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Role of Flightless-I (*Drosophila*) homolog in the transcription activation of type I collagen gene mediated by transforming growth factor beta

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ABSTRACT

Flightless-I (*Drosophila*) homolog (FLII) is a nuclear receptor coactivator that is known to interact with other transcriptional regulators such as the SWI/SNF complex, an ATP-dependent chromatin-remodeling complex, at the promoter or enhancer region of estrogen receptor (ER)-α target genes. However, little is known about the role of FLII during transcription initiation in the transforming growth factor beta (TGFβ)/SMAD-dependent signaling pathway. Here, we demonstrate that FLII functions as a coactivator in the expression of type I collagen gene induced by TGFβ in A549 cells. FLII activates the reporter gene driven by *COL1A2* promoter in a dose-dependent manner. Co-expression of GRIP1, CARM1, or p300 did not show any synergistic activation of transcription. Furthermore, the level of *COL1A2* expression correlated with the endogenous level of *FLII* mRNA level. Depletion of FLII resulted in a reduction of TGFβ-induced expression of *COL1A2* gene. In contrast, over-expression of FLII caused an increase in the endogenous expression of *COL1A2*. We also showed that FLII is associated with Brahma-related gene 1 (BRG1) as well as SMAD in A549 cells. Notably, the recruitment of BRG1 to the *COL1A2* promoter region was decreased in FLII-depleted A549 cells, suggesting that FLII is required for TGFβ-induced chromatin remodeling, which is carried out by the SWI/SNF complex. Furthermore, formaldehyde-assisted isolation of regulatory elements (FAIRE)-quantitative polymerase chain reaction (qPCR) experiments revealed that depletion of FLII caused a reduction in chromatin accessibility at the *COL1A2* promoter. These results suggest that FLII plays a critical role in TGFβ/SMAD-mediated transcription of the *COL1A2* gene through its role in recruiting the SWI/SNF complex to facilitate chromatin accessibility.

1. Introduction

Flightless I (*Drosophila*) homolog (FLII) is an evolutionarily conserved member of the gelsolin family that possesses actin binding and severing capabilities [1,2]. In *Drosophila*, partial loss-of-function mutations of *FLII* result in a loss of flight ability, while more severe mutations lead to impaired cellularization and gastrulation of the embryo [2]. The human *FLII* gene is located within the Smith–Magenis syndrome region on chromosome 17p, which is linked to a genetic disease that causes developmental and behavioral abnormalities [3]. Homologous mutation of *FLII* during *Drosophila* embryogenesis initiates uterine implantation but the embryo fails to develop [4]. Both *Drosophila* and human *FLII* contain two functional domains: an N-terminal leucine-rich repeat (LRR) domain and a C-terminal gelsolin-like domain (Gel), each of which is involved in protein–protein interactions. Similar to other gelsolin family proteins such as gelsolin and villin, the C-terminal gelsolin-like domain of FLII consists of two large tandem repeats, each of which contains three smaller repeats. [5,6].

The role of FLII in transcriptional activation was first studied for nuclear receptor-mediated gene expression in MCF-7 cells [5–7]. Previously it has been shown that FLII binds to nuclear receptors (NR), transcription coactivators, and the SWI/SNF chromatin-remodeling complex during nuclear receptor-mediated transcription initiation. FLII interacts with the ligand-binding domain (LBD) of ERα through its leucine-rich repeat located in the G3 fragment (the third repeat of the three repetitive subregions of GelA). The G3 fragment contains an LXXLL motif (where L represents leucine and X denotes any amino acid), which is observed in many coactivators that interact with hormone activated NRs [6]. Unlike wild type FLII, mutant FLII (LL to AA), which is not able to bind to ERα, failed to further activate ERα-mediated transcription, suggesting that the interaction between FLII and ERα is critical for the coactivator function of FLII. In addition, FLII directly interacts with BAF53, an actin-related component of the SWI/SNF chromatin-remodeling complex, facilitating the recruitment of the SWI/SNF complex to the promoter or enhancer regions of ERα target genes.

