Science, Mannheim, Germany). Slides were counterstained by nuclear fast red, and examined under BX50 light microscope (OLYMPUS, Tokyo, Japan).

Cell cultures

Human dermal fibroblasts were cultured from the affected areas (dorsal forearm) of three female dcSSc patients (23). Age- and sex-matched control fibroblasts were obtained from healthy human subjects. Institutional review board approval with written informed consent was obtained according to the Declaration of Helsinki. Monolayer cultures isolated from different individuals independently were maintained at 37°C in 5% CO_2 in air. Fibroblasts between the third and six subpassages were used for experiments. Before all experiments, cells were serum-starved for 24 hours.

Cell lysis and immunoblotting

Cultured fibroblasts were washed twice with PBS and lysed in Denaturing Cell Extraction Buffer (Biosource International, Camarillo, CA). Aliquots of cell lysates were separated by electrophoresis on SDS-polyacrylamide gels, and transferred onto polyvinylidene difluoride filters. The filters were incubated with primary antibodies (24). Primer antibody for type I collagen-UNLB or β-actin were obtained from Southern Biotechnologies (Birmingham, AL) or Santa Cruz Biotechnology (Santa Cruz, CA), respectively. After the incubation with secondary antibody, the immunoreactive bands were visualized using ECL system (Amersham Biosciences, Arlington Heights, IL).

Transient transfection

For reverse transfection, siRNAs (10nM) were mixed with Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA), and then added when the cells were plated, followed by incubation at 37°C in 5% CO_2. siRNA against TSIX or TGF-β and control siRNA were purchased from Qiagen and Santa Cruz Biotechnology, respectively (25,26). After the transfection, cells were incubated for 96 hours following the manufacture's protocol (http://www.eposters.net/pdfs/long-term-gene-silencing-in-mammalian-cells-using-sirna.pdf) and previous reports (25, 27).

Statistical analysis

Data presented as scatter plots are obtained by at least three experiments. The statistical analysis was performed by Mann-Whitney U test for the comparison of the medians, and by Fisher's exact probability test for the analysis of the frequency. The $P < 0.05$ were considered to be significant.

Results

The expression of lncRNAs in SSc skin

As an initial experiment, we investigated the expression levels of XIST and TSIX in SSc skin. There was no significant difference in XIST expression between normal skin, SLE skin, lcSSc skin and dcSSc skin (Fig.1a). XIST levels in the skin of dcSSc patients was slightly increased compared to those in lcSSc patients, but not statistically significant. On the other hand, TSIX levels were significantly higher in dcSSc skin than normal skin. lcSSc skin showed similar TSIX levels to normal skin. No difference was found between male and female SSc patients (data not shown). HAR1A, another lncRNA randomly chosen as a control, was not detected in most of normal skin, SLE skin or SSc skin, and we could not find significant difference among them.

Next, we tried to determine which kinds of cells overexpress TSIX in dcSSc skin. In situ hybridization revealed signal for TSIX was hardly detected in normal skin, while detected in spindle-shaped fibroblasts in dcSSc skin (Fig.1b). The number of TSIX-positive cells was significantly increased in dcSSc skin, and no difference was found between male and female SSc patients (data not shown). Accordingly, we focused on TSIX overexpression in dcSSc fibroblasts.

Cultured dermal fibroblasts derived from involved skin of dcSSc patients also overexpressed TSIX compared to normal fibroblasts in vitro (Fig.1c). Because no difference was found in TSIX levels between male and female SSc fibroblasts (data not shown), we used female normal and dcSSc fibroblasts in the following experiments. Furthermore, exogenous TGF-β1 stimulation induced TSIX expression significantly in normal dermal fibroblasts, while it induced TSIX in SSc fibroblasts only slightly, probably because TGF-β1 is already saturated as described in Introduction. When intrinsic TGF-β1 was knocked down by the siRNA (Fig.1d), the overexpressed TSIX was reduced significantly in SSc fibroblasts. Taken together, these results suggested that TSIX was overexpressed in

SSc fibroblasts in vivo and in vitro, probably due to the stimulation by intrinsic TGF-β activation.

The function of TSIX in normal and SSc dermal fibroblasts

When SSc skin was divided into three groups (high, medium and low TSIX expression), SSc cases with high TSIX levels showed significantly increased mRNA expression of α1(I) and α2(I) collagen compared to those with low TSIX (Fig.2a). Given that cultured SSc dermal fibroblasts expressed increasing amount of collagen (Fig.2b) as described in the Introduction, as well as TSIX, we hypothesized that TSIX contributes to the collagen regulation in dermal fibroblasts.

Consistently, TSIX siRNA reduced the mRNA expression of α1(I) and α2(I) collagen as well as α1(III) collagen expression in both normal and SSc dermal fibroblasts (Fig.2c). We confirmed that the transfection of TSIX siRNA also down-regulated expression of α1(I) and α2(I) chain of type I collagen at the protein levels using immunoblotting (Fig.2d). On the other hand, forced down-regulation of TSIX did not have significant effects on the expression of major disease-related cytokines or growth factors (IL-6, PDGF as well as TGF-β) (Fig.2e), suggesting that TSIX directly contribute to the regulatory mechanism of type I collagen expression.

