Biochemistry and Biophysics Reports 7 (2016) 246–252

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Transforming growth factor-β-induced CUX1 isoforms are associated with fibrosis in systemic sclerosis lung fibroblasts

Tetsurou Ikeda [a,b,c,1,*], Maria Fragiadaki [b], Xu Shi-wen [a], Markella Ponticos [a], Korsa Khan [a], Christopher Denton [a], Patricia Garcia [a], George Bou-Gharios [b], Akio Yamakawa [c], Chikao Morimoto [c], David Abraham [a]

[a] Royal Free and University College Medical School, London, UK
[b] Imperial College School of Medicine, London, UK
[c] University of Tokyo, Institute of Medical Science, Tokyo, Japan

ARTICLE INFO

Article history:
Received 8 April 2016
Received in revised form
23 June 2016
Accepted 24 June 2016
Available online 2 July 2016

Keywords:
Transforming growth factor-β
CUX1 isoforms
Fibrosis
Cathepsin L inhibitor

ABSTRACT

In the enhancer region of the human type I collagen alpha 2 (*COL1A2*) gene, we identified cis-elements for the transcription factor CUX1. However, the role of CUX1 in fibrosis remains unclear. Here we investigated the role of CUX1 in the regulation of COL1 expression and delineated the mechanisms underlying the regulation of *COL1A2* expression by CUX1 in systemic sclerosis (SSc) lung fibroblasts. The binding of CUX1 to the *COL1A2* enhancer region was assessed using electrophoretic mobility shift assays after treatment with transforming growth factor (TGF)-β. Subsequently, the protein expression levels of CUX1 isoforms were determined using Western blotting. Finally, the expression levels of COL1 and fibrosis-related cytokines, including CTGF, ET-1, Wnt1 and β-catenin were determined. The binding of CUX1 isoforms to the *COL1A2* enhancer region increased after TGF-β treatment. TGF-β also increased the protein levels of the CUX1 isoforms p200, p150, p110, p75, p30 and p28. Moreover, SSc lung fibroblasts showed higher levels of CUX1 isoforms than normal lung fibroblasts, and treatment of SSc lung fibroblasts with a cathepsin L inhibitor (IW-CHO) decreased COL1 protein expression and reduced cell size, as measured using immunocytochemistry. In SSc and diffuse alveolar damage lung tissue sections, CUX1 localised within α-smooth muscle actin-positive cells. Our results suggested that CUX1 isoforms play vital roles in connective tissue deposition during wound repair and fibrosis.

1. Introduction

Scleroderma, or systemic sclerosis (SSc), is a connective tissue disease characterised by excessive type I collagen (COL1) deposition in many organs including the lungs, kidneys and skin [1]. Approximately 40% patients with diffuse SSc experience pulmonary fibrosis, a major cause of mortality [1]. Although the aetiology of this disease is poorly understood, tissue scarring occurs after injury following the release of several mediators including TGF-β, PDGF, CTGF, Wnt1 and ET-1 [2–6]. These factors stimulate fibroblasts to differentiate into myofibroblasts, which are characterised by the expression of α-SMA and excessive production of extracellular matrix molecules such as COL1 [7,8]. Overexpression of these cytokines results in chronic fibroblast stimulation (fibrosis). Moreover, genetically modified mice with altered TGF-β signalling have been reported to exhibit fibrogenic pathology similar to that observed in patients with SSc, indicating a key role of this cytokine in the pathogenesis of fibrosis [9–11]. Elucidation of pivotal mediators or key signalling pathways that are overactive in fibrosis is crucial for designing better therapeutic strategies for SSc and related disorders.

TGF-β is a potent pro-fibrotic cytokine that promotes myofibroblast differentiation, migration, extracellular matrix synthesis and apoptosis resistance [12–14]. TGF-β induces the expression of the gene encoding human collagen type I alpha 2 (*COL1A2*) via a Smad-dependent pathway that acts on a TGF-β responsive element (TbRE) in the human *COL1A2* proximal promoter [15,16]. COL1 expression *in vivo* is exclusively controlled by an enhancer sequence that contains several DNase I hypersensitive sites (HSs). These encompass pivotal regulatory sites conferring tissue-, temporal-, cell- and growth factor-specific expression of COL1 [15,16].

Abbreviations: TGF-β, transforming growth factor-β; CTGF, connective tissue growth factor; ET-1, endothelin-1; Wnt1, wingless-type MMTV integration site family member 1; PDGF, platelet-derived growth factor; α-SMA, α-smooth muscle actin

* Correspondence to: Clinical Immunology, Institute of Medical Science, University of Tokyo, Tokyo, 4-6-1, Shirokanedai, Minato-ku, Japan.
E-mail address: ikedatetsurou@kochi-u.ac.jp (T. Ikeda).
[1] Present, Kochi Medical School, Kochi, Japan.

http://dx.doi.org/10.1016/j.bbrep.2016.06.022

The results of our recent study illustrated that TGF-β activates *COL1A2* via a non-canonical Smad-independent pathway, which requires enhancer/promoter cooperation. Moreover, we identified a novel TbRE in the human *COL1A2* enhancer region and found that it is necessary for *COL1A2* activation [16]. Further, we reported that high doses of TGF-β increased CUX1 binding in the proximal promoter and suppressed COL1 expression [17].

