p-Akt (P = .0055) in DFSP components of FS-DFSPs.

The cases with activation of the Akt-mTOR pathway were as follows (Supplementary Table 2): 15/43 cases (34.8%) in ordinary DFSP components, 7/19 cases (36.8%) in DFSP components of FS-DFSPs, and 11/22 cases (50%) in FS components. The cases with coactivation of the Akt-mTOR pathway and PDGFR were as follows: 12/43 cases (27.9%) in ordinary DFSPs, 6/19 cases (31.5%) in DFSPs of FS-DFSPs, and 5/22 cases (22.7%) in FSs. The association of PDGFRβ phosphorylation with Akt-mTOR pathway activation was significant in ordinary DFSPs and DFSPs of FS-DFSPs at P = .0264 (OR = 5.33, RR = 4) in ordinary DFSPs, at P = .0198 (OR = 18, RR = 6) in DFSPs of FS-DFSPs, and at P = .6749 (OR = 1.67, RR = 1.39) in FSs. The cases with none of the phospho-Akt-mTOR pathway proteins were as follows: 17/43 cases (39.5%) in ordinary DFSPs, 7/19 cases (36.8%) in DFSPs of FS-DFSPs, and 4/22 cases (18.2%) in FSs.

3.3. Western blotting

The results of Western blot analysis are presented in Fig. 3. This analysis for p-PDGFRα, p-PDGFRβ, p-Akt, p-mTOR, p-4EBP1, and p-S6RP confirmed the phosphorylation of each protein as determined by immunohistochemistry. p-PDGFRα was excluded from this study because all of the cases subjected to Western blot analysis were immunohistochemically negative for it.

The results of Western blotting approximately corresponded to the immunohistochemical data, which confirmed the validity of the immunohistochemical findings.

4. Discussion

FS is a potentially metastasizing component of DFSP, so its identification is important when making decisions on which therapy to apply. At present, the identification of FS depends on the morphology, and its molecular background is still unknown.

The Akt-mTOR pathway is highly activated in various malignant tumors, and previous studies have demonstrated the activation of this pathway in several sarcomas [9,11-15]. However, the expression profiles of the Akt/mTOR pathway–associated proteins have not been analyzed sufficiently in DFSPs in a large study [7,16]. In our study, Akt-mTOR pathway–associated proteins and upstream RTKs, PDGFRα and PDGFRβ, were phosphorylated at various rates, and their phosphorylation were correlated with each other. This confirmed the previously proposed hypothesis that the PDGFB-PDGFRβ paracrine-autocrine system contributes to the tumorigenesis of DFSP [6,14]; moreover, it was suggested that various degrees of activation of the Akt-mTOR pathway are also associated with the tumorigenesis of DFSP. Note that activation of PDGFRα/β and Akt-mTOR was not demonstrated in all tumors used in this investigation, which was not apparently consistent with the hypothesis of PDGFB high expression and autoactivation of PDGFR. However, immunohistochemical stain of phosphoproteins detected only sufficiently

phosphorylated cases; in fact, negative cases possibly expressed phosphoproteins in a lower level than detection sensitivity. Considering the above, it was supposed that the stronger activation of PDGFR occurred in a tumor, the stronger influence was given to the downstream Akt-mTOR pathway.

To the best of our knowledge, there is no previous investigation about activation of the Akt-mTOR pathway in fibrosarcomatous DFSP. Focusing on the relationship between histological features and signaling activation, the Akt-mTOR pathway was found to be activated in both DFSP and FS components, and it was slightly highly activated in FS components. Moreover, the phosphorylation of Akt-mTOR pathway proteins was intimately correlated with the phosphorylation of PDGFRβ in ordinary DFSP components; meanwhile, the pathway was less correlated with p-PDGFRβ in DFSP components of FS-DFSP and p-PDGFRβ in FS components. The proportions of cases with PDGFRβ phosphorylation differed little among the 3 groups; therefore, it is considered that another signaling pathway has a dominant influence on the Akt-mTOR pathway in FS components. Moreover, activation of Akt-mTOR seemed to influence the tumor growth of DFSP positively, whereas this was not evident in FS-DFSP. The functional conversion of the Akt-mTOR pathway in FS-DFSP might cause the negative effect of p-4EBP1 to tumor size.