To examine whether the decrease of type I collagen by TSIX siRNA takes place at the transcriptional level or post-transcriptional level, the stability of type I collagen mRNA and protein was examined. Because the steady-state level of mRNA is controlled by the level of gene transcription and/or the stability of mRNA, the *de novo* mRNA synthesis was stopped by actinomycin D, a RNA synthesis inhibitor, in normal and SSc fibroblasts in the presence or absence of TSIX siRNA to specifically assess the mRNA stability. The decreased ratio of α1(I) and α2(I) collagen mRNA in cells transfected with TSIX siRNA was significantly higher than that with control siRNA (Fig.3a). Thus, TSIX controls collagen mRNA stability in normal and SSc fibroblasts. On the other hand, when *de novo* protein synthesis was inhibited by cycloheximide, TSIX siRNA had no effects on the protein half-lives of collagen (Fig. 3b).

Serum concentrations of TSIX

To validate whether the lncRNA is detectable extracellularly, RNA was extracted from cultured medium, and the levels of TSIX were determined by quantitative real-time PCR

using specific primer set (Fig.4a). SSc fibroblasts released higher amount of TSIX mRNA into culture medium compared to normal fibroblasts.

There has been no report demonstrating the expression of TSIX in cell-free body fluid. The amplification of TSIX was also observed in the sera of normal subjects, and Ct values were increased by the serial dilution of the lncRNA (Fig.4b). Thus, TSIX was thought to be detectable and quantitative in the serum using our method. On the other hand, although we previously reported the presence of microRNAs in hair shafts (28), we could not detect TSIX expression in the hair (data not shown).

As shown in Fig.4c, serum TSIX levels in dcSSc patients were significantly higher than those in normal subjects. In addition, patients with SSD, who did not fulfill the ACR criteria of SSc but were thought to develop SSc in the future based on the proposed criteria (15-17), also showed significantly increased serum TSIX levels, suggesting that serum TSIX levels were elevated in SSc patients at the early stage. On the other hand, serum TSIX levels were slightly increased in SLE patients compared to normal subjects, but not statistically significant. When the cut-off value was set at mean+2SD of the normal subjects, increased serum concentrations of TSIX were found in 21 of the 36 (58.3%) SSc patients and 5 of the 10 (50%) SSD patients. XIST and HAR1A were hardly detected in serum samples.

Supplemental Table2 shows the association of serum TSIX levels with the clinical features in SSc patients. SSc patients with increased serum TSIX levels showed significantly higher ratio of dcSSc: lcSSc (13:8 vs 0:12, $p<0.0005$) and higher MRSS (15.9 vs 3.5, $p<0.001$) compared to those with normal levels: The correlation between serum TSIX levels and MRSS was mild but significant ($r=0.58$, $p=0.0006$). Accordingly, serum TSIX levels are correlated with skin sclerosis, which is consisted with the results of experiments using skin samples and cultured fibroblasts of SSc.

Discussion

Role of lncRNAs in rheumatic diseases has been poorly investigated. Most recently, down-regulated Hotair levels in differentiated osteoclasts and rheumatoid synoviocytes were reported (29). The authors also showed high expression level of Hotair in blood mononuclear cells and serum exosome of patients with rheumatoid arthritis. Our study first demonstrated the role of lncRNAs in collagen expression and its contribution to the pathogenesis of SSc by several major findings.

Among lncRNAs, we focused on the increase of TSIX in SSc. Although expression of XIST, antisense gene of TSIX which is well implicated in the X-chromosome inactivation,

was reported to be lost in several human diseases including female breast or ovarian cancers (30), our study showed XIST expression was not changed in rheumatic diseases including SSc. We expected that TSIX has other function than the inhibitor of XIST, and found new lncRNA-target interactions in dermal fibroblasts: forced down-regulation of TSIX leads to decrease of type I collagen, probably via inhibited mRNA stability. The regulation of mRNA half-life by lncRNAs has been reported in breast cancer (31), and our study is first to describe similar phenomenon in autoimmune diseases. Our results also suggested exogenous TGF-β stimulation induces TSIX expression in normal fibroblasts. Although Smads are known to be key intermediates in the TGF-β signaling, recent researches have identified other pathways such as PKC-δ, phosphatidylcholine-specific phospholipase C, p38 mitogen-activated protein kinase or geranylgeranyl transferase I, as the participants in the regulatory mechanisms of various ECM expression by TGF-β (32,33). TSIX is another downstream target to mediate the effect of TGF-β on collagen. The up-regulation of TSIX seen in SSc fibroblasts may result from activated endogenous TGF-β signaling, and may play a role in the constitutive up-regulation of collagen in these cells.

Furthermore, we investigated serum lncRNA levels in SSc. This is the first report showing that TSIX is detectable and quantitative in the serum using our method. Our results indicate that serum TSIX levels are significantly increased in SSc patients, and that SSc patients with elevated serum TSIX levels had significantly higher ratio of dcSSc: lcSSc and significantly higher MRSS. Thus, serum TSIX levels can be a disease marker, reflecting the activity of type I collagen production. Given that cultured SSc fibroblasts released increasing amount of TSIX mRNA into conditioned medium compared to normal fibroblasts, dermal fibroblasts may be one of the main sources of increased serum TSIX levels in SSc patients.

Conventional treatments against severe skin fibrosis of SSc such as steroid, cyclophosphamide and methotrexate have limited effects. In addition, various significant adverse effects need to be considered. Given the significant effects of TSIX siRNA on collagen expression, further studies about the regulatory mechanism of tissue fibrosis by lncRNA in SSc skin may lead to a better understanding of the pathogenesis, new diagnostic methods, and new therapeutic approaches of SSc.