In this study, we identified CUX1 binding sites near this TbRE of the *COL1A2* enhancer region using *in silico* analyses. These sites exist at identity island 4 (IS4) near HS4 in the enhancer region [18] In addition, this study demonstrated that CUX1 responds to TGF-β stimulation and is a potential activator of COL1. Based on these findings, we characterised the role of human CUX1 in the regulation of COL1 expression and subsequent release of pro-fibrotic cytokines. We demonstrated that some isoforms of human CUX1 are strongly induced after TGF-β stimulation, which results in the up-regulation of CTGF, Wnt1, ET-1, β-catenin and COL1 *in vitro*. In addition, we investigated the expression pattern of human CUX1 isoforms in lung fibroblasts derived from normal cells and patients with SSc. In lung fibroblasts derived from patients with SSc, protein levels of the CUX1 isoforms p200, p150, p110, p75, p30 and p28 were increased compared with those in normal cells. Using electrophoretic mobility shift assays (EMSAs) with nuclear extracts from these cells, we demonstrated that CUX1 binding to the *COL1A2* enhancer region was increased. Cleavage sites for cathepsin L have been found between CR1 and CR2 and those for caspases have been identified between CR3 and HR of CUX1 [19]. Therefore, we confirmed whether cathepsin L inhibitor (IW-CHO) can regulate COL1. IW-CHO inhibited COL1 in both normal and SSc lung fibroblasts. Taken together, our data strongly suggest that CUX1 isoforms up-regulate COL1 and regulate key pro-fibrotic activities of TGF-β. Our results could provide novel insights into CUX1-mediated regulation of *COL1A2* in human lung fibroblasts. These results are an important contribution to the understanding of processes involved in SSc-associated fibrosis.

2. Materials and methods

2.1. Cell culture

Cells were maintained in DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin and cultured in a humidified atmosphere of 5% CO_2. We isolated lung fibroblasts as previously described [20]. The cells were cultured under standard conditions in DMEM containing 10% FBS. Pulmonary fibroblasts were obtained from patients who fulfilled the criteria of the American College of Rheumatology for the diagnosis of SSc with lung involvement. Informed consent and ethical approval were obtained. None of the patients was receiving immunosuppressive medication or corticosteroids at the time of biopsy.

2.2. Western blotting

Nuclear extracts and cytosolic fractions were prepared as previously described [16]. The cells were washed with phosphate-buffered saline (PBS) and treated with Laemmli sample buffer. To analyse collagen, the medium was removed and adjusted to 20% (v/v) ammonium sulphate, followed by incubation at 4 °C overnight. The samples were centrifuged, and the pellet was re-suspended in Laemmli sample buffer with β-mercaptoethanol for SDS-PAGE, followed by Western blotting. The following antibodies were used: lamin A/C (sc20681, Santa Cruz), COL1 (rabbit anti-mouse collagen 1, ref 20151, Novotec), ET-1 (T4049, Peninsula), CUX1 (M-222, sc-13024, Santa Cruz), CTGF (L-20, sc14939, Santa Cruz), GAPDH (ab8245-100, Abcam), β-catenin (ab6302-100, Abcam) and Wnt1 (anti-human WNT1 S#500-p250, PeproTech, Inc.).

N-(1-naphthalenylsulfonyl)-Ile-Trp-aldehyde (IW-CHO, 0.2 μM, Alexis Biochemicals) was used as IW-CHO. TGF-β (R&D Systems) was used at 4 ng/ml. The cells were serum-starved for 12 h and incubated with or without TGF-β at 4 ng/ml for a further 24 h.

2.3. Plasmid transfection

Cells were seeded in six-well plates before transfection and 24 h later were transfected using FuGENE 6 (Roche, Basel, Switzerland), according to the manufacturer's instructions; this method was used to transfect sh-CUX1 vector as described previously [17].

2.4. EMSAs

Nuclear extracts were prepared from fibroblasts obtained from patients with SSc using a previously described method [16]. Briefly, double-stranded oligonucleotides were synthesised, end-labelled with ^{32}P-gamma-ATP using T4 kinase and used in binding reactions with nuclear extracts from the cells. Competition was performed with unlabelled oligonucleotides (1.75 pmol/μl). Oligonucleotides for the CUX1-19.51 kb probe (5′-attggcagtgagttacttagta-3′) were used. We employed the standard conditions recommended by the kit manufacturer (Promega) and separated the reaction on a 4% polyacrylamide gel at 4 °C and 160 V. Consensus oligonucleotides for CUX1 (CDP, sc2593, Santa Cruz) and SP1 (sc-2502, Santa Cruz) were used.

2.5. Immunocytochemistry

The cells were washed in ice-cold PBS twice and fixed with 3% paraformaldehyde in PBS for 15 min at room temperature. The cells were blocked with 3% BSA for 30 min at 25 °C, followed by incubation with CUX1 antibody (0.2 mg/ml) and monoclonal anti-α-SMA clone 1a Cy3-conjugated antibody (0.7 mg/ml) (Sigma-Aldrich) overnight at 4 °C. Subsequently, the cells were washed with ice-cold PBS twice, incubated with an Alexa 488 secondary antibody overnight at 4 °C, washed with ice-cold PBS twice and incubated with DAPI (Roche) for 30 min at room temperature. Slides were viewed and photographed using a Zeiss Axioscope light microscope (Carl Zeiss, Göttingen, Germany) with Axiovision software.

2.6. Immunohistochemistry

For formalin-fixed paraffin-embedded specimens, tissues were fixed and hydrated as previously described [10]. For immunohistochemistry, sections were pre-treated with methanol (VWR, Lutterworth, UK), followed by antigen retrieval in heated 10 mM citrate buffer (pH 6). The primary antibodies were CUX1 antibody (0.2 mg/ml) and monoclonal anti-α-SMA clone 1a Cy3-conjugated antibody (0.7 mg/ml). Sections were sequentially incubated with a 1/200 dilution of an Alexa 488 secondary antibody. Slides were viewed and photographed using a Zeiss Axioscope light microscope with Axiovision software.

