Letter to the Editor

Possible contribution of PDGF-BB-induced autophagy in dermatofibrosarcoma protuberans: Autophagy marker Atg5 could be a differential marker between dermatofibrosarcoma protuberans and dermatofibroma

Dermatofibrosarcoma protuberans (DFSP) is a locally aggressive and invasive neoplasm of intermediate malignancy with frequent recurrence. Dermatofibroma (DF) is a commonly occurring benign fibrohistiocytic cutaneous tumor that has a very low risk of local recurrence. Since their biological behavior differs greatly, it is essential to differentiate DFSP and DF. However, we sometimes encounter difficulties in distinguishing DFSP from DF. It is well known that immunohistochemical staining of CD34 and factor XIIIa can be helpful to differentiate DFSP and DF [1]. Nakamura et al. reported that platelet-derived growth factor-BB (PDGF-BB) was expressed in 93% of DFSP, while in 5% of DF, suggesting that immunohistochemical staining of PDGF-BB might be a useful diagnostic tool for DFSP [2].

Autophagy is an intracellular degradation system with various roles in human diseases and physiology [3]. In cancer cells, autophagy has been recognized to enable the continuous tumor growth by energy production, and inhibit the expression of genes that induce tumor cell death [3]. Atg5 is a key regulator of autophagy that is required for autophagosome formation [4]. Atg5 expression was increased in endothelial cells in pyogenic granuloma, liver and prostate carcinoma tissues [5,6]. The objective was to investigate the expression of Atg5 in DFSP and DF, as well as the role of autophagy in the pathogenesis of DFSP.

We herein examined the expression of Atg5, PDGF-BB and CD34 of 10 cases of DF and 12 cases of DFSP by immunohistochemical staining. Additionally, the relationship between Atg5 and PDGF-BB was analyzed using human dermal fibroblasts *in vitro*. The study was approved by Institutional Review Board of Gunma University (#1584).

The result of immunohistological analyses in DFSP and DF was summarized in Table 1, and representative results were shown in Fig. 1A. All cases of DFSP (100%; 12/12 cases) revealed weak to strong Atg5 positivity in tumor cells. We confirmed that control IgG was negative for tumors (data not shown). In contrast, Atg5 was weakly positive for tumor cells in 30% (3/10 cases) of DF cases. The clinical and pathological differences between $Atg5^{+}$ and $Atg5^{-}$ DF were not detected. The frequency of Atg5 positivity in DFSP was significantly higher than those in DF ($P < 0.05$). The expressions of Atg5 in the blood vessels in DF and DFSP were very weak or negative. Additionally, Atg5 was very strongly positive in the epidermis of DF and DFSP. Most cases of DFSP (91.7%; 11/12 cases) showed moderate or strong staining of PDGF-BB. In contrast, PDGF-BB was not expressed in all cases of DF. Consistent with previous study [2], the frequency of PDGF-BB positivity in DFSP was significantly higher than those in DF ($P < 0.05$). Additionally, the area stained for PDGF-BB was almost consistent with the area stained for Atg5. Most cases of DFSP which had *COL1A1-PDGFB* fusion genes expressed both Atg5 and PDGF-BB (90%; 9/10 cases) (Table 1). Most cases of DFSP revealed CD34 positive (83.3%; 10/12 cases) and all tumors of DF revealed CD34 negative. In two cases of DFSP having *COL1A1-PDGFB* fusion gene which CD34 and/or PDGF-BB were negative (DFSP11, 12), Atg5 was positive. These results suggest that the expressions of Atg5 and PDGF-BB might be enhanced in tumor cells of DFSP compared to DF, and that the combination of Atg5, PDGF-BB and CD34 staining in tumor cells may allow more accurate diagnosis of DFSP.

