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INVESTIGATIVE REPORT

Serum Angiopoietin-like Protein 3 Levels: Possible Correlation with Progressive Skin Sclerosis, Digital Ulcers and Pulmonary Vascular Involvement in Patients with Systemic Sclerosis

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Angiopoietin-like protein 3 (ANGPTL3), which is part of a family of secreted glycoproteins that are structurally similar to angiopoietins, is principally expressed in the liver and is involved in lipid metabolism and angiogenesis. The aim of this study was to determine the clinical significance of serum ANGPTL3 levels, measured with a specific enzyme-linked immunosorbent assay, in patients with systemic sclerosis. Serum ANGPTL3 levels correlated positively with skin score in diffuse cutaneous systemic sclerosis with a disease duration ≤6 years. Furthermore, the prevalence of digital ulcers was significantly higher in patients with elevated serum ANGPTL3 levels than in other patients. Moreover, among patients excluding diffuse cutaneous systemic sclerosis with disease duration ≤6 years, serum ANGPTL3 levels correlated positively with estimated right ventricular systolic pressure. In conclusion, ANGPTL3 may contribute to the development of progressive skin sclerosis and proliferative obliterative vasculopathy, such as digital ulcers and pulmonary vascular involvement leading to pulmonary arterial hypertension, in systemic sclerosis. ***Key words: systemic sclerosis; angiopoietin-like protein 3; angiogenesis; skin sclerosis; digital ulcers; pulmonary arterial hypertension.***

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Systemic sclerosis (SSc) is a multisystem autoimmune disease characterized by initial vascular injuries and resultant fibrosis of the skin and certain internal organs. Although fibroblast activation is the final consequence of SSc, autoimmune attacks and vascular injuries prior to the onset of the fibrotic response appear to play central roles in the pathogenesis of this complicated disorder (1).

A family of secreted glycoproteins structurally similar to angiopoietins was identified and designated as angiopoietin-like proteins (ANGPTLs), which consist of an N-terminal coiled-coil domain and C-terminal fibrinogen-like domain. Despite the structural similarity to angiopoietins, ANGPTLs do not bind to endothelial tyrosine kinase receptors Tie1 and Tie2, suggesting that these molecules are functionally different from angiopoietins (2). Angiopoietin-like protein 3 (ANGPTL3), a member of the ANGPTLs, is expressed principally in the liver during development and in adults (3). ANGPTL3 is involved in lipid metabolism and angiogenesis in a domain-dependent manner. The N-terminal coiled-coin domain regulates serum lipid levels by inhibiting lipolysis of triglyceride-rich lipoproteins in mice (4, 5), while the C-terminal fibrinogen-like domain induces endothelial cell adhesion and migration and *in vivo* angiogenesis by interacting with endothelial integrin αVβ3 (6). In humans, serum ANGPTL3 levels correlate positively with the intima-media thickness of carotid and femoral arteries in healthy subjects, but not with triglyceride, low-density lipoprotein, high-density lipoprotein, and total cholesterol levels. More importantly, serum ANGPTL3 levels still show a positive association with arterial wall thickness after adjustment for other possible confounding variables, suggesting that the angiogenic property of ANGPTL3 explains its association with arterial wall thickness (7). Thus, unlike animal models, the role of ANGPTL3 in lipid metabolism is controversial in humans, but it definitely contributes to the development of atherosclerosis, probably by activating angiogenesis.

Given that aberrant activation of angiogenesis underlies the pathogenesis of vasculopathy and subsequent fibroblast activation in SSc, ANGPTL3 may be involved in the mechanism responsible for the pathological process in this disorder. In an initial study of this issue, we investigated serum ANGPTL3 levels and their association with clinical features in SSc.

MATERIALS AND METHODS

Patients

Serum samples, frozen at –80ºC until assayed, were obtained from 51 patients with SSc (48 women, 3 men; age, median [25–75 percentile]: 59 years [51.5–66.5]; disease duration, 3 years [2–10]) and 18 healthy individuals (16 women, 2 men; age, 54.5 years [48.3–61.8]), who visited our department between July 2009 and June 2011. Patients treated with corticosteroids or other immu-