2.7. Data analysis

Data are expressed as the mean ± S.E of at least three independent experiments. Statistical analysis was performed using Student's *t*-test. Values of $P < 0.05$ were considered significant.

3. Results

3.1. CUX1 binds to the enhancer region of human COL1A2

The CUX1 cis-element CCAAT (Fig. 1A) was identified at IS4 near enhancer-containing DNase I HS4 using *in silico* analyses. Cut repeats and homeodomains bind to this cis-element (Fig. 1A) [21]. To determine whether CUX1 interacts with the enhancer region of human *COL1A2*, we performed EMSAs using transcription factor-binding consensus oligonucleotides, CUX1 antibody and sh-CUX1. In these experiments, we not only verified the binding of CUX1 at CUX1 (−19.51 kb) probe sites but also demonstrated the binding of several other transcription factors, including JunD (data not shown). Nuclear extracts from normal lung fibroblasts incubated with or without TGF-β were used, and CUX1 consensus oligonucleotides, CUX1 antibody and sh-CUX1 decreased the intensity of CUX1 bands (Fig. 1B). Furthermore, the binding of CUX1 at CUX1 (−19.51 kb) probe sites increased post-TGF-β treatment in both normal and SSc lung fibroblasts (Fig. 2A).

3.2. TGF-β up-regulates the expression of CUX1 isoforms

CUX1, also known as CCAAT displacement protein (CDP), CUTL1, CUT or Cux, belongs to a family of homeobox transcription factors that are involved in the regulation of cell growth and differentiation [22]. It is evolutionarily conserved and comprises four DNA binding domains, three of which are known as Cut repeats and one is known as the Cut homeodomain [21]. CUX1 mainly acts as a transcriptional repressor, but it can also act as an activator [23–25]. Transgenic mice with CUX1 overexpression have been reported to exhibit multi-organ hyperplasia and organomegaly [26]. Moreover, the CUX1 isoforms p200 and p110 can regulate motility and invasiveness through a TGF-β-dependent mechanism [27,28]. However, the role of CUX1 isoforms in the development of fibrosis remains unclear. We performed Western blotting to determine whether the expression levels of CUX1 isoforms were induced by TGF-β. We found that the expression levels of CUX1 isoforms significantly increased in both normal and SSc lung fibroblasts post-TGF-β treatment (Fig. 2B, C). TGF-β treatment resulted in the induction of p200 and p150 in normal lung fibroblasts. Moreover, TGF-β treatment resulted in the induction of p200, p150, p110, p75, p30 and p28 in SSc lung fibroblasts. These results suggested that TGF-β stimulate the expression of CUX1 isoforms. We also verified whether the expression levels of pro-fibrotic proteins were up-regulated. ET-1, COL1, Wnt1, CTGF and β-catenin levels increased post-TGF-β treatment in normal and SSc lung fibroblasts (Fig. 2D).

3.3. Effects of cathepsin L inhibition on lung fibroblasts derived from patients with SSc

Several studies have illustrated that CUX1 is cleaved into p150, P110, p75 and other subunits by nuclear cathepsin L. To confirm the effects of cathepsin L inhibition on SSc lung fibroblasts, we measured the expression levels of COL1 in both normal and SSc lung fibroblasts. IW-CHO (0.2 μM) was used because its IC50 is 1.9 nM. IW-CHO decreased the expression levels of COL1 in normal and SSc lung fibroblasts (Fig. 2E). To further confirm the effects of cathepsin L on SSc lung fibroblasts, we used immunocytochemistry to study CUX1 localization in these