As the limitation of this study, we did not clarity the mechanism of how TSIX levels are increased in SSc fibroblasts, how TSIX would influence the stability of collagen mRNA levels, and how TSIX is influenced by TGF-β1: for example, TGF-β1 might address its effects on type I collagen levels at least in part through XIST. Also, we did not determine

whether we obtain same results using cultured dermal fibroblasts of lcSSc patients or whether fibroblasts obtained from normal or less affected regions of SSc patients show lower levels of TSIX. The time-course effects of siRNAs were not evaluated in this study. The expression and function of XIST should also be determined in the future. In addition, we should determine the possibility that TSIX is secreted by fibroblasts and is integrated by surrounding cells to modulate type I collagen mRNAs, and that an overexpression of TSIX or incubations of serum with high levels of TSIX would increase the collagen expression in normal fibroblasts. The possibility that TSIX inside or outside the cells promotes the expression of disease-related cytokines in other cell types than fibroblasts should also be assessed.

Acknowledgements

This study was supported in part by a grant for scientific research from the Japanese Ministry of Education, Science, Sports and Culture, and by project research on intractable diseases from the Japanese Ministry of Health, Labour and Welfare. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions

ZW performed the experimental work, analyzed the data and wrote the paper. MJ contributed to experimental work and critically revised the manuscript. HI supervised the research study and critically revised the manuscript. KN, MH, HK, WN, KI and TN contributed to experimental design and manuscript preparation. TN, NH and SF contributed to study design.

Conflict of interest

The authors have declared no conflicting interests.

References

1. LeRoy EC. Increased collagen synthesis by scleroderma skin fibroblasts in vitro: a possible defect in the regulation or activation of the scleroderma fibroblast. J Clin Invest 1974:54:880-889.

2. Jimenez SA, Feldman G, Bashey RI et al. Co-ordinate increase in the expression of type I and type III collagen genes in progressive systemic sclerosis. Biochem J 1986:237:837-843.

3. Massagué J. Transforming growth factor-β family. Ann Rev Cell Biol 1990:6:597-641.

4. LeRoy EC, Smith EA, Kahaleh MB et al. A strategy for determining the pathogenesis of systemic sclerosis. Is transforming growth factor β the answer? Arthritis Rheum 1989:32:817-825.

5. Kikuchi K, Hartl CW, Smith EA et al. Direct demonstration of transcriptional activation of collagen gene expression in systemic sclerosis fibroblasts. Biochem Biophys Res Commun 1992:187:45-50.

6. Ihn H, Yamane K, Kubo M, Tamaki K. Blockade of endogenous transforming growth factor β signaling prevents up-regulated collagen synthesis in scleroderma fibroblasts: association with increased expression of transforming growth factor β receptors. Arthritis Rheum 2001:44:474-480.

7. Asano Y, Ihn H, Yamane K et al. Impaired Smad7-Smurf-mediated negative regulation of TGF-β signaling in scleroderma fibroblasts. J Clin Invest 2004:113:253-264.

8. Asano Y, Ihn H, Yamane K et al. Phosphatidylinositol 3-kinase is involved in α2(I) collagen gene expression in normal and scleroderma fibroblasts. J Immunol 2004:172:7123-7135.

9. Sarkar SP, Adshead G. Whose DNA is it anyway? European court, junk DNA, and the

problem with prediction. J Am Acad Psychiatry Law 2010:38:247-250.

10. Li G, Zhang H, Wan X et al. Long Noncoding RNA plays a key role in metastasis and prognosis of hepatocellular carcinoma. Biomed Res Int 2014:2014:780521.

11. Bhan A, Mandal SS. Long Noncoding RNAs: Emerging stars in gene regulation, epigenetics and human disease. ChemMedChem 2014:9:1932-1956.

12. Eades G, Zhang YS, Li QL et al. Long non-coding RNAs in stem cells and cancer. World J Clin Oncol 2014:5:134-141.

13. Luo Y, Wang Y, Wang Q et al. Systemic sclerosis: genetics and epigenetics. J Autoimmun 2013:41:161-167.

14. Lian X, Xiao R, Hu X et al. DNA demethylation of CD40l in CD4+ T cells from women with systemic sclerosis: a possible explanation for female susceptibility. Arthritis Rheum 2012:64:2338-2345.

15. Maricq HR, Weinrich MC, Keil JE et al. Prevalence of scleroderma spectrum disorders in the general population of South Carolina. Arthritis Rheum 1989:32:998-1006.

16. Maricq HR, McGregor AR, Diat F et al. Major clinical diagnoses found among patients with Raynaud phenomenon from the general population. J Rheumatol 1990:17:1171-1176.

17. Ihn H, Sato S, Tamaki T et al. Clinical evaluation of scleroderma spectrum disorders using a points system. Arch Dermatol Res 1992:284:391-395.

18. Arakawa H, Jinnin M, Muchemwa FC et al. Adiponectin expression is decreased in the

involved skin and sera of diffuse cutaneous scleroderma patients. Exp Dermatol 2011: 20:764-766.

19. Moriya C, Jinnin M, Yamane K et al. Expression of matrix metalloproteinase-13 is controlled by IL-13 via PI3K/Akt3 and PKC-δ in normal human dermal fibroblasts. J Invest Dermatol 2011:131:655-661.

20. Martin MM, Buckenberger JA, Jiang J et al. The human angiotensin II type 1 receptor +1166 A/C polymorphism attenuates microRNA-155 binding. J Biol Chem 2007:282:24262-24269.

21. Nuovo GJ. In situ detection of precursor and mature microRNAs in paraffin embedded, formalin fixed tissues and cell preparations. Methods 2008:44:39-46.

22. Honda N, Jinnin M, Kira-Etoh T et al. miR-150 down-regulation contributes to the constitutive type I collagen overexpression in scleroderma dermal fibroblasts via the induction of integrin β3. Am J Pathol 2013:182:206-216.