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nosuppressants prior to their first visit were excluded. There were no significant differences in age and disease duration between men and women (age 59 [26–66] vs. 59 years [51.3–67], $p = 0.66$, Mann–Whitney U test; disease duration, 1 year [1–2] vs. 3.5 years [2–10], $p = 0.066$, Mann–Whitney U test). Patients were grouped by the LeRoy's classification system (8): 24 patients with limited cutaneous SSc (lcSSc) (all women; age, 65 years [56.5–70.3]; disease duration, 8 years [2.8–15]) and 27 with diffuse cutaneous SSc (dcSSc) (24 women, 3 men; age 57 years [46–61]; disease duration, 2 years [2–4]). dcSSc patients were significantly younger than lcSSc patients ($p = 0.0073$, Mann–Whitney U test) and disease duration was much shorter in dcSSc patients than in lcSSc patients ($p = 0.036$, Mann–Whitney U test). All dcSSc and 22 lcSSc patients fulfilled the criteria proposed by the American College of Rheumatology (9). Two lcSSc patients not meeting these criteria had sclerodactyly and at least 2 other features of SSc, such as calcinosis, Raynaud's phenomenon, oesophageal dysfunction, and telangiectasia. The study was performed according to the Declaration of Helsinki and approved by the ethics committee of the University of Tokyo Graduate School of Medicine. Written informed consent was obtained from all patients and healthy controls.

Measurement of serum ANGPTL3 levels

Specific enzyme-linked immunosorbent assay kits were used to measure serum ANGPTL3 levels (R&D Systems, Minneapolis, USA). Briefly, polystyrene 96-well plates coated with antibodies against ANGPTL3 were incubated with 100 µl 50-fold diluted serum at room temperature for 2 h. The wells were then washed and incubated at room temperature for 2 h with horseradish peroxidase conjugated antibodies against ANGPTL3. Next, the wells were washed again, added with tetramethylbenzidine, and incubated at room temperature for 30 min. Finally, sulphuric acid was added to terminate the reaction and the absorbance at 450 nm was measured. Serum ANGPTL3 levels were calculated using standard curve.

Clinical assessment

Disease onset was defined as the first clinical event of SSc other than Raynaud's phenomenon. Disease duration was defined as the interval between onset and time of blood sampling. The clinical and laboratory data were obtained when the blood samples were drawn. Skin score was measured using modified Rodnan total skin thickness score (MRSS) (10). The degree of interstitial lung disease (ILD) was evaluated by the percentage of predicted vital capacity (%VC) and the percentage of predicted diffusion lung capacity for carbon monoxide (%DLco) on pulmonary function test. Ground-glass opacity on chest computed tomography was scored by 2 independent readers, as previously reported (11) and the mean estimate of the 2 readers was used as ground glass score. Elevated right ventricular systolic pressure (RVSP) was defined as 35 mmHg or more on echocardiogram. Scleroderma renal crisis (SRC) was defined as malignant hypertension and/or rapidly progressive renal failure.

Statistical analysis

The statistical analysis used for each experiment is described in the figure legends and Results section. Statistical significance was defined as $p < 0.05$.

RESULTS

Serum ANGPTL3 levels in patients with systemic sclerosis

No significant difference was found between serum ANGPTL3 levels in SSc patients and control subjects (median [25–75 percentiles]; 2.24 ng/ml [0.21–5.30] vs. 2.69 ng/ml [2.12–3.27], $p = 0.51$, Welch's t-test, respectively). There was also no significant difference in serum ANGPTL3 levels among healthy controls, dcSSc (2.12 ng/ml [0.21–3.60]), and lcSSc patients (3.16 ng/ml [0.56–5.87]) ($p = 0.42$ by Kruskal-Wallis test) (Fig. 1). However, close examination of the distribution of serum ANGPTL3 levels in SSc patients, revealed 2 subgroups of SSc patients: those with quite low levels and those with highly elevated levels of serum ANGPTL3. This observation suggests that serum ANGPTL3 levels are linked to certain clinical features in patients with SSc.