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Chromatin accessibility analysis on ERα-mediated transcriptional activity using formaldehyde-assisted isolation of regulatory elements (FAIRE)-qPCR has shown that FLII regulates the expression of ERα target genes by facilitating E2-induced chromatin accessibility to estrogen response elements (EREs) [5,7].

A recent study revealed that FLII is an important regulator of wound repair. Reduced type I collagen, a transforming growth factor (TGF)β-responsive gene, was observed in fibroblasts from *FLII*-deficient (+/−) mice [8]. However, the mechanisms by which the activation of collagen genes is regulated by FLII remain unclear. TGFβ is a member of a superfamily of multifunctional cytokines that functions during wound repair, scar formation, and various developmental processes. TGFβ regulates the transcription of target genes using the canonical SMAD-dependent pathway, and non-canonical SMAD-independent pathway through TGFBR2 [9]. In the SMAD-dependent pathway, binding of TGFβ to type II receptors causes phosphorylation and incorporation of the type I receptor, forming a heterotetrameric complex with the ligand. The type II receptor phosphorylates serine residues of the type I receptor, which then recruits and phosphorylates receptor-regulated SMAD (R-SMAD). Type I collagen, one of the TGFβ-regulated genes, is a heterotrimer composed of two α1 chains (encoded by *COL1A1* gene) and one α2 chain (encoded by *COL1A2* gene).

Diverse functional protein–protein interactions have been reported in the SMAD pathway *in vitro* and *in vivo* [10,11]. Notably, the TGFβ-dependent interaction between SMAD2 and Brahma-related gene 1 (BRG1), which is a component in the SWI/SNF complex, and the requirement of BRG1 for TGFβ-induced expression of SMAD target genes have been reported [12]. Most recently, a genome-wide analysis to define the requirement for BRG1 in TGFβ-responsive genes revealed that BRG1 is required for the activation of the most TGFβ-responsive genes [13], suggesting a critical role of the SWI/SNF chromatin-remodeling complex in the TGFβ signaling pathway.

Our recent finding that FLII directly interacts with NRs and BAF53 in MCF-7 cells and plays a critical role in the recruitment of the SWI/SNF complex to ERα-target genes led us to propose that FLII might be responsible for recruitment of the SWI/SNF complex to the promoters of SMAD target genes in the TGFβ signaling pathway [5,6]. In the current study, we therefore investigated whether depletion of FLII affected TGFβ-dependent expression of endogenous *COL1A2* and recruitment of SWI/SNF complex to *COL1A2* promoter region. Our findings indicate that FLII plays a critical role in the TGFβ/SMAD-mediated transcription of *COL1A2* through its role in interacting with the SWI/SNF complex to regulate chromatin accessibility of target genes.

2. Materials and methods

2.1. Plasmids

The following plasmids were described previously [5,6]: pSG5-GRIP1, pSG5-CARM, pCMV-p300, pCDNA-FLII, pGEX4T1-LRR (a.a. 1–494), pGEX4T1-GelA (a.a. 495–822), and pGEX4T1-GelB (a.a. 825–1269).

2.2. Cell culture and reporter gene assay

A549 cells were maintained in RPMI 1640 medium with 2 mM L-glutamine and 10% fetal bovine serum (FBS) at 37 °C and in an atmosphere containing 5% CO_2. For reporter gene assays, A549 cells were plated in RPMI 1640 medium with 10% FBS at a density of 1×10^5 cells/well in 12-well plates the day before transfection. Transfection of pCOL1A2-luc and other expression plasmids was performed using Lipofectamine 2000 (Invitrogen, Carlsbad CA), according to the manufacturer's protocol. After transfection, cells were grown in RPMI 1640 with 10% FBS for 24 h in the presence or absence of 5 ng/ml TGFβ1 (KOMA Biotech, Seoul, Korea). Cell lysis and luciferase assays on cell extracts were performed with a luciferase assay kit (Promega, Madison, WI). The results are presented as mean ± S.D. of three transfected wells and are representative of at least three independent experiments.