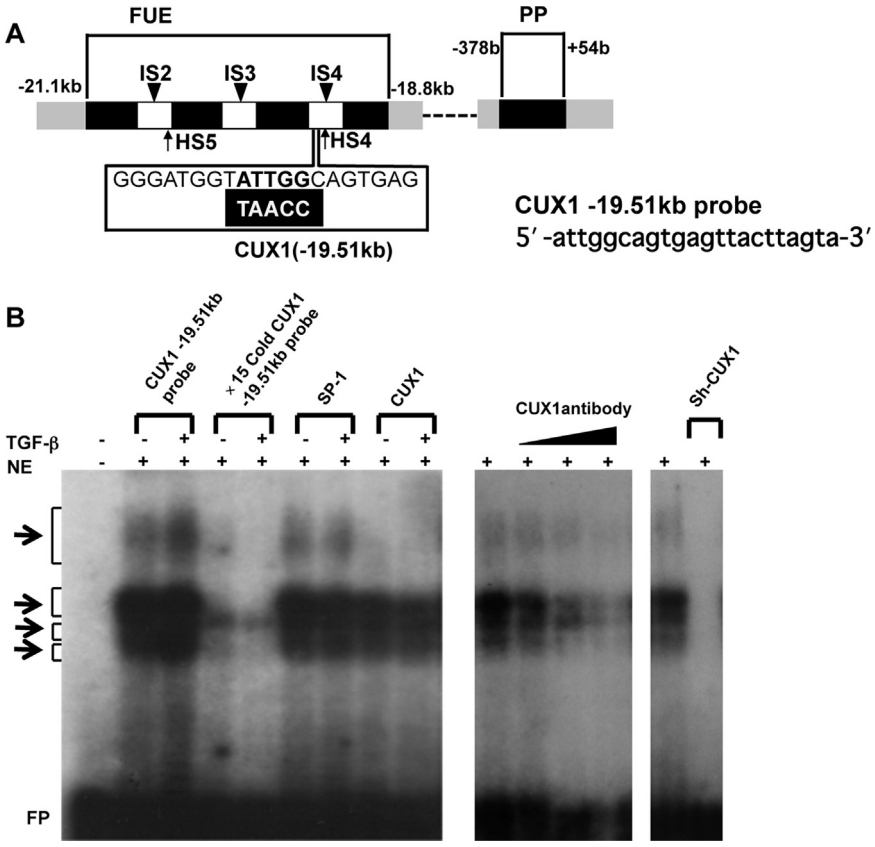

Fig. 1. Electrophoretic mobility shift assays (EMSAs) revealed that CUX1 binds to the human type I collagen alpha 2 gene (*COL1A2*) enhancer. A. Schematic diagrams of the far upstream enhancer region (FUE) with arrows indicating known DNase hypersensitive sites; the white box indicates the known identity islands of human *COL1A2*. CUX1 cis-elements are indicated by bold letters. B. EMSA using the CUX1 (−19.51 kb) probe. NE, nuclear extract from normal lung fibroblasts. The right-hand graph of Fig. 1B shows several bands detected using P^{32}-labelled oligos in Lanes 2 and 3. Bands were confirmed using a cold competitor. Unlabelled competing oligo probes are shown in Lanes 4 and 5. Consensus oligo for CUX1, Lanes 8–9. Consensus oligo for SP1, Lanes 6–7. Antibody for CUX1, Lanes 11–13. Sh-CUX1, Lanes 15. FP, free probe.

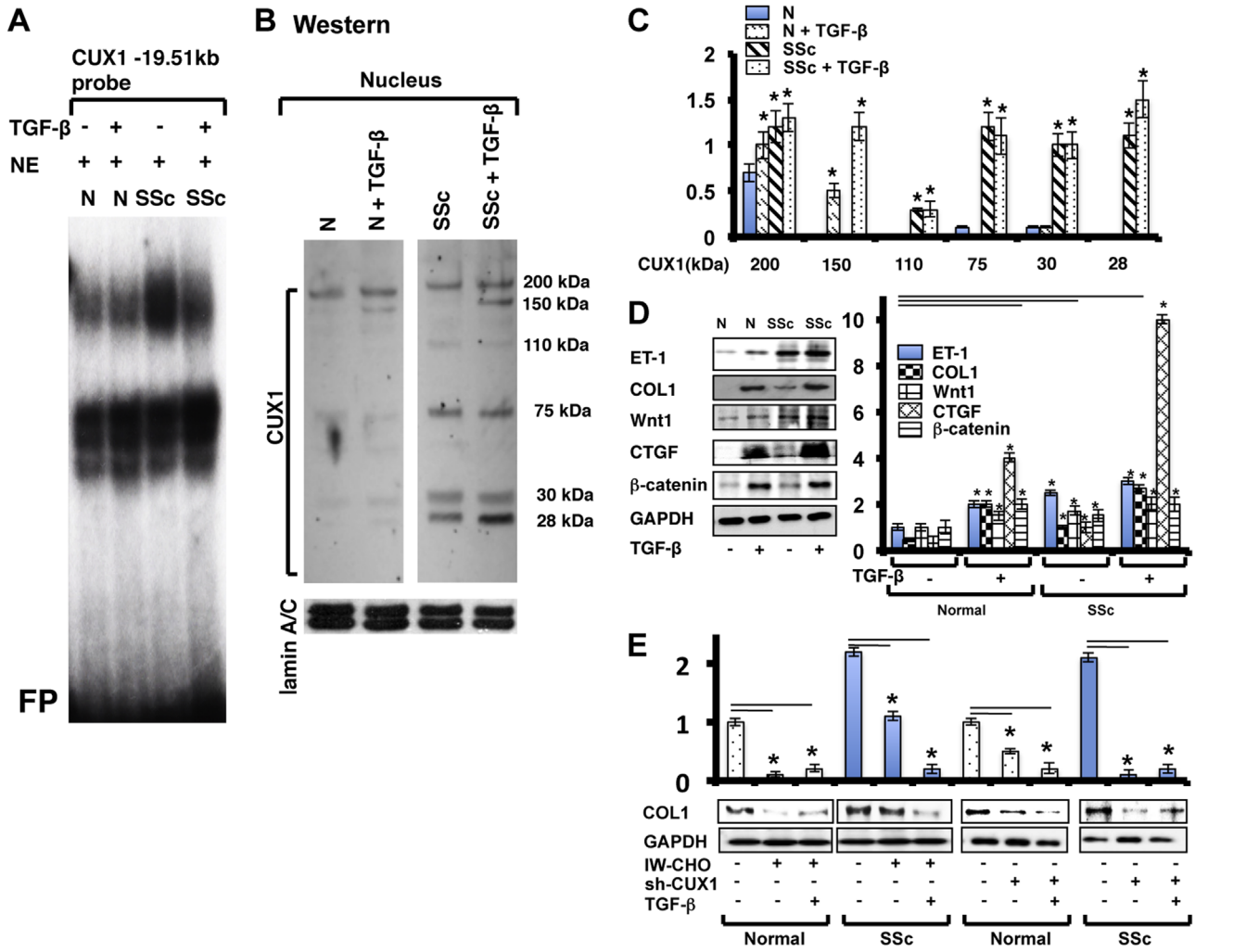

Fig. 2. CUX1 isoforms in normal human and systemic sclerosis (SSc) lung fibroblasts. A. Electrophoretic mobility shift assay using normal lung fibroblast (N) nuclear extract (NE) (Lanes 1–2) and SSc lung fibroblast NE (Lanes 3–4) treated with transforming growth factor (TGF)- β (Lanes 2 and 4) or untreated (Lanes 1 and 3) for the CUX1 (− 19.51 kb) probe. FP, free probe. B. Effects of TGF-β on the expression of CUX1 isoforms as assessed using Western Blotting. TGF-β (4 ng/ml) was added to the fibroblasts after 12 h of serum starvation. Then, the cells were cultured for 24 h. C. Graphs represent the results of Fig. 2B. D. Effects of TGF-β on the expression of collagen type I and fibrosis-related proteins. Several fibrosis-related cytokines and proteins were detected using the cytosolic fraction as well as the medium and NEs from both normal and SSc lung fibroblasts with or without TGF-β treatment. E. Effects of a cathepsin L inhibitor (IW-CHO, 0.2 μM) and sh-CUX1 on the expression of type I collagen (COL1) in normal and SSc lung fibroblasts. Values represent mean ± S.E.M., n = 3. *, significantly different from control, $P < 0.05$ by Student's *t*-test. GAPDH and lamin A/C were used as internal controls.