Serum ANGPTL3 levels correlated positively with MRSS in dcSSc patients with disease duration ≤6 years

In order to investigate the clinical association of serum ANGPTL3 levels, we initially studied the correlation of serum ANGPTL3 levels with dermal and pulmonary fibrotic markers, including MRSS, %VC, and %DLco, because activation of angiogenic process is potentially linked to the initiation and progression of fibrosis in SSc. Since severe organ damage occurs within 5–6 years of disease onset in most cases of dcSSc, and progression to severe skin thickening seldom occurs afterwards (12), the correlation of serum ANGPTL3 levels with MRSS was also assessed in dcSSc patients with disease duration ≤6 years in addition to dcSSc and lcSSc patient groups. As

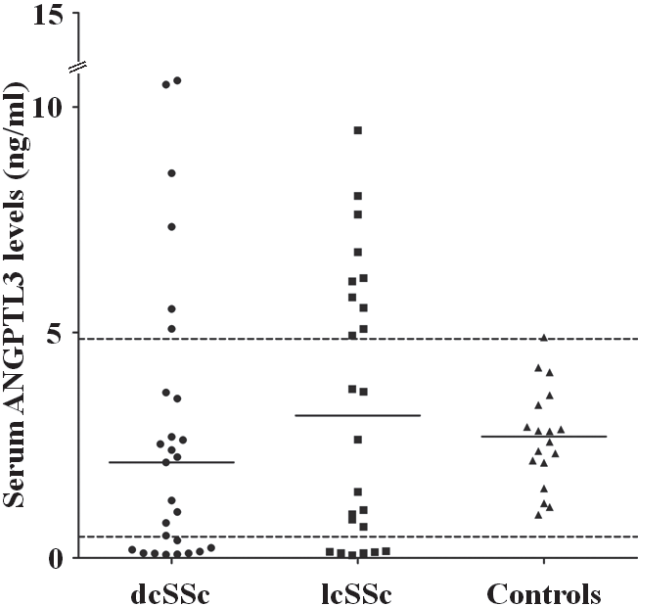

Fig. 1. Serum angiopoietin-like protein 3 (ANGPTL3) levels in systemic sclerosis (SSc) and healthy controls. Serum ANGPTL3 levels were determined by a specific enzyme-linked immunoassay (ELISA). Multiple comparison was carried out with a Kruskal–Wallis test. Since serum ANGPTL3 levels were normally distributed in healthy controls ($p = 0.76$, Shapiro–Wilk test), but not in dcSSc patients ($p = 0.0001$, Shapiro-Wilk test) and lcSSc patients ($p = 0.012$, Shapiro-Wilk test), horizontal bars indicate median for each group. The dotted lines represent mean ± 2 SD of serum AGPTL3 levels in healthy controls, which were used as cut-off values in the following analyses.

shown in Fig. 2A, serum ANGPTL3 levels correlated significantly with MRSS in dcSSc patients with disease duration ≤6 years ($r=0.53$, $p=0.022$; Spearman's rank correlation coefficient), but not in dcSSc and lcSSc patients ($r=0.39$, $p=0.06$, and $r=0.38$, $p=0.11$, respectively). Given that there was no correlation between serum ANGPTL3 levels and MRSS in dcSSc with disease duration >6 years ($r=-0.31$, $p=0.56$), serum ANGPTL3 levels may reflect the activation of angiogenic process during the development of skin sclerosis in dcSSc. On the other hand, regarding %VC and %DLco, no significant correlation with serum ANGPTL3 levels was detected in these 3 groups. We also evaluated the association of serum ANGPTL3 levels with ground glass score in these 3 groups because ground glass opacity reflects inflammation and vasculopathy associated with ILD better than %VC and %DLco, but did not see any significant correlation. Collectively, ANGPTL3 is potentially involved in the mechanism underlying skin sclerosis, but not ILD, in dcSSc.

Clinical features of SSc patients with elevated serum ANGPTL3 levels

We next investigated the association of serum ANGPTL3 levels with clinical features related to SSc vasculopathy. Since there was a subset of SSc patients with quite high serum ANGPTL3 levels, we first set the cut-off value at 4.86 ng/ml (mean +2 SD) of healthy controls) and classified SSc patients into 2 groups; patients with elevated serum ANGPTL3 levels (31% of SSc patients; 6 of 27 dcSSc patients and 10 of 24 lcSSc patients) and the other patients. Patient information and the prevalence of clinical features associated with SSc vasculopathy in these 2 groups are shown in the left-hand columns of Table I. There was no significant difference between these 2 groups in terms of sex, age, disease duration, and the frequency of dcSSc. Regarding the frequency of cutaneous vascular manifestations, including Raynaud's phenomenon, nail-fold bleeding, telangiectasia, and pitting scars, there was no significant difference between these 2 groups. As for organ involvements associated with proliferative obliterative vasculopathy, the frequency of digital ulcers was significantly greater in patients with increased serum ANGPTL3 levels than in the other patients (40 % vs. 11%, $p=0.048$), while the frequencies of SRC and elevated RVSP were comparable between these 2 groups. Thus, the increase in serum ANGPTL3 levels is associated with the development of digital ulcers, which are attributable to intimal proliferation and luminal narrowing or occlusion of small digital arteries