2.3. RNA interference

Small interfering RNA (siRNA) experiments were performed according to previously published methods [6]. The sequences of siRNA used were as follows: siFLII, 5′-CAACCUGACCACGCUUCAUdTdT-3′ (sense) and 5′-AUGAAGCGUGGUCAGGUUGdTdT-3′ (antisense) and nonspecific siRNA (siNS), 5′-UUCUCCGAACGUGUCACGUdTdT-3′ (sense) and 5′-ACGUGACACGUUCGGAGAAdTdT-3′ (antisense). siRNAs were transfected into A549 cells by using Oligofectamine (Invitrogen, Carlsbad CA), according to the manufacturer's protocol. For lentivirus production, pCMV-ΔR8.91 (packing vector), pMD.G1 (envelop plasmid), and pHRCMVpuro-Sin8 (transfer vector) were used as described previously [14]. Forward primers used for PCR are as follows: shRNA with a nonspecific targeting sequence (shNS), 5′-CTTGTGGAAAGGACGAAACACCGGGTAGGTTCAACTAGCAAGACTTCTTCAAGAGAGAAGTCTTGCTAGTCGAACCTACCCTTTTTCTGCAGTTTT-3′; and shRNA directed against *FLII* mRNA (shFLII), 5′-CTTGTGGAAAGGACGAAACACCGCTGCCACAGATCAACTACTTTCTTCAAGAGAGATTGTAGTTGATCTGTGGCAGCTTTTTCTGCAGTTTT-3′.

2.4. Quantitative RT-PCR

Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA) after TGFβ1 treatment as indicated, and subjected to reverse transcription by Promega Go-Taq DNA polymerase [15]. Real-time PCR amplification of this cDNA was performed on a Roche LightCycler 480 II by using SYBR Green master mix (Roche, Indianapolis, IN). The primers used were as follows: *FLII*, 5′-CCT CCTACAGCTAGCAGGTTATCAAC-3′ (forward) and 5′-GCATGTG CTGGATATATACCTGGCAG-3′ (reverse); *COL1A2*, 5′-TCCAA GGACAAGAAACAC-3′ (forward) and 5′-GCAGCCATCTACAAGAAC-3′ (reverse); and *18S*, 5′-GAGGATGAGGTGGAACGTGT-3′ (forward) and 5′-TCTTCAGTCGCTCCAGGTCT-3′ (reverse). Relative expression levels were normalized to *18S* mRNA levels. The results are from a single experiment that is representative of at least three independent experiments conducted on different days.

2.5. Protein interaction assays and immunoblotting

The procedure for GST pull-down assays was described previously [6]. GST-fused LRR, GelA, and GelB protein were expressed in *Escherichia coli* BL21(DE3) strain and purified by incubation with glutathione-Sepharose beads and washing with NETN buffer (300 mM NaCl, 1 mM EDTA, 20 mM Tris–HCl (pH 8.0), and 0.01% Nonidet P-40). For co-immunoprecipitation assays, the extracts from A549 treated with TGFβ1 or vehicle were prepared in 1.0 ml of radioimmunoprecipitation assay (RIPA) buffer. Immunoblotting was performed as described previously by using the following antibodies: anti-FLII (Santa Cruz Biotechnology, Dallas, TX), anti-SMAD3, anti-BRG1 (Abcam), and anti-β-actin (Thermo Scientific, Waltham, MA).

2.6. Chromatin immunoprecipitation assay (ChIP)

ChIP was performed as previously described [7,16]. A549 cells were transfected with siRNA targeting *FLII* (siFLII) or non-specific siRNA (siNS), and then cultured for 3 days in RPMI 1640 medium

with 10% FBS. Cells at approximately 95% confluency were treated with TGFβ1 (5 ng/ml) for 4 h. Depletion of endogenous level of FLII was measured by western immunoblotting. The primers used were as follows: *COL1A2* promoter region, 5′-GCGGAGGTATGCAGA-CAACG-3′ (forward) and 5′-GGGCTGGCTTCTTAAATTG-3′ (reverse). The immunoprecipitated DNA signal was normalized to the signal from DNA prepared from the same amount of chromatin before immunoprecipitation (input).