fibroblasts. As shown in Fig. 3, immunofluorescence analysis indicated that SSc lung fibroblasts were CUX1- and α-SMA-positive, with positivity increasing post-TGF-β treatment. CUX1 localised to the nucleus and cytosol. In addition, SSc lung fibroblasts were larger than normal lung fibroblasts (data not shown). IW-CHO decreased the CUX localization in the nucleus and reduced the large size of SSc lung fibroblasts with or without TGF-β treatment (Fig. 3).

3.4. *Immunohistochemical analyses of SSc and diffuse alveolar damage (DAD) lung tissue sections stained with antibodies against CUX1 and α-SMA*

Immunocytochemistry revealed the presence of α-SMA-positive fibrotic loci (Fig. 4), which is characteristic of patients with SSc and DAD. CUX1 localised at alveolar cells and fibrotic loci. In addition, CUX1 localised within α-SMA-positive cells (Fig. 4, merged).

4. Discussion

This study demonstrated the binding of CUX1 to the IS4 domain of the *COL1A2* enhancer region in both normal and SSc lung fibroblasts. Further, our results demonstrated that TGF-β-induced CUX1 isoforms function as transcriptional activators that increase the expression of COL1 proteins. Previous studies implicated CUX1 in both the activation and suppression of several genes [23–25]. Our previous study found that CUX1 bound to the proximal promoter of *COL1A2* after treatment with high doses of TGF-β [22]. In this study, we provide evidence that CUX1 also plays a major role in *COL1A2* activation both *in vitro* and *ex vivo* via the IS4 domain of the *COL1A2* enhancer.

Our data clearly demonstrated that TGF-β induces the expression of CUX1 isoforms, some of which were also up-regulated in SSc lung fibroblasts. In association with this change, the binding of CUX1 to the enhancer region of human *COL1A2* increased in response to TGF-β treatment in normal and SSc lung fibroblasts. These results suggested that TGF-β up-regulates CUX1 protein expression, resulting in an increased binding of CUX1 to the human *COL1A2* enhancer and COL1 up-regulation.