To examine whether PDGF-BB induces Atg5 expression in tumor cells, we examined the expression of Atg5 in human dermal fibroblasts treated by PDGF-BB *in vitro*. We found that mRNA and protein levels of Atg5 were increased by PDGF-BB treatment (Fig. 1B, C). Microtubule-associated protein light chain 3 (LC3) is widely used to monitor autophagy [7,8]. The cytosolic form of LC3 (LC3-I) is conjugated to phosphatidylethanolamine to form LC3-phosphatidylethanolamine conjugate (LC3-II), which is recruited to autophagosomal membranes. Since the amount of LC3-II is correlated with the number of autophagosomes, one approach of the quantification of autophagy is to detect LC3 conversion (LC3-I to LC3-II) by immunoblot analysis [7,8]. We found that PDGF-BB stimulation enhanced LC3-II formation (Fig. 1C). However, it is known that the amount of LC3 at a certain time point does not

Table 1
Summary of Atg5, PDGF-BB and CD34 staining in tumor cells of DFSP and DF.

Case	Age	Sex	Site	Size (mm)	*COL1A1-PDGFB*	CD34	PDGF-BB	Atg5
DFSP1	64	M	Lower leg	22	Exon 34	+++	+++	+++
DFSP2	23	F	Abdomen	Scar (30)	Exon 5	+++	++	+
DFSP3	35	M	Abdomen	60 × 45	Exon 32	+++	+++	++
DFSP4	31	F	Back	92 × 85	Exon 9	+++	+++	+++
DFSP5	55	F	Lower leg	Scar (10)	ND	+++	+++	++
DFSP6	37	M	Abdomen	Scar (9)	ND	+++	++	+++
DFSP7	59	M	Chest	40 × 35	Exon 11	+++	+++	+++
DFSP8	27	F	Upper leg	20 × 10	Exon 25	+++	+++	++
DFSP9	30	M	Abdomen	Scar (20)	Exon 29	++	++	+
DFSP10	35	F	Abdomen	80 × 37	Exon 25	+++	+++	+++
DFSP11	51	M	Scalp	22 × 20	Exon47	–	+++	+++
DFSP12	34	M	Face	10 × 8	Exon44	–	–	++
DF1	29	M	Back	10 × 10		–	–	+
DF2	54	F	Back	10 × 10		–	–	–
DF3	37	F	Lower leg	ND		–	–	+
DF4	59	F	Finger	5		–	–	–
DF5	9	F	Chest	8		–	–	–
DF6	59	M	Lower leg	5		–	–	–
DF7	35	F	Lower leg	15 × 10		–	–	–
DF8	43	M	Lower leg	10 × 8		–	–	–
DF9	53	M	Hand	14 × 12		–	–	+
DF10	34	F	Lower leg	15 × 15		–	–	–

-, negative; +, slight staining; ++, staining; +++, strong staining; ND, not determined.