23. Ihn H, LeRoy EC, Trojanowska M. Oncostatin M stimulates transcription of the human α2(I) collagen gene via the Sp1/Sp3-binding site. J Biol Chem 1997: 272:24666-24672.

24. Jinnin M, Medici D, Park L et al. Suppressed NFAT-dependent VEGFR1 expression and constitutive VEGFR2 signaling in infantile hemangioma. Nat Med 2008: 14:1236-1246.

25. Honda N, Jinnin M, Kajihara I et al. TGF-β-mediated downregulation of microRNA-196a contributes to the constitutive upregulated type I collagen expression in scleroderma dermal fibroblasts. J Immunol 2012:188:3323-3331.

26. Kajihara I, Jinnin M, Honda N et al. Scleroderma dermal fibroblasts overexpress vascular endothelial growth factor due to autocrine transforming growth factor β signaling. Mod Rheumatol 2013:23:516-524.

27. Ghatak S, Bogatkevich GS, Atnelishvili I, et al. Overexpression of c-Met and CD44v6 receptors contributes to autocrine TGF-β1 signaling in interstitial lung disease. J Biol Chem 2014:289:7856-7872.

28. Wang Z, Jinnin M, Kudo H et al. Detection of hair-microRNAs as the novel potent biomarker: evaluation of the usefulness for the diagnosis of scleroderma. J Dermatol Sci 2013:72:134-141.

29. Song J, Kim D, Han J et al. PBMC and exosome-derived Hotair is a critical regulator and potent marker for rheumatoid arthritis. Clin Exp Med 2015:15:121-126.

30. Weakley SM, Wang H, Yao Q, Chen C. Expression and function of a large non-coding RNA gene XIST in human cancer. World J Surg 2011:35:1751-1756.

31. Griseri P, Pagès G. Regulation of the mRNA half-life in breast cancer. World J Clin Oncol 2014:5:323-334.

32. Kucich U, Rosenbloom JC, Shen G et al. Requirement for geranylgeranyl transferase I and acyl transferase in the TGF-β-stimulated pathway leading to elastin mRNA stabilization. Biochem Biophys Res Commun 1998:252:111-116.

33. Kucich U, Rosenbloom JC, Shen G et al. TGF-β1 stimulation of fibronectin transcription in cultured human lung fibroblasts requires active geranylgeranyl transferase I,

phosphatidylcholine-specific phospholipase C, protein kinase C-δ, and p38, but not erk1/erk2. Arch Biochem Biophys 2000:374:313-324.

LEGENDS

Figure 1. The expression of TSIX in SSc skin.

(a) The levels of lncRNA expression in skin tissues of normal subjects (NS, n=5), SLE (n=3), diffuse cutaneous SSc (D, n=8) and limited cutaneous SSc (L, n=5) were determined by real-time PCR. Relative lncRNA levels normalized by GAPDH levels are shown on the ordinate. Bars show means. The mean value in NS was set at 1. *$P<0.05$* by Mann-Whitney U test.

(b) (left) In situ detection of TSIX in paraffin-embedded, formalin-fixed tissues of female NS skin and dcSSc skin. Nucleus was counterstained with nuclear fast red. TSIX stained blue. The arrow indicates cells positive for TSIX staining. One experiment representative of three independent experiments is shown. (right) The number of TSIX-positive cells by in situ hybridization was counted at five high-power fields in the skin specimens from three female NS and dcSSc skin. *$p < 0.05$ as compared with the values in NS skin.

(c) Human dermal fibroblasts cultured from female NS and dcSSc patients were incubated with or without TGF-β1 (2ng/ml) for 12 hours, and total RNA was extracted. Quantitative real-time PCR determining mRNA expression of TSIX was performed. Data are expressed as scatter plots (n=3). The mean value in untreated NS fibroblasts was set at 1. *$P<0.05$ compared to untreated fibroblasts.

(d) Female dcSSc fibroblasts were transfected with control or TGF-β1 siRNA for 96 hours. The mRNA levels of TGF-β1 (white circles) and TSIX (black circles) were quantitated by real-time PCR (n=3). The mean values in control fibroblasts were set at 1.*$P<0.05$.

Figure 2. Role of TSIX in collagen regulation of NS and SSc fibroblasts.

(a) SSc skin was divided into three groups: low (n=5), medium (n=4), and high (n=4) TSIX expression. α1(I) and α2(I) collagen mRNA levels in each groups were determined by real-time PCR. The mean values in low TSIX expression group were set at 1. *$P<0.05$* compared to low TSIX expression group by Mann-Whitney U test.

(b) Female NS and dcSSc fibroblasts were cultured independently under the same conditions. Cell lysates were subjected to immunoblotting with antibody for type I collagen. The same

membrane was reprobed with anti-β-actin antibody as a loading control. One experiment representative of three independent experiments is shown.

(c) Female NS and dcSSc fibroblasts were transfected with control or TSIX siRNA. The mRNA levels of indicated genes were quantitated by real-time PCR (n=3). The mean values in control fibroblasts were set at 1. *P<0.05.

(d) (Upper panels) Lysates from female NS and dcSSc fibroblasts treated with or without TSIX siRNA were subjected to immunoblotting with antibody against type I collagen or β-actin. (Lower panels) Protein levels of type I collagen quantitated by scanning densitometry and corrected for β-actin levels in the same samples are shown relative to the level in cells transfected with control siRNA (n=3). The mean values in control fibroblasts were set at 1. *P<0.05.