2.7. Formaldehyde-assisted isolation of regulatory elements (FAIRE)-qPCR

FAIRE-qPCR was performed as previously described [16]. A549 cells were transfected with siRNA and cultured for 3 days in RPMI 1640 supplemented with 5% charcoal–dextran–stripped FBS. At approximately 90% confluency, the cells were treated with TGFβ1 (5 ng/ml) or ethanol for 4 h. Results shown depict the mean and range of variation of duplicate PCR reactions performed on the same DNA sample. Results are expressed as the percent of input chromatin (Input DNA) and were derived from a single experiment that is representative of at least two independent experiments.

3. Results

3.1. FLII enhances TGFβ-dependent gene expression

To address whether FLII could modulate TGFβ-mediated transcription, we monitored the effect of FLII over-expression on the transcriptional activity of a reporter gene driven by the promoter of *COL1A2*, which is a TGFβ target gene. Over-expression of FLII by transient transfection in A549 cells increased the transcription of the pCOL1A2-luc reporter gene (Fig. 1B). In the presence of TGFβ, transfection of increasing amounts of FLII expression plasmid led to a dose-dependent rise in the TGFβ-dependent transcription of the reporter gene. These results suggest that FLII functions as a coactivator for expression of TGFβ-mediated genes. To test the effects of other coactivators that are known to bind to FLII [5], a plasmid encoding GRIP1, CARM1, or p300 was transfected along with the FLII expression plasmid in the presence of the pCOL1A2-luc reporter gene. Ectopic expression of GRIP1 or CARM1 with FLII further enhanced the increase of TGFβ-mediated transcriptional activation; however, no synergistic increase of transcription was observed by simultaneous expression of these coactivators.

3.2. Regulation of COL1A2 expression by FLII

To conduct further studies of FLII in a physiologically relevant situation, we investigated the function of FLII in the expression of endogenous TGFβ target genes. First, A549 cells were transiently transfected with pCDNA-FLII or empty vector and grown for 48 h. Total RNA was analyzed for *FLII* and *COL1A2* mRNA by RT-qPCR (Fig. 2A). Both mRNA and protein level of FLII were enhanced by transient transfection with pCDNA-FLII (Fig. 2A, left panel and Fig. 2B). In addition, the expression of *COL1A2* was increased by TGFβ treatment in control A549 cells. Over-expression of FLII further enhanced *COL1A2* expression in the presence or absence of TGFβ (Fig. 2A). In contrast, when FLII was depleted from A549 cells by transient transfection with siRNA targeting *FLII* (siFLII), TGFβ-induced expression of *COL1A2* was greatly reduced (Fig. 2C and D) compared to that observed in cells transfected with non-specific siRNA (siNS). The reduction of *FLII* mRNA level by a lentiviral shRNA system targeting a different part of the coding region of *FLII* resulted in a similar inhibition of *COL1A2* expression in A549 cells (Fig. 2E). Thus, FLII is required for the TGFβ-mediated expression of endogenous *COL1A2* in A549 cells.

3.3. FLII is associated with BRG1 and SMAD3 in A549 cells

We previously showed that FLII recruits the SWI/SNF complex to ERα target genes in MCF-7 breast cancer cells [6]. Therefore, we examined the possible functional relationship between FLII and the SWI/SNF complex in the TGFβ signaling pathway. A549 cells were transiently transfected with a full-length FLII expression plasmid and immunoprecipitated with anti-FLII antibody or anti-SMAD3 antibody (Fig. 3A and B). Anti-SMAD3 antibody effectively precipitated FLII. Moreover, co-immunoprecipitation of FLII was increased by TGFβ treatment, suggesting that the interaction of FLII