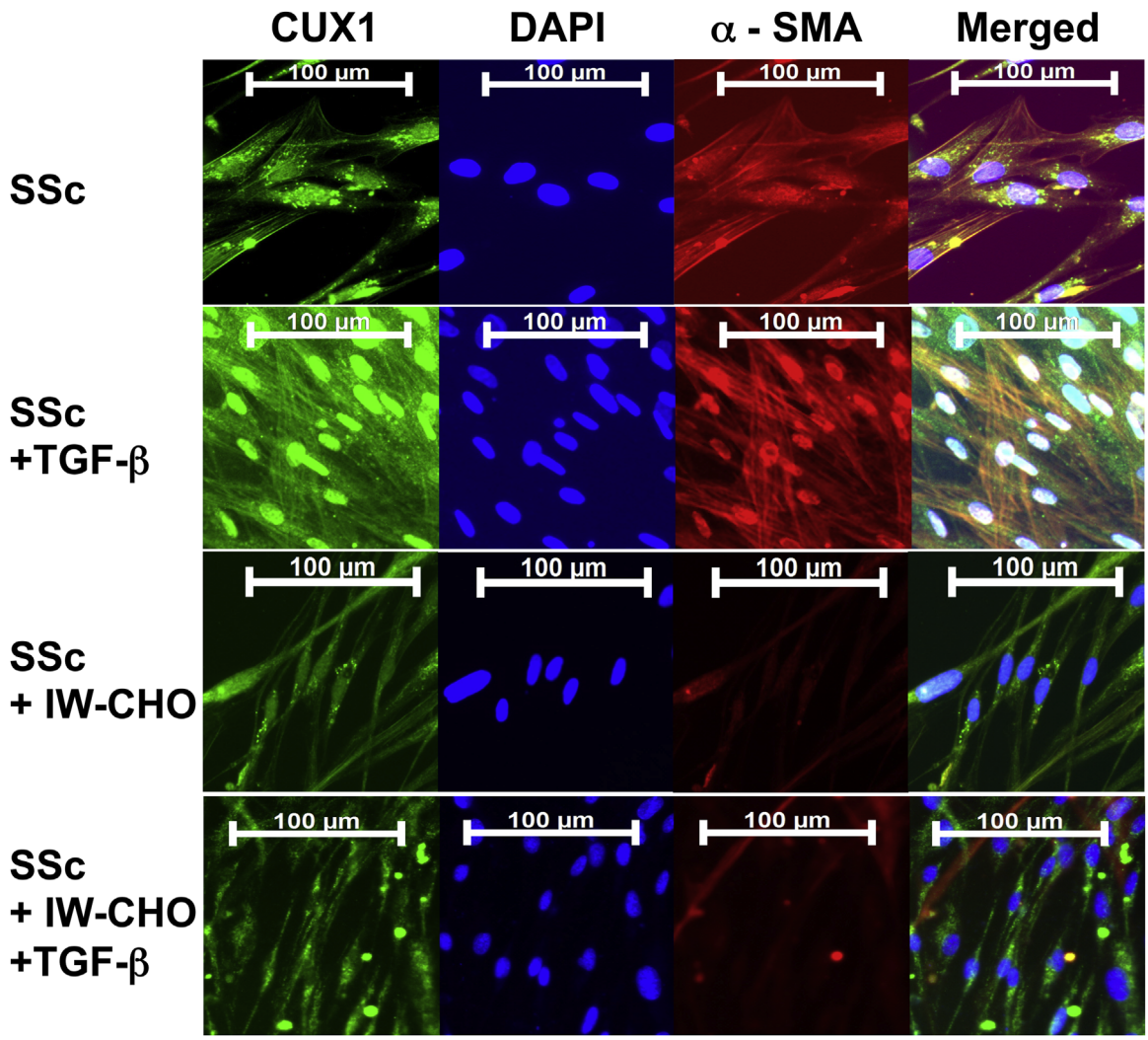

Fig. 3. Effects of IW-CHO on systemic sclerosis (SSc) lung fibroblasts. Immunofluorescence analysis performed using SSc lung fibroblasts treated with or without TGF-β. Cells were stained using CUX1 antibody, DAPI and α-SMA antibody. IW-CHO treatment decreased the CUX localization in the nucleus and reduced the size of lung fibroblasts in patients with SSc. In addition, in the presence of TGF-β, IW-CHO treatment reduced the size of lung fibroblasts in patients.

Another important finding of this study was that TGF-β induces the expression of the CUX1 isoforms p200, p150, p110, p75, p30 and p28 in SSc lung fibroblasts. Cleavage sites have been found for cathepsin L between CR1 and CR2 and for caspase between CR3 and HR of CUX1 [19]. Previous studies have revealed the induction of HA-tagged CUX-1 p200 and p110 in certain cell lines [19,29]. The CUX-1 antibody M-222 simultaneously recognises several CUX1 isoforms in normal and SSc lung fibroblasts following TGF-β treatment. As the molecular weight patterns observed in our study are similar to those previously reported, we believe that all bands observed in this study were specific for CUX1 isoforms.

Our data provide further evidence that CUX1 isoforms may play crucial roles in *COL1A2* regulation. First, the levels of all CUX1 isoforms increased in SSc lung fibroblasts compared with the levels in normal lung fibroblasts. Moreover, compared with the findings in normal lung fibroblasts, data from EMSA revealed significantly increased binding of CUX1 at CUX1 (− 19.51 kb) probe sites of human *COL1A2* in SSc lung fibroblasts. Several reports studies have illustrated that CUX1 isoforms have different DNA binding affinities and functions [19,23–25,30]. Second, COL1 expression decreased by cathepsin L inhibition in normal and SSc lung fibroblasts, highlighting the importance of CUX1 isoforms in fibrosis. Third, CUX1 localised at the nucleus and cytosol (Fig. 3). CUX1 and α-SMA co-localised in the cytosol in SSc lung fibroblasts. IW-CHO decreased the CUX localization in the nucleus and repressed the large size of SSc lung fibroblasts with or without TGF-β treatment (Fig. 3). In addition, in SSc and DAD lung sections, CUX1 localised around fibrotic loci and alveolar cells. Some of these cells were stained by both CUX1 and α-SMA (Fig. 4). Furthermore, in the SSc skin and idiopathic pulmonary fibrosis (IPF) sections, CUX1 localised within and around α-SMA positive cells (See Figs. 1 and 2, reference [31]). These results indicated that CUX1 isoforms are necessary for fibrosis.

In future, investigations on the mutagenesis of CUX1 cleavage sites are required to determine which CUX1 isoforms are crucial. In addition, we need to verify the effects of CUX1 isoforms on the promoter region of α-SMA and pro-fibrotic cytokines. We speculate that some specific features of CUX1 isoforms are important for the activation of human *COL1A2* via the IS4 domain, which is located in the *COL1A2* enhancer.

In summary, our study demonstrated that the up-regulation of CUX1 isoforms is associated with fibrosis *in vitro* in SSc lung fibroblasts as well as in tissue sections from patients with SSc and DAD. Hence, these data indicate that the expression of CUX1 isoforms is possibly important for fibrosis and could be relevant to the pathogenesis of SSc.