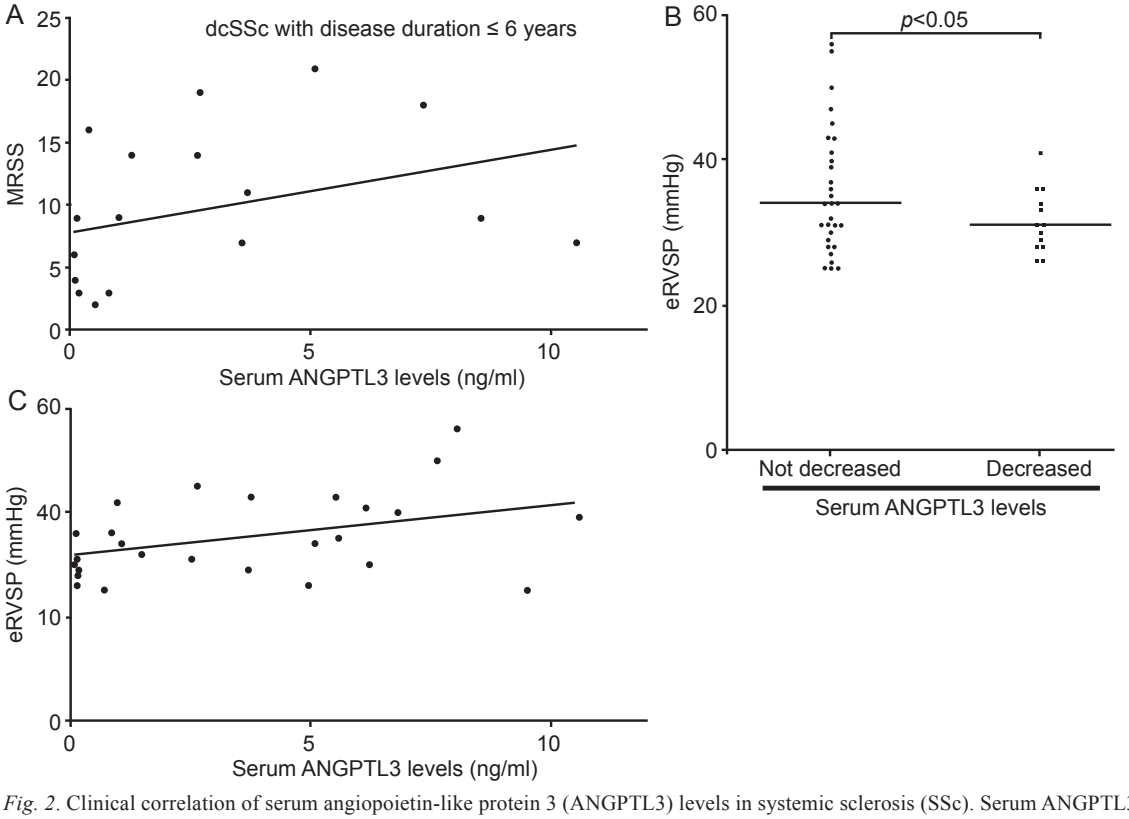

Fig. 2. Clinical correlation of serum angiopoietin-like protein 3 (ANGPTL3) levels in systemic sclerosis (SSc). Serum ANGPTL3 levels correlated with modified Rodnan total skin thickness score (MRSS) in dcSSc patients with disease duration ≤6 years (A; $r=0.53$, $p=0.022$, Spearman's rank correlation coefficient) and with right ventricular systolic pressure (RVSP) in SSc patients excluding dcSSc with disease duration ≤6 years (C; $r=0.39$, $p=0.044$, Spearman's rank correlation coefficient). The solid line represents the regression line. The values of RVSP were significantly higher in SSc patients with normal and elevated serum ANGPTL3 levels than in those with descreased levels. (B; $p=0.036$, Welch's *t*-test). Since serum ANGPTL3 levels were normally distributed in SSc patients with decreased serum ANGPTL3 levels ($p=0.02$, Shapiro-Wilk test), but not in patients with normal and elevated ANGPTL3 levels ($p=0.54$, Shapiro-Wilk test), median was shown as horizontal bars.

