Suppressive effect of SATB1 on hepatic stellate cell activation and liver fibrosis in rats

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A R T I C L E   I N F O

Article history:
Received 24 December 2014
Revised 24 March 2015
Accepted 8 April 2015
Available online 17 April 2015

Keywords:
Liver fibrosis
Hepatic stellate cell
SATB1
Ras/Raf-1/MEK/ERK/Ets-1

A B S T R A C T

Liver fibrosis is a worldwide clinical issue. Activation of hepatic stellate cells (HSCs) is the central event during liver fibrosis. We investigated the role of SATB1 in HSC activation and liver fibrogenesis. The results show that SATB1 expression is reduced during HSC activation. Additionally, SATB1 inhibits HSC activation, proliferation, migration, and collagen synthesis. Furthermore, CTGF, a pro-fibrotic agent, is also significantly inhibited by SATB1 through the Ras/Raf-1/MEK/ERK/Ets-1 pathway. In vivo, SATB1 deactivates HSCs and attenuates fibrosis in TAA-induced fibrotic rat livers. These data indicate that SATB1 plays an important role in HSC activation and liver fibrosis.

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1. Introduction

Liver fibrosis, a major cause of high morbidity and mortality worldwide [1], is characterized by excessive and abnormal deposition of extracellular matrix (ECM), which leads to liver damage and seriously threatens human health [2]. Therefore, a better understanding of the reversible steps in the fibrotic response may lead to identification of new therapeutic targets.

Hepatic stellate cells (HSCs) play a central role in the development of liver fibrosis [3]. HSCs are in a quiescent state in normal liver, and their main function is storing retinoids [4]. After continued liver damage, these quiescent HSCs transdifferentiate into fibrogenic, proliferative, and contractile myofibroblast-like phenotypes, as represented by the expression of α-SMA and by the production of excessive collagen and by enhanced proliferation and migration, thereby accelerating the progression of liver fibrosis [5,6]. Understanding the molecular mechanisms that drive this phenotype switch may provide new insights into the pathogenesis of liver fibrosis.

In the special AT-rich binding protein 1 (SATB1), a nuclear matrix attachment region binding protein, acted as a molecular switch that could modulate gene expression by binding with MARs [7–9]. Under physiological conditions, SATB1 is expressed abundantly in thymocytes [8]. It is necessary during T-cell development, orchestrating the spatial and temporal expression of multiple genes [10], and is involved in early Th2 cell differentiation [11]. Since the seminal article on its role in breast cancer [12], a large number of studies conducted in recent years have shown that SATB1 abundantly expresses in various types of tumor and positively correlates with prognostic and clinico-pathological properties [12–14]. However, in colorectal cancer [15] and lung squamous cell carcinoma [16], it is loss of expression rather than overexpression of SATB1 that is associated with poor prognosis. This supports the tissue-specific organizational role of SATB1. Thus far, the role of SATB1 in HSCs and liver fibrosis has remained unknown.
2. Materials and methods

See Supplementary materials for detailed experimental materials and methods.

2.1. Ethics statement

All animal works were performed in accordance with the relevant national and international guidelines. The protocol was approved by the Committee on the Ethics of Animal Experiments of Tongji Medical College and monitored by the Department of Experimental Animals of Tongji Medical College.

2.2. HSCs isolation and cell lines

The TAA-induced liver fibrosis procedure was described previously [17]. Male Sprague–Dawley (SD) rats weighting approximately 250–270 g were purchased from the Department of Experimental Animals of Tongji Medical College (Wuhan, China). They were kept on a standard rat diet with free access to tap water and food, with a 12-h light–dark cycle. Rats were randomly divided into two groups, 3 each, injected intraperitoneally with either thioacetamide (TAA; dissolved in saline, 200 mg/kg B.W.) twice a week for up to 7 weeks or with the same amount of saline. Then primary rat HSCs were isolated from these rats and were cultured in DMEM/20% fetal bovine serum (FBS) containing penicillin and streptomycin. The immortalized human hepatic stellate cell line LX-2 [18] was cultured in DMEM/10% FBS.

2.3. SATB1-knockdown cells

Chemically synthesized siRNA directed against SATB1 were transfected into the cells and the level of knockdown was determined by real-time PCR and Western blot (Fig. S1).