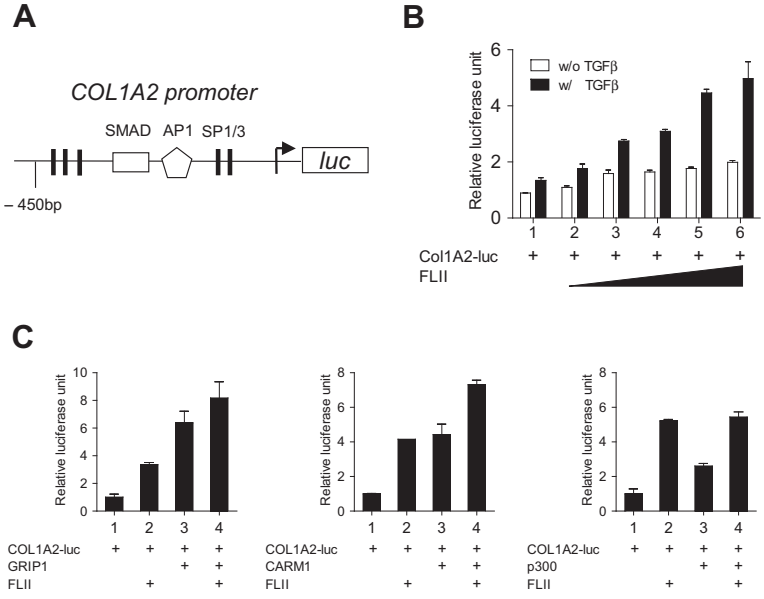

Fig. 1. Activation of *COL1A2*-driven reporter gene expression by FLII. (A) Structure of pCOL1A2-luc reporter gene construct. (B) Activation of transcription of *COL1A2*-driven reporter gene by FLII expression. A549 cells were transfected with pCOL1A2-luc reporter plasmid (200 ng) and expression plasmids encoding full-length FLII (10–200 ng). Transfected cells were grown for 2 days and harvested for luciferase assays. (C) A549 cells were transfected with GRIP1, CARM1, or p300 (100 ng) along with the expression plasmids encoding full-length FLII.