Table I. *Clinical features of systemic sclerosis (SSc) patients with increased or decreased serum angiopoietin-like protein 3 (ANGPTL3), levels*

	Serum ANGPTL3 levels			
	Increase		Decrease	
	Yes ($n=16$)	No ($n=35$)	Yes ($n=15$)	No ($n=36$)
Sex, male:female, *n*	0:16	4:31	3:12	1:35
Age, years, median [25–75 percentiles]	58 [54.5–68]	59 [48.8–65]	59 [54–66.5]	58.5 [48.8–65]
Disease duration, years, median [25–75 percentiles]	6 [1.9–16]	3 [1.5–10]	2.6 [1.8–7]	4 [1.5–10.8]
dcSSc:lcSSc, *n*	6:10	21:14	9:6	18:18
Cutaneous vascular symptoms, % (*n*)				
Raynaud's phenomenon	88 (14/16)	88 (30/34)	80 (12/15)	91 (32/35)
Nail-fold bleeding	80 (12/15)	76 (25/33)	71 (10/14)	79 (27/34)
Telangiectasia	46 (6/13)	48 (13/27)	33 (4/12)	54 (15/28)
Pitting scars	38 (5/13)	38 (13/34)	27 (4/15)	44 (14/32)
Organ involvements associated with proliferative vasculopathy, % (*n*)				
Digital ulcers	40* (6/15)	11 (4/35)	13 (2/15)	26 (9/35)
Scleroderma renal crisis	6 (1/16)	6 (2/35)	7 (1/15)	6 (2/36)
Elevated RVSP	44 (7/16)	26 (9/35)	20 (3/15)	39 (14/36)

dcSSc: diffuse cutaneous SSc; lcSSc: limited cutaneous SSc; RVSP: right ventricular systolic pressure. Statistical analysis was carried out with Fisher's exact probability test. *$p=0.048$ (Yes compered with No).

and larger arteries (the palmar arch and radial and ulnar arteries) (13), in SSc patients.

Clinical features of patients with systemic sclerosis with decreased serum ANGPTL3 levels

The other feature of serum ANGPTL3 levels in patients with SSc was the presence of another patient subset with quite low serum ANGPTL3 levels. Therefore, we also set another cut-off value at 0.47 ng/ml (mean –2 SD of healthy controls) and classified SSc patients into 2 groups; patients with decreased serum ANGPTL3 levels (29% of SSc patients; 9 of 27 dcSSc patients and 6 of 24 lcSSc patients) and the other patients. Similar analyses were carried out between these 2 groups (right-hand columns in Table I). There was no significant difference in patients' backgrounds (sex, age, disease duration, and frequency of dcSSc). In contrast to the former analysis, we did not detect any clinical features associated with SSc vasculopathy in patients with decreased serum ANGPTL3 levels. However, when the RVSP values were evaluated, they were found to be significantly higher in SSc patients with normal and elevated serum ANGPTL3 levels than in those with decreased levels (34 mmHg [29–41.3] vs. 31 mmHg [28.3–33.8], $p=0.036$; Fig. 2B). Collectively, these results suggest that decreased serum ANGPTL3 levels serve as a marker for SSc patients at low risk of pulmonary vascular involvement leading to pulmonary arterial hypertension (PAH).

Serum ANGPTL3 levels correlated positively with right ventricular systolic pressure in SSc patient group excluding dcSSc patients with disease duration ≤6 years

As described above, RVSP values were significantly higher in SSc patients with normal and elevated serum ANGPTL3 levels than in those with decreased levels, while there was no significant correlation between serum ANGPTL3 levels and RVSP ($r=0.19$, $p=0.22$). Given that serum ANGPTL3 levels correlate with MRSS in dcSSc patients with disease duration ≤6 years, this may affect the relationship between serum ANGPTL3 levels and RVSP. Therefore, we re-evaluated the association of serum ANGPTL3 levels with RVSP in SSc patient group excluding dcSSc patients with disease duration ≤6 years. As expected, serum ANGPTL3 levels positively correlated with RVSP in this patient group ($r=0.39$, $p=0.044$; Spearman's rank correlation coefficient) (Fig. 2C), but not in dcSSc patients with disease duration ≤6 years ($r=–0.19$, $p=0.42$; Spearman's rank correlation coefficient). These results suggest the potential association of ANGPTL3 with the mechanism responsible for the development of pulmonary vascular involvement leading to PAH in SSc.