DFSP is thought to be intimately associated with the PDGFB paracrine-autocrine system, as to the tumorigenesis [3,17-19], and the excessive secretion of PDGFB is considered to be a result of *COL1A1-PDGFB* fusion gene products. Imatinib, a tyrosine kinase inhibitor, which is well known as a specific treatment for gastrointestinal stromal tumors, has been adopted for recurrent or unresectable cases of DFSP [5,17], having a therapeutic effect on 36% to 50% of these tumors [4,5]. The National Comprehensive Cancer Network guidelines currently recommend that imatinib be considered in cases of DFSP recurrence after resection or in cases deemed unresectable [20]. Imatinib has been reported to show efficacy against DFSP [3-5,21-24], and a recent analysis of 2 phase II trials also reported its promising clinical activity [5]. However, imatinib-resistant cases have also been reported, and progressive disease tends to be observed more in FS-DFSP cases than in classical DFSP ones. Interestingly, one previously reported FS-DFSP case developed an imatinib-resistant tumor and sensitivity to everolimus, an mTOR inhibitor [16]. One unfavorable DFSP case developed lung metastases and died of a tumor in which there was activation of several RTKs (PDGFRα, PDGFRβ, EGFR, MCSFR, IR, and IGF1R) [3]. The PDGFRβ-Akt-mTOR pathway alteration described above could also be responsible for the resistance of DFSP components of FS-DFSP to imatinib therapy.

The progression of DFSP has been examined by histopathological and biomolecular methods in some studies, and one investigation showed the association of microsatellite instability and p53 mutation with FS change in DFSPs [25]. In this study, we added a new concept that the profile of RTK phosphorylation can change according to tumor progression, possibly contributing to the resistance to RTK inhibitors in DFSP cases (Supplementary Fig. 1). Moreover, it is proposed that the Akt-mTOR pathway is a potential therapeutic target in imatinib-resistant DFSP or FS.

In conclusion, we have revealed that alteration between Akt-mTOR pathway proteins and PDGFR may be associated with the progression of DFSP. We also propose that the Akt-mTOR pathway is a potential therapeutic target in imatinib-resistant DFSP or FS.

Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.humpath.2017.07.001.

Acknowledgments

We appreciate the technical assistance of The Research Support Center, Kyushu University Graduate School of Medical Sciences.

The authors thank J. Motoshita (Hamanomachi Hospital, Fukuoka), S. Toyoshima and S. Tamiya (Kitakyushu Municipal Medical Center, Kitakyushu), Y. Nakashima and K. Nishiyama (Japanese Red Cross Fukuoka Hospital, Fukuoka), H. Nakane (National Hospital Organization Fukuoka-higashi Medical Center, Fukuoka), M. Diguchi (Yokohama Rosai Hospital, Yokohama), N. Iida (Japanese Red Cross Akita Hospital, Akita), K. Tsuchiya (Kyushu Kouseinenkin Hospital, Fukuoka), Y. Yamamoto (Wakayama Medical University Hospital, Wakayama), and K. Higaki (St. Mary's Hospital, Fukuoka) for providing clinical and histological data.

References

[1] Fletcher JA, Fletcher CDM, Bridge JA, Lee J-C. Dermatofibrosarcoma protuberans. In: CDM Fletcher, Bridge JA, PCW Hogendoorn, Mertens F, editors. World Health Organization classification of tumours. Pathology and genetics of tumours of soft tissue and bone. Lyon, France: IARC Press; 2013. p. 77-9.

[2] Llombart B, Monteagudo C, Sanmartin O, et al. Dermatofibrosarcoma protuberans a clinicopathological immunohistochemical genetic (COL1A1-PDGFB) and therapeutic study of low-grade versus high-grade (fibrosarcomatous) tumors. J Am Acad Dermatol 2011;65:564-75.

[3] Ugurel S, Mentzel T, Utikal J, et al. Neo-adjuvant imatinib in advanced primary or locally recurrent dermatofibrosarcoma protuberans: a multicenter phase-II DeCOG trial with long-term follow-up. Clin Cancer Res 2014;20:499-510.

[4] Kerob D, Porcher R, Verola O, et al. Imatinib mesylate as a preoperative therapy in dermatofibrosarcoma: results of a multicenter phase II study on 25 patients. Clin Cancer Res 2010;16:3288-95.

[5] Rutkowski P, Van Glabbeke M, Rankin CJ, et al. Imatinib mesylate in advanced dermatofibrosarcoma protuberans: pooled analysis of two phase II clinical trials. J Clin Oncol 2010;28:1772-9.

[6] Ruvinsky I, Meyuhas O. Ribosomal protein S6 phosphorylation: from protein synthesis to cell size. Trends Biochem Sci 2006;31:342-8.