(e) Female NS and dcSSc fibroblasts were transfected with control or TSIX siRNA. The mRNA levels of indicated genes were quantitated by real-time PCR (n=3).

Figure 3. The mechanism of TSIX-mediated collagen expression.

(a) Female NS (left panels) and dcSSc (right panels) fibroblasts were incubated in the presence or absence of TSIX siRNA for 48 hours before the addition with 2.5μg/ml actinomycin D for 6 or 12 hours. mRNA expression of α1 (upper panels) and α2(I) collagen (lower panels) was analyzed by real-time PCR (n=3). The values were expressed as a percentage of the value at time 0 and plotted on a scale. The solid line indicates mean levels in fibroblasts transfected with control siRNA, and the dotted line indicates those in cells with TSIX siRNA. *P<0.05.

(b) Female NS (left panels) and dcSSc (right panels) fibroblasts were incubated in the presence or absence of TSIX siRNA for 72 hours before the addition of cycloheximide (10μg/ml). Cells were harvested at 2 or 4 hours after cycloheximide was administrated, and immunoblotting using antibody for type I collagen and β-actin was performed. A representative result of three independent experiments is shown. The levels of type I collagen quantitated by scanning densitometry was expressed as a percentage of the value at time 0 and plotted on a scale (n=3).

Figure 4. Serum TSIX levels in patients with rheumatic diseases.

(a) Serum TSIX expression in the cultured media obtained from female NS and dcSSc fibroblast were measured by quantitative real-time PCR (n=3). The mean value in NS fibroblasts was set at 1. *P<0.05.

(b) TSIX gene was present in serum sample. Serial dilution of cDNA (as is, 10-fold dilution, 100-fold dilation and 0) synthesized from serum RNAs was used as template for real-time PCR. Amplification curves of gene-specific transcripts are presented to illustrate the process of exponential increase of fluorescence.
(c) Serum TSIX expression measured by quantitative real-time PCR in patients with SLE (n=8), SSD (n=10) or SSc (n=36), and in normal subjects (NS, n=12) are shown. SSc patients were divided into dcSSc (D, n=13) and lcSSc (L n=23). Relative TSIX levels normalized by the levels of GAPDH are shown on the ordinate. The horizontal bars in each group show the mean values. The mean values in NS were set at 1. Dotted line indicates mean+2SD of the values in NS. *P<0.05.