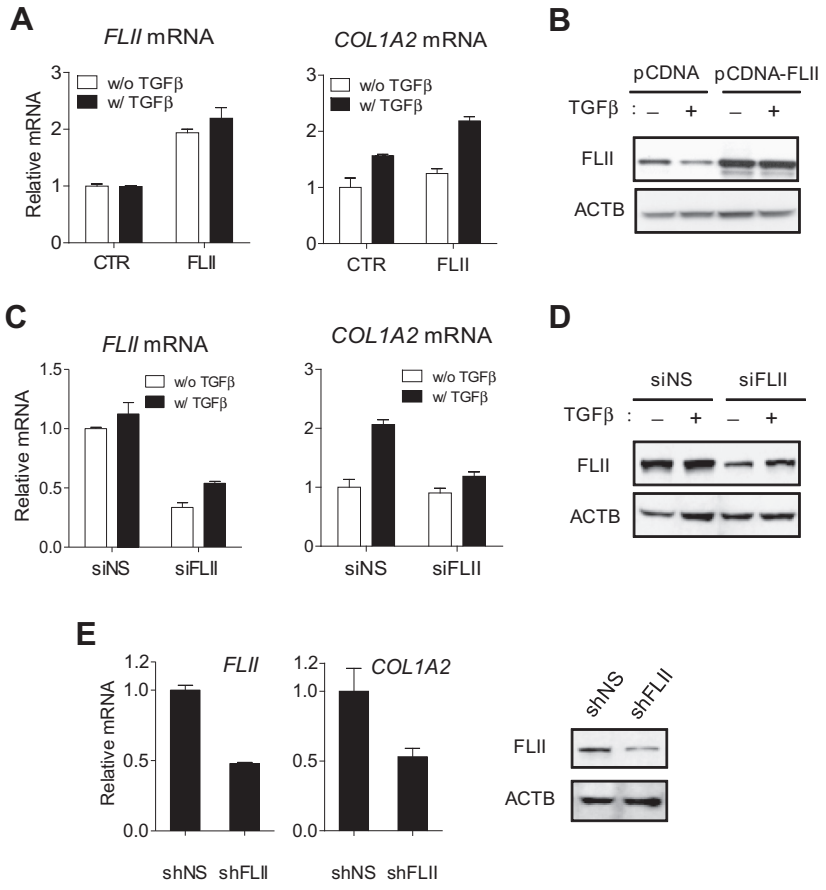

Fig. 2. FLII regulates expression of endogenous *COL1A2*. (A) Overexpression of FLII in A549 cells caused an increase in *COL1A2* expression. A549 cells were transiently transfected with pCDNA-FLII (FLII) or empty vector (CTR) and grown for 48 h. Total RNA was analyzed for *FLII* and *COL1A2* mRNA by RT-qPCR and normalized to the level of *18S* mRNA. (B) Protein levels of FLII and β-actin were assessed by immunoblotting. (C) Reduction in the levels of endogenous FLII by siRNA attenuated the expression of *COL1A2*. A549 cells were transiently transfected with siRNA targeting *FLII* (siFLII) or non-specific siRNA (siNS) and grown in media for 72 h. (D) Reduction of endogenous FLII protein level by siFLII was monitored by western immunoblotting. (E) Expression of endogenous *COL1A2* in shFLII-infected A549 cells by using Lentiviral shRNA system.

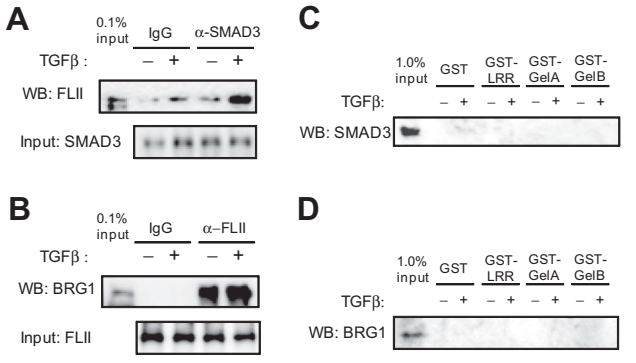

Fig. 3. FLII is associated with BRG1 and SMAD3 in A549 cells. (A and B) Co-immunoprecipitation was performed with total cell extracts from A549 cells transfected with pCDNA-FLII. At 48 h after transfection, cell extracts were prepared in RIPA buffer. Immunoprecipitation was performed using normal mouse or rabbit IgG, anti-SMAD3 antibody, and anti-FLII antibody. Immunoblotting was performed using anti-FLII antibody or anti-BRG1 antibody. (C and D) GST pull-down assays were performed by incubating total A549 cell lysates with 25 μg of GST-fused FLII fragments, GST-LRR, GST-GelA, and GST-GelB. Bound protein was analyzed by immunoblotting with anti-SMAD3 antibody (C) or anti-BRG1 (D) antibody.

with SMAD3 is TGFβ-dependent. We also observed co-immunoprecipitation of FLII with BRG1, however, normal IgG failed to bring down BRG1. To examine whether FLII directly interacts with SMAD3 and BRG1, we performed GST pull-down assays by using different domains of FLII. *E. coli* expressed LRR domain (amino acids 1–494), GelA domain (amino acids 495–822), or GelB domain (amino acids 825–1269) of FLII fused to GST was incubated with total cell lysates of A549 cells. Unlike in the case of the co-immunoprecipitation assays, purified recombinant FLII domains and full length FLII did not bind to SMAD3 or BRG1 (Fig. 3C and D and Supplementary Fig. S1). Thus, the interaction between FLII and SMAD3 or BRG1 would have to be indirect and could potentially be mediated by other proteins.