DISCUSSION

This study assessed the clinical significance of serum ANGPTL3 levels and enabled speculation about the contribution of this molecule to the developmental process in SSc. Although serum ANGPTL3 levels were comparable among dcSSc, lcSSc and healthy controls, some SSc patients had highly elevated or quite low serum ANGPTL3 levels, suggesting that altered expression of ANGPTL3 contributes to certain pathological process in SSc. Supporting this idea, serum ANGPTL3 levels showed a positive correlation with MRSS in dcSSc patients with disease duration ≤6 years. Furthermore, digital ulcers were seen in SSc patients with elevated serum ANGPTL3 levels much more frequently than in the other patients. Moreover, in the SSc patient group excluding dcSSc patients with disease duration

≤6 years, serum ANGPTL3 levels positively correlated with the values of RVSP. Collectively, the increased expression of ANGPTL3 may play a role in the mechanism responsible for the initiation and progression of skin sclerosis and the development of proliferative obliterative vasculopathy, such as digital ulcers and pulmonary vascular involvement leading to PAH in SSc.

The significant role of ANGPTL3 in lipid metabolism has clearly been shown in animal models. Consistent with the *in vitro* data that ANGPTL3 inhibits the activity of lipoprotein lipase (14), lower plasma triglyceride levels in *Angptl3*-deficient mice are reversed to normal levels by introducing the functioning *Angptl3* gene (4, 14) or by intravenous administration of recombinant human ANGPTL3 protein (4, 15). Furthermore, the development of atherosclerosis in apolipoprotein E knockout mice is suppressed, along with the reduction in triglyceride levels by introducing a recessive mutation of the *Angptl3* gene homozygously (16). Consistent with these findings in animal models, serum ANGPTL3 levels correlate with the thickness of arterial walls in humans. However, serum ANGPTL3 levels do not correlate with triglyceride, high-density lipoprotein, low-density lipoprotein, and total cholesterol levels. Most importantly, the positive association of serum ANGPTL3 levels with intima-media thickness of carotid and femoral arteries is independent of lipids and other possible confounding variables, such as age, sex, smoking, body mass index, systolic blood pressure, plasma glucose, and insulin resistance index (7). Supporting this notion, the association between the *Angptl3* gene polymorphisms and coronary plaque area is independent of lipids and other classical risk factors in survivors of myocardial infarction (17). Collectively, in contrast to animal models, serum ANGPTL3 levels have a significant association with atherosclerotic vascular changes independent of plasma lipid levels in humans. Although the lipid-independent mechanism by which ANGPTL3 promotes atherosclerosis is totally unknown, the direct effect of ANGPTL3 on endothelial cells potentially explains this phenomenon. Camenisch et al. (6) demonstrated that integrin αVβ3 serves as a receptor for ANGPTL3 on endothelial cells and this interaction promotes endothelial cell adhesion and migration. Furthermore, the author revealed that ANGPTL3 induces angiogenesis to a similar extent to that caused by vascular endothelial growth factor (VEGF)-A in rat corneal assay. Given that adventitial angiogenesis induced by the transduction of adenovirus encoding VEGF-A, VEGF-D and VEGF-DΔNΔC correlates positively with the intimal hyperplasia in animal models (18), the angiogenic property of ANGPTL3 may explain the positive association of its serum levels with arterial wall thickness. Thus, ANGPTL3 potentially causes intimal hyperplasia in concert with other pro-angiogenic factors in various vascular diseases.

Vasculopathy associated with SSc is generally classified into 2 subgroups; destructive vasculopathy and proliferative obliterative vasculopathy (19). Destructive vasculopathy is characterized by progressive loss of capillaries, which is clinically related to pitting scars. In contrast, proliferative obliterative vasculopathy is characterized by proliferation of vascular cells resulting in intimal hyperplasia, intimal fibrosis, and luminal narrowing or occlusion of arteries, which is associated with the development of digital ulcers, PAH and SRC. As shown in the present study, the increase in serum ANGPTL3 levels is closely linked to the development of digital ulcers and elevated RVSP. Given that ANGPTL3 is potentially involved in the mechanism by which thickening of arterial walls develops in humans (7), the current data strongly suggest that ANGPTL3 plays a role in the pathogenesis of SSc vasculopathy, especially proliferative obliterative vasculopathy.