Figure 1

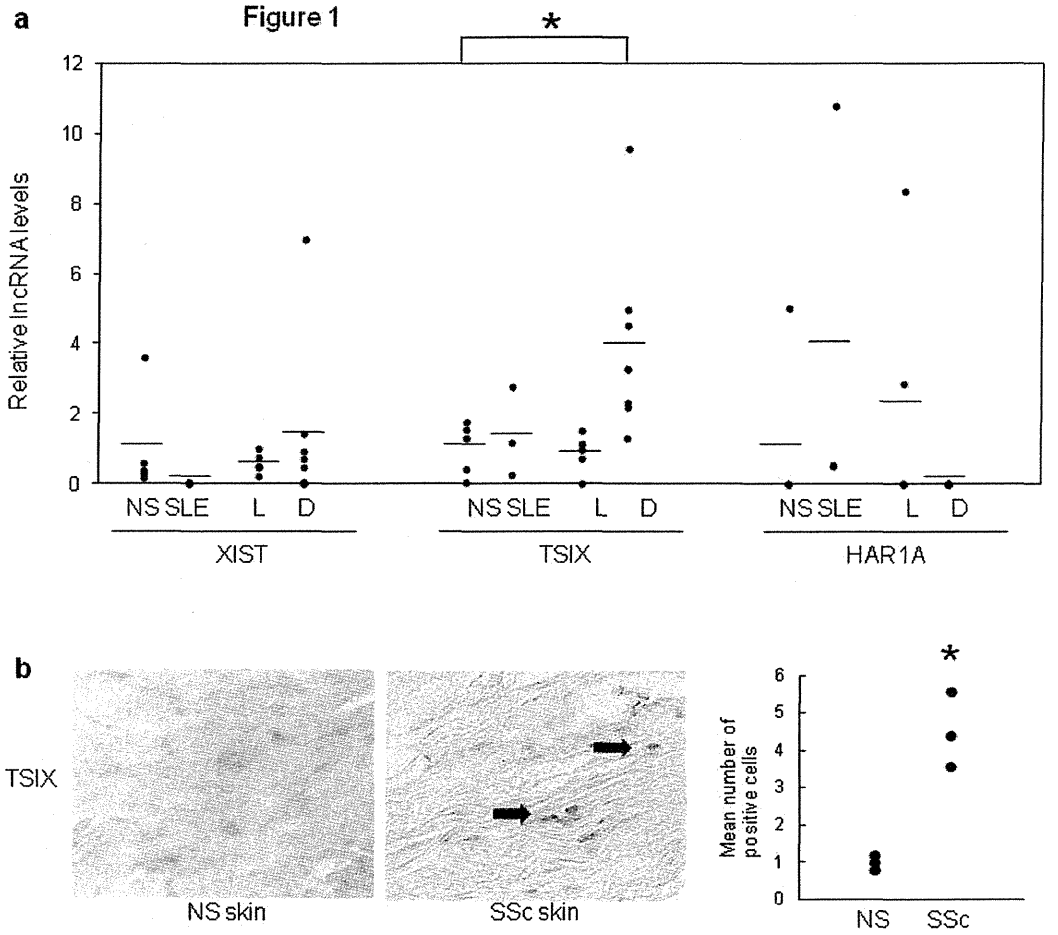


Figure 2

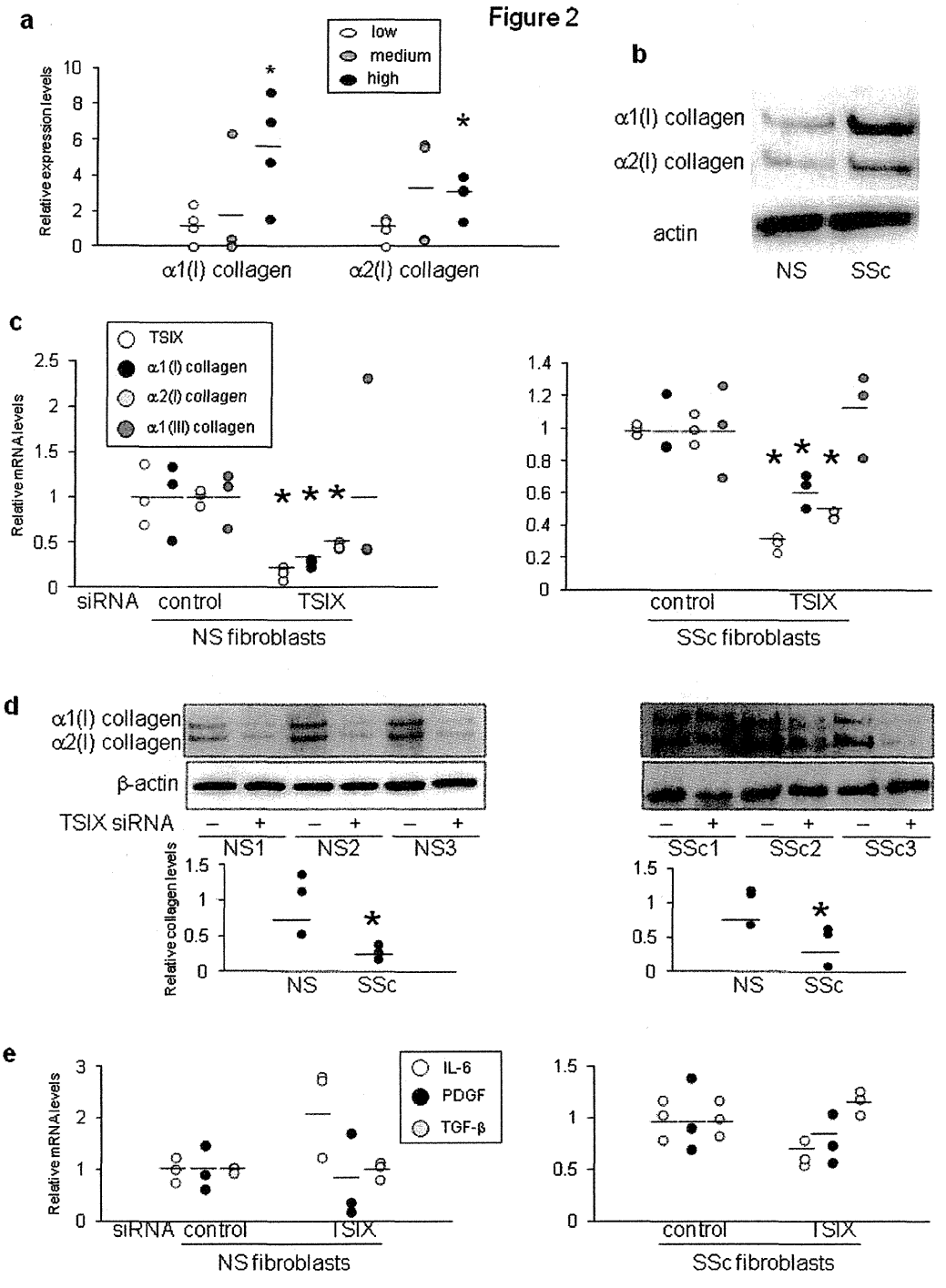