3.4. FLII is required to recruit the SWI/SNF complex to the COL1A2 promoter region

Given that FLII is associated with the SMAD3 and the BRG1 subunits of the SWI/SNF complex in A549 cells, we tested whether FLII is required for the recruitment of the SWI/SNF complex to the promoter region of the SMAD-responsive *COL1A2* gene. siNS- or siFLII-transfected A549 cells were treated with TGFβ (5 ng/ml) or vehicle for 4 h. The recruitment of BRG1 to the *COL1A2* promoter region was increased after 4 h of TGFβ treatment (Fig. 4A). However, reduction of endogenous FLII by transfection with siRNA against FLII resulted in decreased BRG1 recruitment to the *COL1A2* promoter in the presence of TGFβ. These results indicate that FLII is required for the recruitment of BRG1.

Chromatin-remodeling complexes are multiprotein complexes that utilize the energy derived from ATP hydrolysis to alter chromatin architecture at the nucleosome level and increase the accessibility of DNA templates assembled as chromatin in an

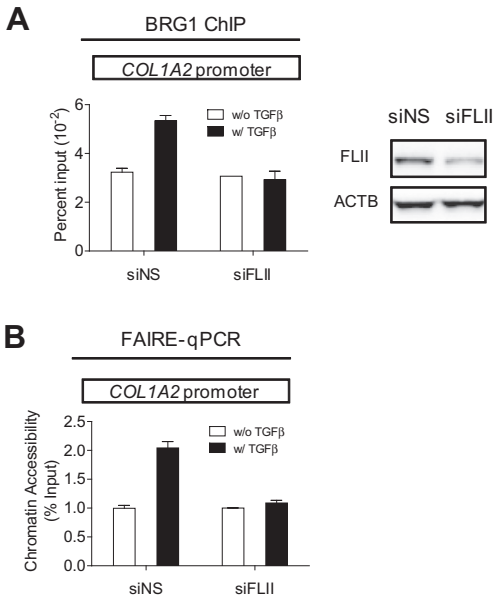

Fig. 4. FLII is required for chromatin remodeling by the SWI/SNF complex. (A) Chromatin immunoprecipitation assays were performed with A549 cells in 150 mm dishes treated with TGFβ (5 ng/ml) or vehicle for 4 h. After immunoprecipitation of cross-linked chromatin fragments with anti-BRG1 antibody, the amount of *COL1A2* promoter fragments was determined by qPCR. (B) FLII is required for chromatin accessibility at the *COL1A2* promoter region. Chromatin accessibility at the *COL1A2* promoter was assessed by FAIRE-qPCR analysis by using chromatin samples prepared from A549 cells transfected with siNS or siFLII and treated with TGFβ (5 ng/ml) or ethanol for 4 h. Data are normalized against non-cross-linked genomic DNA for each primer pair.

ATP-dependent manner [17–20]. Given that FLII is required for the recruitment of the SWI/SNF complex to the *COL1A2* promoter, we used FAIRE-qPCR to investigate whether FLII influences the chromatin accessibility of *COL1A2* promoter regions. FAIRE is one of the methods to identify nucleosome-depleted region within chromatin. Chromatin is crosslinked with formaldehyde *in vivo*, sheared by sonication, and nucleosome-depleted DNA fragments are recovered by phenol–chloroform extraction. The FAIRE-enriched chromatin can be detected using qPCR (FAIRE-qPCR) or sequencing (FAIRE-seq). A549 cells were transfected with siNS or siFLII, and treated with TGFβ for 4 h. siNS-transfected A549 cells showed increased FAIRE signals at the *COL1A2* promoter region by TGFβ treatment (Fig. 4B). However, specific silencing of FLII by siRNA dramatically decreased the incidence of TGFβ-induced FAIRE signals in the *COLIA2* promoter regions, that were previously observed in siNS-transfected cells (Fig. 4B). Thus, these results indicate that FLII is critical for the recruitment of the SWI/SNF complex and subsequently, for the chromatin accessibility of the *COL1A2* gene induced by TGFβ.