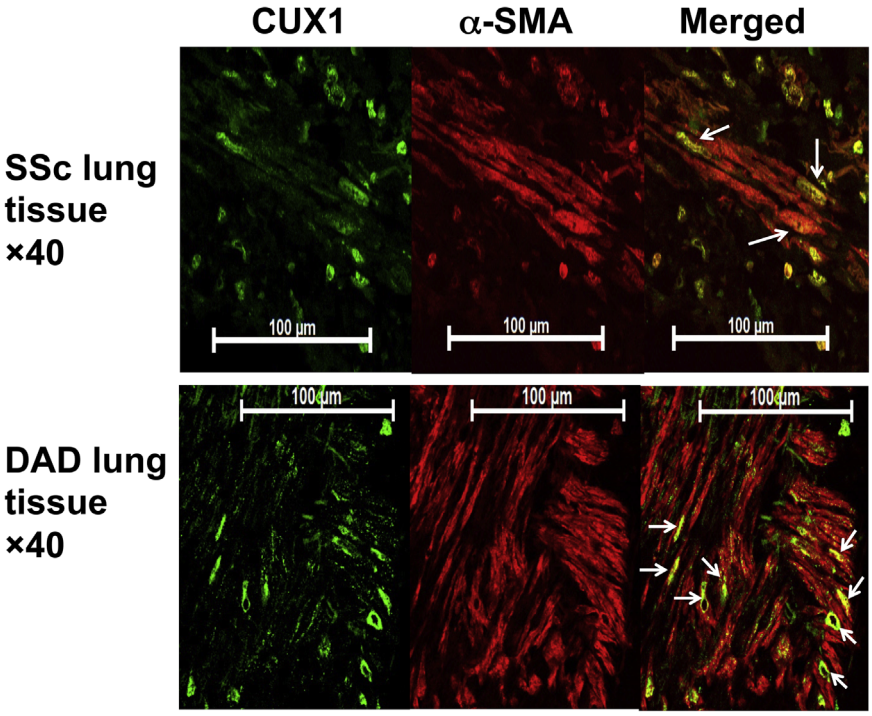

Fig. 4. Systemic sclerosis (SSc) and diffuse alveolar damage (DAD) lung tissue sections stained with antibodies against CUX1 and α-smooth muscle actin (SMA). The figure shows fibrotic loci that were stained by CUX1 and α-SMA antibodies. Alveolar cells around the loci were positive for CUX1 and α-SMA. CUX1 localised within α-SMA-positive cells.

Acknowledgements

This work was supported partly by a grant from the Japan Society for the Promotion of Science (Grant No. 17109011). Further, this work was supported by the Global COE Program 'Center of Education and Research for the Advanced Genome-Based Medicine-For personalized medicine and the control of worldwide infectious disease', MEXT Japan. The authors would like to thank Enago for the English language review.

Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2016.06.022.

References

[1] C.P. Denton, C.M. Black, D.J. Abraham, Mechanisms and consequences of fibrosis in systemic sclerosis, Nat. Clin. Pract. Rheumatol. 2 (2006) 134–144.
[2] X. Shi-Wen, A. Leask, D. Abraham, Regulation and function of connective tissue growth factor/CCN2 in tissue repair, scarring and fibrosis, Cytokine Growth Factor Rev. 19 (2008) 133–144.
[3] S.W. Xu, S.L. Howat, E.A. Renzoni, A. Holmes, J.D. Pearson, M.R. Dashwood, G. Bou-Gharios, C.P. Denton, R.M. du Bois, C.M. Black, A. Leask, D.J. Abraham, Endothelin-1 induces expression of matrix-associated genes in lung fibroblasts through MEK/ERK, J. Biol. Chem. 279 (2004) 23098–23103.
[4] C. Fonseca, G.E. Lindahl, M. Ponticos, P. Sestini, E.A. Renzoni, A.M. Holmes, P. Spagnolo, P. Pantelidis, P. Leoni, N. McHugh, C.J. Stock, X. Shi-Wen, C. P. Denton, C.M. Black, K.I. Welsh, R.M. du Bois, D.J. Abraham, A polymorphism in the CTGF promoter region associated with systemic sclerosis, N. Engl. J. Med. 357 (2007) 1210–1220.
[5] M.L. Whitfield, D.R. Finlay, J.I. Murray, O.G. Troyanskaya, J.T. Chi, A. Pergamenschikov, T.H. McCalmont, P.O. Brown, D. Botstein, M.K. Connolly, Systemic and cell type-specific gene expression patterns in scleroderma skin, Proc. Natl. Acad. Sci. USA 100 (2003) 12319–12324.
[6] J. Yang-Snyder, J.R. Miller, J.D. Brown, C.J. Lai, R.T. Moon, A frizzled homolog functions in a vertebrate Wnt signaling pathway, Curr. Biol. 6 (1996) 1302–1306.
[7] A. Desmouliere, Factors influencing myofibroblast differentiation during wound healing and fibrosis, Cell Biol. Int. 19 (1995) 471–476.
[8] N.K. Harrison, A.C. Argent, R.J. McAnulty, C.M. Black, B. Corrin, G.J. Laurent, Collagen synthesis and degradation by systemic sclerosis lung fibroblasts. Responses to transforming growth factor-beta, Chest 99 (1991) 71S–72S.
[9] C.P. Denton, B. Zheng, L.A. Evans, X. Shi-wen, V.H. Ong, I. Fisher, K. Lazaridis, D. J. Abraham, C.M. Black, B. de Crombrugghe, Fibroblast-specific expression of a kinase-deficient type II transforming growth factor beta (TGFbeta) receptor leads to paradoxical activation of TGFbeta signaling pathways with fibrosis in transgenic mice, J. Biol. Chem. 278 (2003) 25109–25119.
[10] R.K. Hoyles, K. Khan, X. Shiwen, S.L. Howat, G.E. Lindahl, P. Leoni, R.M. du Bois, A.U. Wells, C.M. Black, D.J. Abraham, C.P. Denton, Fibroblast-specific perturbation of transforming growth factor beta signaling provides insight into potential pathogenic mechanisms of scleroderma-associated lung fibrosis: exaggerated response to alveolar epithelial injury in a novel mouse model, Arthritis Rheum. 58 (2008) 1175–1188.
[11] S. Sonnylal, C.P. Denton, B. Zheng, D.R. Keene, R. He, H.P. Adams, C.S. Vanpelt, Y. J. Geng, J.M. Deng, R.R. Behringer, B. de Crombrugghe, Postnatal induction of transforming growth factor beta signaling in fibroblasts of mice recapitulates clinical, histologic, and biochemical features of scleroderma, Arthritis Rheum. 56 (2007) 334–344.
[12] W.A. Border, N.A. Noble, Transforming growth factor beta in tissue fibrosis, N. Engl. J. Med. 331 (1994) 1286–1292.
[13] J.C. Horowitz, D.S. Rogers, V. Sharma, R. Vittal, E.S. White, Z. Cui, V. J. Thannickal, Combinatorial activation of FAK and AKT by transforming growth factor-beta1 confers an anoikis-resistant phenotype to myofibroblasts, Cell Signal. 19 (2007) 761–771.
[14] V.J. Thannickal, D.Y. Lee, E.S. White, Z. Cui, J.M. Larios, R. Chacon, J.C. Horowitz, R.M. Day, P.E. Thomas, Myofibroblast differentiation by transforming growth factor-beta1 is dependent on cell adhesion and integrin signaling via focal adhesion kinase, J. Biol. Chem. 278 (2003) 12384–12389.
[15] T.T. Antoniv, S. Tanaka, B. Sudan, S. De Val, K. Liu, L. Wang, D.J. Wells, G. Bou-Gharios, F. Ramirez, Identification of a repressor in the first intron of the human alpha2(I) collagen gene (COL1A2), J. Biol. Chem. 280 (2005) 35417–35423.
[16] M. Ponticos, C. Harvey, T. Ikeda, D. Abraham, G. Bou-Gharios, JunB mediates enhancer/promoter activity of COL1A2 following TGF-beta induction, Nucleic Acids Res. 37 (2009) 5378–5389.
[17] M. Fragiadaki, T. Ikeda, A. Witherden, R.M. Mason, D. Abraham, G. Bou-Gharios, High doses of TGF-beta potently suppress type I collagen via the transcription factor CUX1, Mol. Biol. Cell 22 (2011) 1836–1844.
[18] T.T. Antoniv, S. De Val, D. Wells, C.P. Denton, C. Rabe, B. de Crombrugghe, F. Ramirez, G. Bou-Gharios, Characterization of an evolutionarily conserved far-upstream enhancer in the human alpha 2(I) collagen (COL1A2) gene, J. Biol. Chem. 276 (2001) 21754–21764.
[19] L. Sansregret, A. Nepveu, The multiple roles of CUX1: insights from mouse models and cell-based assays, Gene 412 (2008) 84–94.