Figure 3

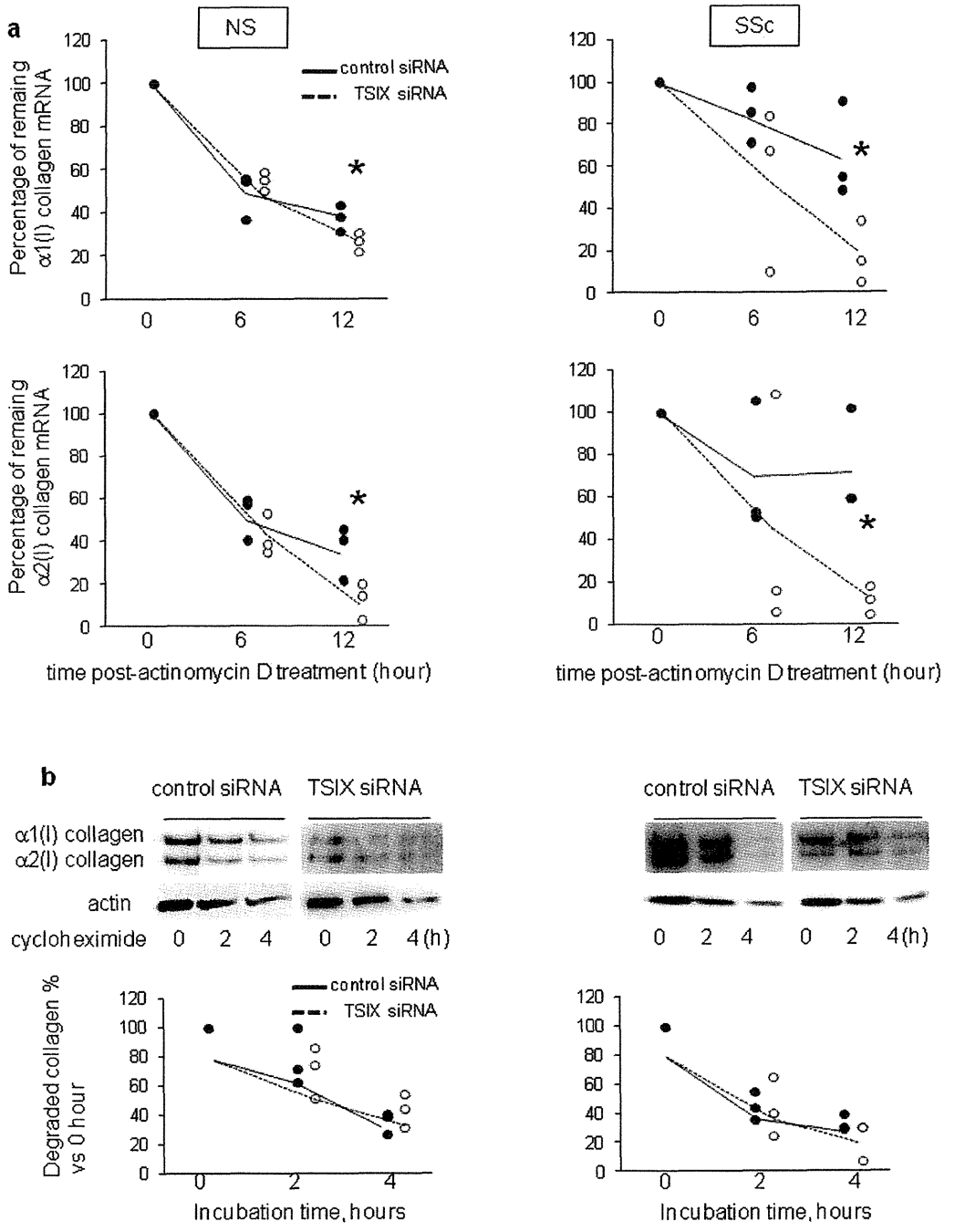
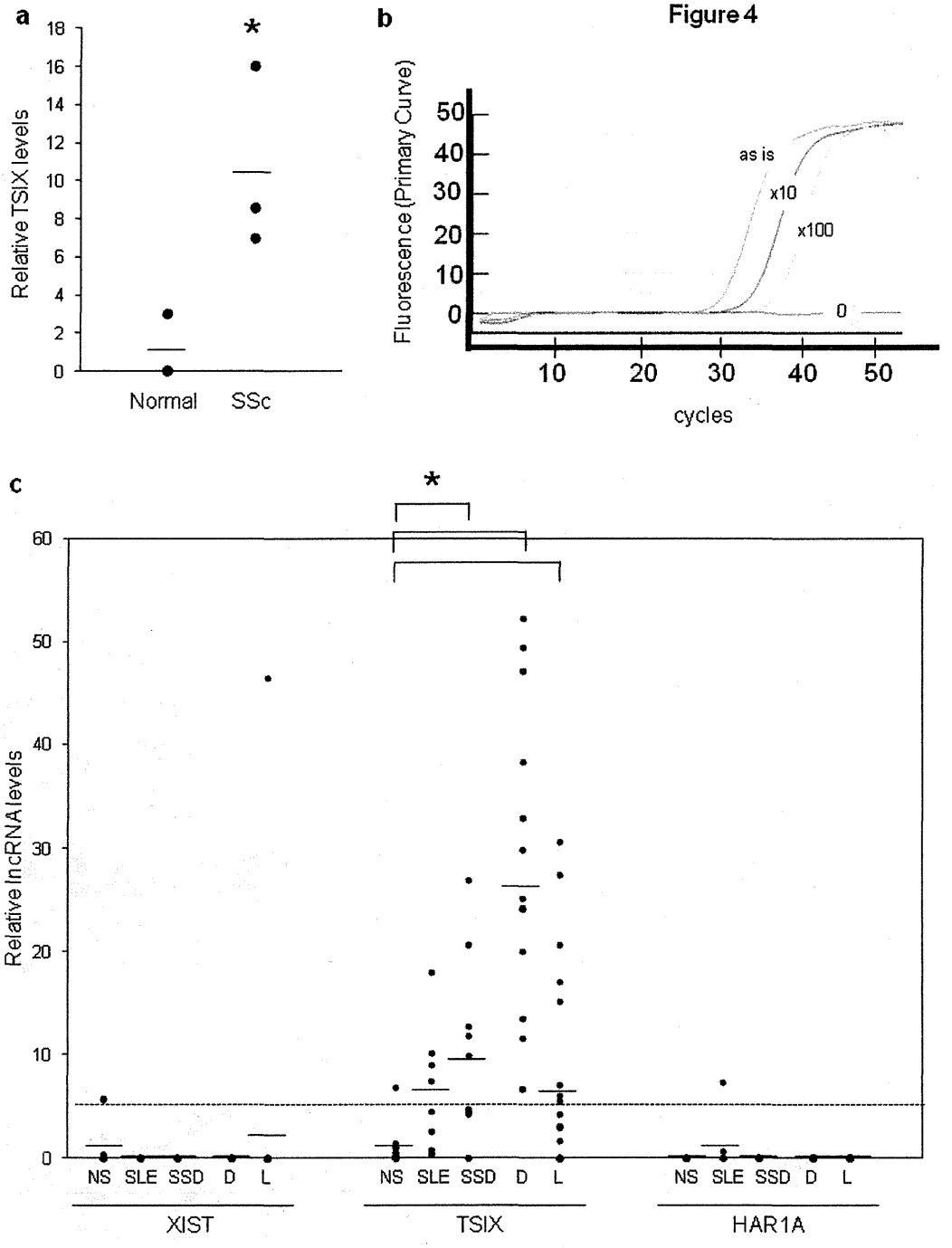