4. Discussion

SMAD proteins are central mediators in the TGFβ-dependent signaling pathway, transducing signals from type I and II TGFβ receptors to target genes [11]. Upon TGFβ stimulation, the type I receptor phosphorylates SMAD2 and SMAD3. Receptor-phosphorylated SMAD proteins (R-SMAD proteins) translocate into the nucleus which potentiate their ability to bind to specific DNA sequences. Subsequently, they recruit multiple proteins, forming transcriptional complexes that pair with other context-dependent transcription factors, and eventually RNA polymerase II is recruited to the target gene promoter and transcription is initiated [21,22]. Recent studies on SMAD proteins and chromatin remodeling have elucidated the function of the SWI/SNF complex in TGFβ-mediated gene expression [13,23]. BRG1, which is a core ATPase, is associated with SMAD2 and SMAD3, and recruited to SMAD-dependent promoters. However, the exact mechanism by which the SWI/SNF complex is recruited to SMAD target genes remained unclear.

While the SWI/SNF complex was previously shown to be critical for TGFβ-dependent expression of the SMAD target genes [12], we showed here that FLII is required for efficient recruitment of the SWI/SNF complex to *COL1A2* promoter. We demonstrated that FLII activates the expression of TGFβ-mediated reporter genes in A549 cells (Fig. 1). In multiple independent experiments, there was a strong correlation between the level of FLII protein and the degree of *COL1A2* expression (Fig. 2). Type I procollagen is composed of an α1(I) chain and an α2(I) chain, and the expression of these two polypeptide chains is under the control of two separate genes. For the expression of *COL1A2*, the TGFβ-stimulated SMAD3 binds to the TGFβ-responsive element (TRE) of the *COL1A2* promoter at the consensus CAGACA sequence located at −263 to −258 bp [24]. Recently, FLII-deficient +/− mice showed improved wound healing with increased epithelial migration, and enhanced wound contraction, suggesting that FLII is an important regulator of wound healing [8]. In the same study, the reduced expression of type I collagen was observed in fibroblasts from *FLII*-deficient (+/−) mice. However, the mechanism by which FLII regulates the collagen gene had not been determined.

Here, we demonstrated that FLII is associated with both SMAD3 and BRG1 in A549 cells (Fig. 3) and that the co-immunoprecipitation of SMAD3 was increased by TGFβ treatment. This suggests that the interaction of FLII with SMAD3 is TGFβ-dependent, and that this interaction might play an important role in the recruitment of the SWI/SNF complex to SMAD target genes. Indeed, the increase in BRG1 recruitment to the *COL1A2* promoter region by TGFβ treatment was remarkably inhibited by transfection with siRNA against FLII, indicating that FLII is critical for the recruitment of BRG1. Thus, our results suggest a model whereby SWI/SNF is recruited to the SMAD-responsive *COL1A2* promoter region through an interaction with FLII. Our FAIRE-qPCR results also support the model for FLII to recruit the SWI/SNF complex to SMAD-bound *COL1A2* promoter. Specific silencing of endogenous FLII by siRNA in A549 cells resulted in significant inhibition of chromatin accessibility of the *COL1A2* promoter (Fig. 4).

Previous reports on FLII interactions with other transcription activators (e.g., ERα) and coactivators (e.g., CARM1, GRIP1 and p300) [5,6] in nuclear receptor-mediated transcription led us to investigate whether FLII has a synergistic effect on the activation of TGFβ-responsive genes. Ectopic expression of GRIP1 or CARM1 further enhanced the TGFβ-mediated transcription of the *COL1A2*-promoter reporter; however, no synergistic increase in transcription was observed by co-expression of FLII. Although our observations suggest that the function of FLII at the *COL1A2* promoter region does not involve direct association with these coactivators, it is still possible that FLII interacts with other proteins (e.g., AP-1 or SP1) that are also recruited to the *COL1A2* promoter.

Collectively, our findings provide a molecular mechanism for the involvement of FLII in TGFβ-mediated gene expression. FLII is a key protein that regulates the expression of *COL1A2* gene by recruiting the SWI/SNF complex to the TRE in a TGFβ-dependent manner, subsequently facilitating TGFβ-induced chromatin accessibility to *COL1A2* gene promoter.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.10.100.

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