[20] X. Shi-Wen, C.P. Denton, A. McWhirter, G. Bou-Gharios, D.J. Abraham, R.M. du Bois, C.M. Black, Scleroderma lung fibroblasts exhibit elevated and dysregulated type I collagen biosynthesis, Arthritis Rheum. 40 (1997) 1237–1244.
[21] R. Harada, G. Berube, O.J. Tamplin, C. Denis-Larose, A. Nepveu, DNA-binding specificity of the cut repeats from the human cut-like protein, Mol. Cell Biol. 15 (1995) 129–140.
[22] A. Nepveu, Role of the multifunctional CDP/Cut/Cux homeodomain transcription factor in regulating differentiation, cell growth and development, Gene 270 (2001) 1–15.
[23] B. Goulet, P. Watson, M. Poirier, L. Leduy, G. Berube, S. Meterissian, P. Jolicoeur, A. Nepveu, Characterization of a tissue-specific CDP/Cux isoform, p75, activated in breast tumor cells, Cancer Res. 62 (2002) 6625–6633.
[24] N.S. Moon, P. Premdas, M. Truscott, L. Leduy, G. Berube, A. Nepveu, S phase-specific proteolytic cleavage is required to activate stable DNA binding by the CDP/Cut homeodomain protein, Mol. Cell Biol. 21 (2001) 6332–6345.
[25] M. Truscott, L. Raynal, P. Premdas, B. Goulet, L. Leduy, G. Berube, A. Nepveu, CDP/Cux stimulates transcription from the DNA polymerase alpha gene promoter, Mol. Cell Biol. 23 (2003) 3013–3028.
[26] A.W. Ledford, J.G. Brantley, G. Kemeny, T.L. Foreman, S.E. Quaggin, P. Igarashi, S.M. Oberhaus, M. Rodova, J.P. Calvet, G.B. Vanden Heuvel, Deregulated expression of the homeobox gene Cux-1 in transgenic mice results in downregulation of p27(kip1) expression during nephrogenesis, glomerular abnormalities, and multiorgan hyperplasia, Dev. Biol. 245 (2002) 157–171.
[27] P. Michl, J. Downward, CUTL1: a key mediator of TGFbeta-induced tumor invasion, Cell Cycle 5 (2006) 132–134.
[28] P. Michl, A.R. Ramjaun, O.E. Pardo, P.H. Warne, M. Wagner, R. Poulsom, C. D'Arrigo, K. Ryder, A. Menke, T. Gress, J. Downward, CUTL1 is a target of TGF (beta) signaling that enhances cancer cell motility and invasiveness, Cancer Cell 7 (2005) 521–532.
[29] S. Ceru, S. Konjar, K. Maher, U. Repnik, I. Krizaj, M. Bencina, M. Renko, A. Nepveu, E. Zerovnik, B. Turk, N. Kopitar-Jerala, Stefin B interacts with histones and cathepsin L in the nucleus, J. Biol. Chem. 285 (2010) 10078–10086.
[30] M. Truscott, L. Raynal, Y. Wang, G. Berube, L. Leduy, A. Nepveu, The N-terminal region of the CCAAT displacement protein (CDP)/Cux transcription factor functions as an autoinhibitory domain that modulates DNA binding, J. Biol. Chem. 279 (2004) 49787–49794.
[31] T. Ikeda, M. Fragiadaki, X. Shi-Wen, M. Ponticos, K. Khan, C. Denton, P. Garcia, G. Bou-Gharios, A. Yamakawa, C. Morimoto, and D. Abraham, Data on the CUX1 isoforms in the Idiopatic Pulmonary Fibrosis (IPF) tissue section and Systemic Sclerosis (SSc) skin tissue section, Data In Brief. (submitted).