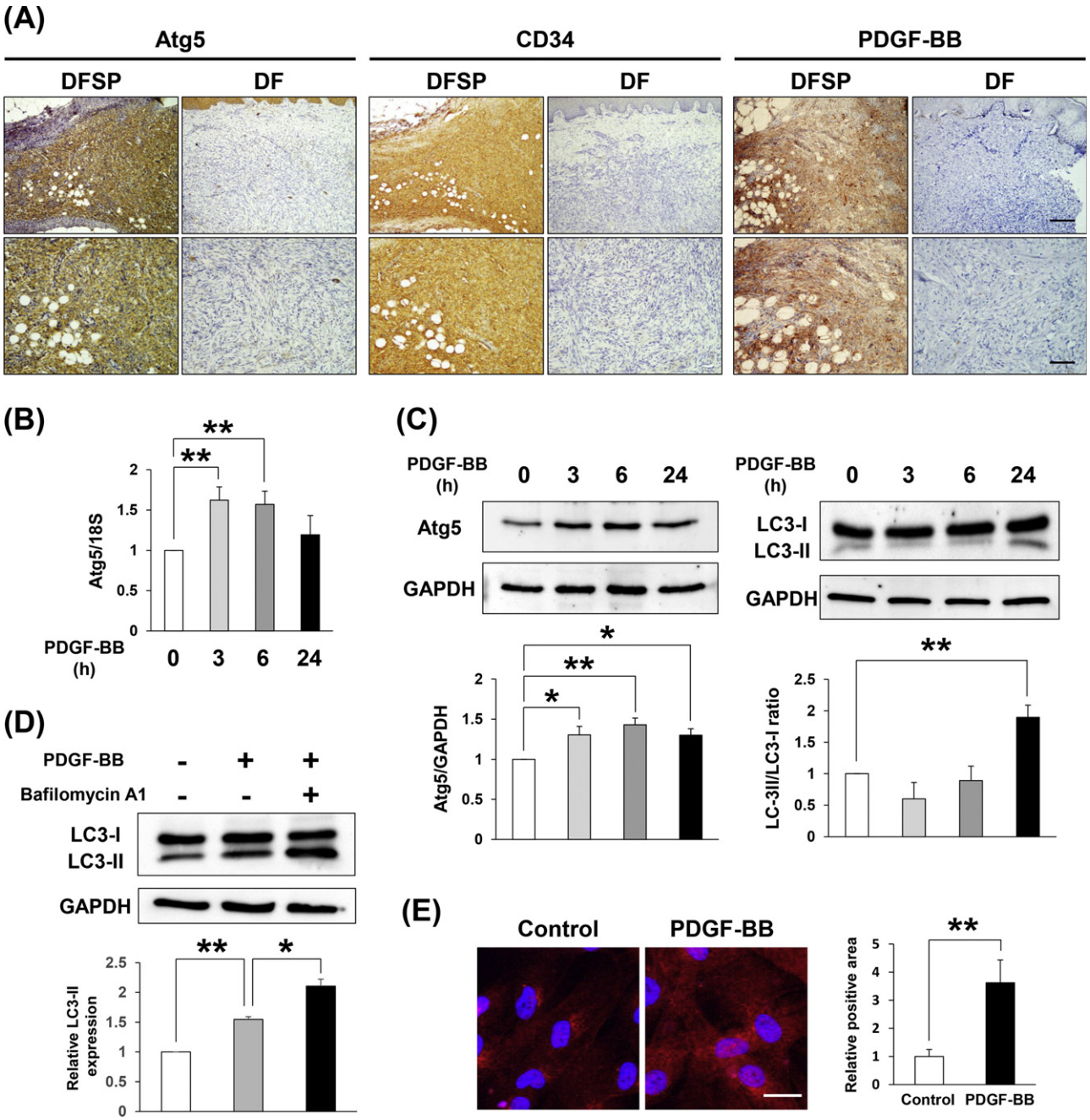

Fig. 1. The expressions of Atg5 and PDGF-BB were enhanced in tumor cells of DFSP compared to DF, and PDGF-BB enhanced the expression of Atg5 and autophagy in human fibroblasts *in vitro*. A) Representative immunohistochemical stainings of Atg5, PDGF-BB and CD34 in DFSP and DF using anti-APG5L/Atg5 antibody (Ab) (abcam, Cambridge, MA), mouse anti-CD34 Ab (Histofine, Nichirei, Tokyo) and anti-PDGF-BB Ab (abcam). Upper panels: Scale bar = 100 μm. Lower panels: Scale bar = 100 μm. (B) mRNA levels of Atg5 expressions in human fibroblasts treated with PDGF-BB (20 ng/ml) (R&D systems, Minneapolis, MN). n = 3 donors. (C) Atg5 expression and the ratio of LC3-II/LC3-I in human fibroblasts treated with PDGF-BB (20 ng/ml). (D) PDGF-BB (20 ng/ml for 24 h)-induced LC3-II formation was enhanced by autophagy inhibitor, bafilomycin A1 (Sigma-Aldrich, 400 nM for the last 4 h of culture). (E) Immunofluorescence staining of LC3 (anti-LC3B Ab (Novus Biologicals)) in fibroblasts treated with PDGF-BB (20 ng/ml) for 24 h. Scale bar = 25 μm. Quantification of the $LC3^{+}$ areas in 8 random microscopic fields per group was performed using the Image J software. Positive area in control was assigned a value of 1. *P*-values were calculated by Student's *t*-test or one-way ANOVA followed by a Bonferroni's posttest for multiple comparisons. All values represent mean ± SEM. $^{**}P < 0.01$, $^{*}P < 0.05$.

indicate autophagic flux, and the difference in the amount of LC3-II between samples with and without autophagy inhibitors represents the level of autophagic flux [7,8]. We identified that PDGF-BB-induced LC3-II formation was enhanced by autophagy inhibitor, bafilomycin A1 (Fig. 1D). In immunofluorescence staining, $LC3^{+}$ autophagosomes were increased in dermal fibroblasts treated by PDGF-BB compared to non-treated cells (Fig. 1E). These results suggest that PDGF-BB might enhance the expression of Atg5 and autophagy in human fibroblasts.