In order to evaluate the association of serum ANGPTL3 levels with pulmonary arterial involvement leading to PAH in SSc, we used RVSP measured by echocardiography. However, it has been reported that RVSP lacks specificity for the diagnosis of PAH in SSc due to the influence of left-heart disease and ILD associated with this disease (20). Therefore, in order to better evaluate the potential role of ANGPTL3 in SSc-PAH, it is necessary to assess the correlation of serum ANGPTL3 levels with mean pulmonary arterial pressure (mPAP) measured by right heart catheterization. Given the relatively low correlation of RVSP with mPAP in SSc, serum ANGPTL3 levels may have a much better correlation with mPAP than RVSP.

It is generally accepted that a certain subset of pro-angiogenic factors serves as a biomarker of disease activity and severity in SSc. For instance, Michalska-Jakubus et al. (21) demonstrated that serum levels of angiopoietin-2, a potent pro-angiogenic factor exerting its biological effect through Tie1 and Tie2 tyrosine kinase receptors, correlate positively with MRSS and inversely with %DLco and are significantly elevated in intermediate/late SSc compared with early SSc. More importantly, serum angiopoietin-2 levels are independently associated with the European Scleroderma Study Group disease activity index score in multivariate regression analysis. Another study by Dunne et al. (22), in which platelet-free plasma samples from relatively severe patients were used, also demonstrated the contribution of the angiopoietin-1/angiopoietin-2/Tie2 system to ongoing vasculopathy in SSc by the following data: (*i*) soluble angiopoietin-1, angiopoietin-2 and Tie2 levels and the ratios of angiopoietin-2/angiopoietin-1 and angiopoietin-2/Tie2 are higher in dcSSc and lcSSc than in healthy controls; (*ii*) angiopoietin-2 levels correlate with RVSP and %DLco in lcSSc; and (*iii*) angiopoietin-2/Tie2 ratio shows a positive association with disease activity in both dcSSc and lcSSc. These results

indicate that the activation of angiogenesis occurs throughout the disease course and is closely related to the activity and severity of SSc. In the present study, serum ANGPTL3 levels correlated with MRSS in dcSSc with disease duration ≤6 years, but not in dcSSc with disease duration >6 years and lcSSc. As for ILD, serum ANGPTL3 levels were associated with neither %VC nor %DLco in the 3 subgroups. These results suggest that ANGPTL3 plays a role in the constitutive activation of angiogenesis, which leads to fibroblast activation, together with other pro-angiogenic factors especially during the progressive stage of skin sclerosis in dcSSc.

In summary, we report here the first study demonstrating the close association of serum ANGPTL3 levels with the development of progressive skin sclerosis and proliferative obliterative vasculopathy, such as digital ulcers and pulmonary vascular involvement leading to PAH, in SSc. However, the present conclusion regarding pulmonary vascular involvement is still preliminary, because RVSP measured by echocardiography lacks specificity for the diagnosis of PAH in SSc due to the influence of left-heart disease and ILD (20). Furthermore, we cannot deny the possibility that the elevation of serum ANGPTL3 levels in SSc is an epiphenomenon because the correlation of ANGPTL3 mRNA levels in dcSSc skin with MRSS was not confirmed due to undetectably low levels of ANGPTL3 expression (data not shown). Regardless of the limitations, this study supports previous data regarding the pro-angiogenic property of ANGPTL3 and provides a useful clue to further understanding of the mechanism responsible for the development of skin sclerosis and vasculopathy in SSc.