2.4. SATB1 over-expressing cells

2.4.1. LX-2

The cells were transfected with pcDNA3.1(–)-SATB1 and pcDNA3.1(–)-vector plasmids. Two single colonies of stable cells with the highest SATB1 expression were used in this study (Fig. S1).

2.4.2. Rat HSCs

Adenoviral vectors expressing rat SATB1 and the negative control (AdGFP) were purchased from Obio Technology Co., Ltd. (Shanghai, China). Cultured rat HSCs were infected with AdSATB1 or AdGFP at a multiplicity of infection (MOI) of 100 (Fig. S1).

2.5. Animal fibrosis models and adenoviral delivery in vivo

Male SD rats weighting about 200 g were randomly divided into four groups, 7 each. TAA was injected intraperitoneally (200 mg/kg, twice a week for 6 weeks) to all groups of rats (group II, III, and IV) except for one group, which were injected intraperitoneally with the same amount of saline (group I). From the beginning of the 6th week, rats were infused with PBS (group I and II), 5 × 10⁹ pfu AdGFP (group III), or AdSATB1 (group IV) via the tail vein twice a week for 2 weeks. At the ending of the 7th week, all of the rats were sacrificed and liver samples were collected (Fig. S6C). RNA and protein were extracted directly as soon as possible. Liver tissues were fixed in 4% buffered paraformaldehyde and then embedded in paraffin for immunohistochemistry analysis.

2.6. Raf-1 membrane translocation and Ras activation assay

The membrane protein was extracted by using the Membrane and Cytosol Protein Extraction Kit (Thermo Fisher scientific, MA, USA). The cytosolic and membrane fraction were immunoblotted

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**Fig. 1.** SATB1 is downregulated in activated HSCs. Real-time PCR and Western blot were used to assess gene expression in rat HSCs. (A and D) Primary HSCs were isolated from normal (N, n = 3) and fibrosis (TAA, n = 3) rats and cultured for 3 days. The mRNA levels of SATB1 (A) and the protein levels of α-SMA and SATB1 (D) was analyzed. *P < 0.05 vs. N. (B and E) Primary HSCs were isolated from normal rats and cultured for 7 days. The expression of α-SMA and SATB1 in culture-activated HSCs were assessed. n = 3. *P < 0.05 vs. 1 d. (C and F) 2 × 10⁵ freshly isolated rat HSCs were seeded in a 6-well-plate well. The next day cells were cultured in serum-free medium for 48 h. Then, PBS or TGF-β1 (10 ng/ml; PeproTech, NJ, USA) was added for 24 h. Levels of SATB1 and α-SMA expression were measured. n = 3. *P < 0.05 vs. PBS. Data are shown as mean ± S.E.M.
with Raf-1 antibody. The activation of Ras was assessed by using a Ras Activation Assay Kit (Millipore) according to the manufacturer’s protocol.

2.7. Statistical analysis

Results are presented as mean ± S.E.M. from three independent experiments. The analysis of Student’s t-test and ANOVA/tukey post hoc test for comparison of normally distributed data between paired data and among the groups respectively. Data not normally distributed were compared using the Mann–Whitney tests. A P-value <0.05 was considered statistically significant.

3. Results

3.1. SATB1 shows an inverse relationship with HSCs activation

To study the expression and distribution of SATB1 in liver tissues, immunohistochemistry was performed on histological sections of rat liver tissues. Positive staining of SATB1 was seen only in the nucleus of liver non-parenchymal cells, almost no staining was observed in the hepatocytes (Fig. S3A). In addition, SATB1 was expressed in rat primary HSCs and was mainly located within the nucleus (Fig. S3B).