It is considered that DFSP tumorigenesis mechanism might involve the powerful promotor activity of the *COL1A1* gene, contributing to constitutive expression of PDGF-BB from tumor cells of DFSP with *COL1A1-PDGFB* fusion genes [2]. Autocrine and paracrine secretion of PDGF-BB from tumor cells might stimulate

PDGF receptor to increase proliferation of DFSP. Interestingly, PDGF:PDGF receptor signaling was recently shown to activate autophagy in vascular smooth muscle cells, resulting in the proliferation and cell survival [9]. Our results suggest that Atg5 expression and autophagy might be enhanced by autocrine and paracrine PDGF-BB secreted from tumor cells of DFSP. However, it has been reported that immunohistochemical staining analysis of Atg5 is not recommended as marker for autophagic activity in tissue [10], suggesting that the increasing Atg5 expression in the tumor lesion of DFSP cannot directly indicate the occurrence of autophagy. Therefore, further examination is necessary to clarify the occurrence of autophagy in DFSP in detail. *COL1A1-PDGFB* fusion genes in tumor cells may be associated with the continuous secretion of PDGF-BB. However, the mechanism by which PDGF-BB activates autophagy remains to be identified.

In conclusion, we for the first time identified the possible link between autophagy and PDGF-BB in DFSP. The use of autophagy inhibitors, such as chloroquine or taxol, may encourage the efficacy of anti-cancer drugs against DFSP. Our results also suggest that the combined analyses of CD34, Atg5 and PDGF-BB expressions in tumor cells might enable more precise diagnosis of DFSP.

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Conflict of interest

The authors state no conflict of interest.

References

[1] J.R. Goldblum, R.J. Tuthill, CD34 and factor-XIIIa immunoreactivity in dermatofibrosarcoma protuberans and dermatofibroma, Am. J. Dermatopathol. 19 (1997) 147–153.
[2] I. Nakamura, Y. Kariya, E. Okada, M. Yasuda, S. Matori, O. Ishikawa, et al., A novel chromosomal translocation associated with COL1A2-PDGFB gene fusion in dermatofibrosarcoma protuberans: PDGF expression as a new diagnostic tool, JAMA Dermatol. 151 (2015) 1330–1337.
[3] N. Mizushima, B. Levine, A.M. Cuervo, D.J. Klionsky, Autophagy fights disease through cellular self-digestion, Nature 451 (2008) 1069–1075.
[4] S. Yousefi, R. Perozzo, I. Schmid, A. Ziemiecki, T. Schaffner, L. Scapozza, et al., Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis, Nat. Cell Biol. 8 (2006) 1124–1132.
[5] M.S. Kim, S.Y. Song, J.Y. Lee, N.J. Yoo, S.H. Lee, Expressional and mutational analyses of ATG5 gene in prostate cancers, APMIS 119 (2011) 802–807.
[6] S. Motegi, C. Fujiwara, S. Yamazaki, A. Sekiguchi, O. Ishikawa, Possible contribution of autophagy in pyogenic granuloma, J. Dermatol. 45 (2018) 1145–1146.
[7] I. Tanida, T. Ueno, E. Kominami, LC3 and autophagy, Methods Mol. Biol. 445 (2008) 77–88.
[8] N. Mizushima, T. Yoshimori, How to interpret LC3 immunoblotting, Autophagy 3 (2007) 542–545.
[9] J.K. Salabei, T.D. Cummins, M. Singh, S.P. Jones, A. Bhatnagar, B.G. Hill, PDGF-mediated autophagy regulates vascular smooth muscle cell phenotype and resistance to oxidative stress, Biochem. J. 451 (2013) 375–388.
[10] W. Martinet, L. Roth, G.R.Y. De Meyer, Standard immunohistochemical assays to assess autophagy in mammalian tissue, Cells 6 (2017) E17.

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