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REFERENCES

1. Asano Y. Future treatments in systemic sclerosis. J Dermatol 2010; 37: 54–70.
2. Hato T, Tabata M, Oike Y. The role of angiopoietin-like proteins in angiogenesis and metabolism. Trends Cardiovasc Med 2008; 18: 6–14.
3. Conklin D, Gilbertson D, Taft DW, Maurer MF, Whitmore TE, Smith DL, et al. Identification of a mammalian angiopoietin-related protein expressed specifically in liver. Genomics 1999; 62: 477–482.
4. Koishi R, Ando Y, Ono M, Shimamura M, Yasumo H, Fujiwara T, et al. Angptl3 regulates lipid metabolism in mice. Nat Genet 2002; 30: 151–157.
5. Ono M, Shimizugawa T, Shimamura M, Yoshida K, Noji-Sakikawa C, Ando Y, et al. Protein region important for regulation of lipid metabolism in angiopoietin-like 3 (ANGPTL3): ANGPTL3 is cleaved and activated in vivo. J Biol Chem 2003; 278: 41804–41809.
6. Camenisch G, Pisabarro MT, Sherman D, Kowalski J, Nagel M, Hass P, et al. ANGPTL3 stimulates endothelial cell adhesion and migration via integrin alpha vbeta 3 and induces blood vessel formation in vivo. J Biol Chem 2002; 277: 17281–17290.
7. Hatsuda S, Shoji T, Shinohara K, Kimoto E, Mori K, Fukumoto S, et al. Association between plasma angiopoietin-like protein 3 and arterial wall thickness in healthy subjects. J Vasc Res 2007; 44: 61–66.
8. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 1988; 15: 202–205.
9. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum 1980; 23: 581–590.
10. Clements PJ, Lachenbruch PA, Seibold JR, Zee B, Steen VD, Brennan P, et al. Skin thickness score in systemic sclerosis: an assessment of interobserver variability in 3 independent studies. J Rheumatol 1993; 20: 1892–1896.
11. Kazerooni EA, Martinez FJ, Flint A, Jamadar DA, Gross BH, Spizarny DL, et al. Thin-section CT obtained at 10-mm increments versus limited three-level thin-section CT for idiopathic pulmonary fibrosis: correlation with pathologic scoring. Am J Roentgenol 1997; 169: 977–983.
12. Steen VD, Medsger TA, Jr. Severe organ involvement in systemic sclerosis with diffuse scleroderma. Arthritis Rheum 2000; 43: 2437–2444.
13. Marvi U, Chung L. Digital ischemic loss in systemic sclerosis. Int J Rheumatol 2010; 2010.
14. Shimizugawa T, Ono M, Shimamura M, Yoshida K, Ando Y, Koishi R, et al. ANGPTL3 decreases very low density lipoprotein triglyceride clearance by inhibition of lipoprotein lipase. J Biol Chem 2002; 277: 33742–33748.
15. Shimamura M, Matsuda M, Kobayashi S, Ando Y, Ono M, Koishi R, et al. Angiopoietin-like protein 3, a hepatic secretory factor, activates lipolysis in adipocytes. Biochem Biophys Res Commun 2003; 301: 604–609.
16. Ando Y, Shimizugawa T, Takeshita S, Ono M, Shimamura M, Koishi R, et al. A decreased expression of angiopoietin-like 3 is protective against atherosclerosis in apoE-deficient mice. J Lipid Res 2003; 44: 1216–1223.
17. Korstanje R, Eriksson P, Samnegård A, Olsson PG, Forsman-Semb K, Sen S, et al. Locating Ath8, a locus for murine atherosclerosis susceptibility and testing several of its candidate genes in mice and humans. Atherosclerosis 2004; 177: 443–450.
18. Bhardwaj S, Roy H, Heikura T, Ylä-Herttuala S. VEGF-A, VEGF-D and VEGF-D(DNDC) induced intimal hyperplasia in carotid arteries. Eur J Clin Invest 2005; 35: 669–676.
19. Horn A, Distler JH. Vascular alterations upon activation of TGFb signaling in fibroblasts - implications for systemic sclerosis. Arthritis Res Ther 2010; 12: 125.
20. Kowal-Bielecka O, Avouac J, Pittrow D, Huscher D, Behrens F, Denton CP, et al. Echocardiography as an outcome measure in scleroderma-related pulmonary arterial hypertension: a systematic literature analysis by the EPOSS group. J Rheumatol 2010; 37: 105–115.
21. Michalska-Jakubus M, Kowal-Bielecka O, Chodorowska G, Bielecki M, Krasowska D. Angiopoietins-1 and -2 are differentially expressed in the sera of patients with systemic sclerosis: high angiopoietin-2 levels are associated with greater severity and higher activity of the disease. Rheumatology (Oxford) 2011; 50: 746–755.
22. Dunne JV, Keen KJ, Van Eeden SF. Circulating angiopoietin and Tie-2 levels in systemic sclerosis. Rheumatol Int 2013; 33: 475–484.