[7] West MJ, Stoneley M, Willis AE. Translational induction of the c-myc oncogene via activation of the FRAP/TOR signalling pathway. Oncogene 1998;17:769-80.

[8] Dormond O, Madsen JC, Briscoe DM. The effects of mTOR-Akt interactions on anti-apoptotic signaling in vascular endothelial cells. J Biol Chem 2007;282:23679-86.
[9] Dobashi Y, Suzuki S, Sato E, Hamada Y, Yanagawa T, Ooi A. EGFR-dependent and independent activation of Akt/mTOR cascade in bone and soft tissue tumors. Mod Pathol 2009;22:1328-40.
[10] Yamada Y, Kohashi K, Fushimi F, et al. Activation of the Akt-mTOR pathway and receptor tyrosine kinase in patients with solitary fibrous tumors. Cancer 2014;120:864-76.
[11] Endo M, Yamamoto H, Setsu N, et al. Prognostic significance of AKT/mTOR and MAPK pathways and antitumor effect of mTOR inhibitor in NF1-related and sporadic malignant peripheral nerve sheath tumors. Clin Cancer Res 2013;19:450-61.
[12] Subbiah V, Brown RE, Jiang Y, et al. Morphoproteomic profiling of the mammalian target of rapamycin (mTOR) signaling pathway in desmoplastic small round cell tumor (EWS/WT1), Ewing's sarcoma (EWS/FLI1) and Wilms' tumor (WT1). PLoS One 2013;8:e68985.
[13] Smith KB, Tran LM, Tam BM, et al. Novel dedifferentiated liposarcoma xenograft models reveal PTEN down-regulation as a malignant signature and response to PI3K pathway inhibition. Am J Pathol 2013;182:1400-11.
[14] Shimizu A, OBrien KP, Sjoblom T, et al. The dermatofibrosarcoma protuberans-associated collagen type Ialpha1/platelet-derived growth factor (PDGF) B-chain fusion gene generates a transforming protein that is processed to functional PDGF-BB. Cancer Res 1999;59:3719-23.
[15] Edelweiss M, Malpica A. Dermatofibrosarcoma protuberans of the vulva: a clinicopathologic and immunohistochemical study of 13 cases. Am J Surg Pathol 2010;34:393-400.
[16] Stacchiotti S, Pedeutour F, Negri T, et al. Dermatofibrosarcoma protuberans-derived fibrosarcoma: clinical history, biological profile and sensitivity to imatinib. Int J Cancer 2011;129:1761-72.
[17] Simon MP, Navarro M, Roux D, et al. Structural and functional analysis of a chimeric protein COLIA1-PDGFB generated by the translocation t(17;22)(q22;q13.1) in dermatofibrosarcoma protuberans (DP). Oncogene 2001;20:2965-75.
[18] Gibson S, Sebire NJ, Anderson J. Platelet-derived growth factor receptors and ligands are up-regulated in paediatric fibromatoses. Histopathology 2007;51:752-7.
[19] Zhang H, Bajraszewski N, Wu E, et al. PDGFRs are critical for PI3K/Akt activation and negatively regulated by mTOR. J Clin Invest 2007;117:730-8.
[20] Bogucki B, Neuhaus I, Hurst EA. Dermatofibrosarcoma protuberans: a review of the literature. Dermatol Surg 2012;38:537-51.
[21] Rubin BP, Schuetze SM, Eary JF, et al. Molecular targeting of platelet-derived growth factor B by imatinib mesylate in a patient with metastatic dermatofibrosarcoma protuberans. J Clin Oncol 2002;20:3586-91.
[22] McArthur GA, Demetri GD, van Oosterom A, et al. Molecular and clinical analysis of locally advanced dermatofibrosarcoma protuberans treated with imatinib: imatinib target exploration consortium study B2225. J Clin Oncol 2005;23:866-73.
[23] Wicherts DA, van Coevorden F, Klomp HM, et al. Complete resection of recurrent and initially unresectable dermatofibrosarcoma protuberans downsized by imatinib. World J Surg Oncol 2013;11:59.
[24] Jeon IK, Kim JH, Kim SE, Kim SC, Roh MR. Successful treatment of unresectable dermatofibrosarcoma protuberans on finger with imatinib mesylate and Mohs microsurgery. J Dermatol 2013;40:288-9.
[25] Takahira T, Oda Y, Tamiya S, et al. Microsatellite instability and p53 mutation associated with tumor progression in dermatofibrosarcoma protuberans. Hum Pathol 2004;35:240-5.