Figure 4



Received Date: 23-Jul-2015

Revised Date: 15-Dec-2015

Accepted Date: 22-Dec-2015

Article Type: Original Article (BJD)

The role of PSMB9 up-regulated by interferon signature in the pathophysiology of cutaneous lesions of dermatomyositis and lupus erythematosus

2) The running head: PSMB9 up-regulation in DM and LE skin

4) K. Nakamura*, M. Jinnin*, H. Kudo*, K. Inoue*, W. Nakayama*, N. Honda*, I. Kajihara*, S. Masuguchi*, S. Fukushima*, H. Ihn*

5) * Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan.

6) Corresponding author:
Masatoshi Jinnin MD, PhD, Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, Honjo 1-1-1, Kumamoto 860-8556, Japan.
Tel +81 96 373 5233
Fax +81 96 373 5235
E-mail: mjin@kumamoto-u.ac.jp

7) This study was supported in part by project research on intractable diseases from the Japanese Ministry of Health, Labour and Welfare.

8) All authors state no conflict of interest.

9) What's already known about this topic?

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bjd.14385

- Dermatomyositis and systemic lupus erythematosus have common skin features, including dermal mucin deposition and interferon signature.

What does this study add?

- PSMB9 and versican V1, a core protein for glycosaminoglycan, were up-regulated while type I collagen was down-regulated in both DM and SLE skin.
- Interferon signature reduces collagen in dermal fibroblasts of DM and SLE skin, while PSMB9 overexpressed by interferon induces TGF-β2/β3 in epidermal keratinocytes, which results in the versican overexpression in dermal fibroblasts.
- The TGF-β2-ΔDiHS-diS1 pathway is the specific molecular changes to DM skin.

Summary

Background

Dermatomyositis (DM) and systemic lupus erythematosus (SLE) have common skin features, including dermal mucin deposition and interferon signature, although their roles are unknown.

Objective

To identify common or specific molecular changes in DM and SLE skin.

Methods

Proteomic analysis was performed utilizing DM and normal subject (NS) skin. Glycosaminoglycans were analyzed by high-performance liquid chromatography.

Results

The expressions of 61 proteins were upregulated or downregulated in DM skin compared to NS skin in the proteomic analysis. Among those proteins, PSMB9, an immunoproteasome subunit, was up-regulated in the epidermis of DM and SLE, but not in other skin diseases. Furthermore, versican V1, a core protein for glycosaminoglycans, was upregulated while type I collagen was downregulated in the dermis of DM and SLE. Interferon stimulated PSMB9 expression in cultured keratinocytes and reduced collagen expression in dermal fibroblasts, but did not affected on versican expression. The PSMB9 knockdown in keratinocytes led to the significant suppression of TGF-β2 and TGF-β3, inducers of versican synthesis. TGF-β3 expression was upregulated in both DM and SLE, while TGF-β2 expression was increased only in the DM epidermis. ΔDiHS-diS1, a component of heparan sulfate, was significantly increased only in DM. TGF-β2 expression significantly increased the ΔDiHS-diS1 expression in dermal fibroblasts in vitro.

Conclusions

The interferon signature in DM and SLE skin reduces collagen in dermal fibroblasts, whereas overexpression of PSMB9 induced by interferon stimulates versican inducers in epidermal keratinocytes. In addition, the TGF-β2-ΔDiHS-diS1 pathway may be the specific molecular change of the skin with DM.

Introduction

Dermatomyositis (DM) and systemic lupus erythematosus (SLE) are autoimmune disorders characterised by acute or chronic inflammation. They have several common features in the skin, including erythematous eruptions, photosensitivity and the Köbner phenomenon.[1-3] Recent studies have indicated that the gene expression profiles in DM and SLE patients are remarkably similar. For example, interferon (IFN)-related genes were reported to be overexpressed in the skin, muscle, and peripheral blood of DM and SLE patients.[4,5] Although IFN may be a key signal in the pathogeneses of both DM and SLE skin, the specific targets of the signaling have yet to be elucidated.

The histopathological findings from skin with DM and SLE include liquefaction degeneration of basal layer, mild infiltration of lymphocytes, and dermal mucin deposition,[6,7] but these findings are not specific to these diseases. Glycosaminoglycans (GAGs), histologically known as dermal mucin, usually exist by binding with core proteins, and the resulting complex is called proteoglycan. Excess mucin deposition in the skin with DM and SLE is speculated to be caused by increased GAGs production by dermal fibroblasts after some immunological stimulation or by decreased hyaluronidase-mediated resorption.[8] However, the detailed mechanism is still unknown.

Accordingly, DM and SLE are sometimes difficult to clinically and histopathologically distinguish in terms of cutaneous eruptions. Several previous reports attempted to identify specific changes in each disease, including microvasculopathy or immunocomplex deposition, but such candidates have not been clinically utilised. Compared with the many research studies that used muscle tissues, the kidney, or peripheral blood,[5,9,10] the number of researches that examined the skin of DM or SLE patients is limited. The aim of present study was to identify common molecular changes of the skin with DM and SLE, or specific molecular changes of the skin with DM, especially focusing on IFN signaling and mucin deposition.