To explore the relationship between SATB1 expression and HSCs activation, HSCs were isolated from both the normal and TAA rats (n = 3 rats/group). The HSCs were cultured for 3 days and then the expression of SATB1 and α-SMA (a well-established marker of activated HSCs) were measured. Compared with normal rats, lower expression of SATB1 along with higher expression of α-SMA was found in HSCs isolated from TAA rats (Fig. 1A and D). Similar results were also observed in two models of in vitro activation of rat HSCs. When rat HSCs were freshly isolated, they were in a quiescent state [4] and then were activated by culture [5]. In our study, they were freshly isolated and cultured for up to 7 days (Fig. S2). The expression of α-SMA was increased, while SATB1 expression dramatically decreased (Fig. 1B and E). Additionally, we treated rat HSCs with TGF-β1, as TGF-β1 was the strongest stimulator for HSCs activation (Fig. 1C and F) [19]. As expected, TGF-β1 significantly induced increased α-SMA expression compared with the untreated group, while SATB1 expression was inhibited. Taken together, these results indicate that there was a strong negative correlation between SATB1 expression and HSCs activation.
3.2. SATB1 inhibits the activation of HSCs in vitro

Next, we investigated whether SATB1 was involved in the process of HSCs activation. We first delivered siRNA against SATB1 into LX-2 (siSATB1-1 or siSATB1-2). As activated HSCs exhibited [20], knockdown of SATB1 resulted in a significant increase in the expression of α-SMA (Fig. 2A). In the meantime, other fibrosis-related genes, including CTGF, COL1A1, and COL3A1 were also upregulated (Fig. 2B and C). Furthermore, we established SATB1-overexpressing cells. As expected, upregulation of SATB1 resulted in deactivation of LX-2 cells, as the expression of α-SMA was significantly reduced (Fig. 2A). The levels of CTGF, COL1A1, and COL3A1 were also decreased (Fig. 2D and E). Similar results were observed in rat HSCs, except that the expression of COL3A1 did not change significantly in rat HSCs treated with AdSATB1 (Fig. S4). These findings suggest that SATB1 could inhibit HSCs activation and collagen synthesis.

3.3. SATB1 inhibits the proliferation and migration of HSCs in vitro

Previous research demonstrated that activated HSCs have the ability to proliferate and migrate into the site of injury, which contributes to the progression of liver fibrosis [21]. Therefore, cell proliferation and migration were analyzed. Compared to NC, LX-2 cells transfected with siSATB1 (siSATB1-1 or siSATB1-2) had a greater proliferative capacity and higher absorbance value (Fig. 3A). On the other hand, upregulation of SATB1 significantly suppressed proliferation of LX-2 cells (Fig. 3C). Furthermore, real-time PCR
analysis revealed that SATB1 negatively regulated the expression of the G1-S transition promoter cyclin E1 (Fig. 3B and D). The results indicate that SATB1 may suppress HSCs proliferation by downregulating cyclin E1 expression. The migration ability significantly increased after SATB1 silencing compared with NC, and more cells migrated through the pores (Fig. 3E); inversely, the migration capability was inhibited after enhanced expression of SATB1 (Fig. 3F).

3.4. SATB1 inhibits CTGF expression through the MAPK pathway

Emerging evidence has remarkably established the important role of CTGF in driving the activated phenotype in HSCs [22]. Stimulation of HSCs with CTGF caused an acceleration of a pattern of gene expression that involved in HSCs activation, proliferation, migration, and collagen production[23–25]. Therefore, we investigated the molecular mechanism of the regulation of CTGF expression by SATB1 next.

3.4.1. SATB1-dependent downregulation of CTGF involves Ets-1 inhibition

Two transcriptional factors, Smad and Ets-1, are known as essential players that regulate CTGF expression [26,27]. Therefore, we analyzed the regulatory effect of SATB1 on the transcriptional activity of Smad and Ets-1. Our results revealed that SATB1 had no effect on the expression of Smad2/3/4/7 in LX-2 cells (Fig. S5A). At the same time, Smad3 phosphorylation and Smad4 nuclear translocation were also unchanged (Fig. S5B). After SATB1 expression was silenced, surprisingly, the level of phospho-Ets-1 (Thr38) and its accumulation in the nucleus was significantly elevated, while the total Ets-1 expression was nearly unchanged; inversely, the level of phospho-Ets-1 significantly decreased when SATB1 expression was enhanced (Figs. 4A, B and 5G, H). In addition, we used Ets-1 siRNA in SATB1-knockdown cells. The results showed that inhibition of Ets-1 antagonized the elevation of CTGF expression in SATB1-knockdown cells (Fig. 4C). Considered together, these results suggest that Ets-1 activation is involved in the regulation of CTGF expression by SATB1.

3.4.2. SATB1-dependent downregulation of CTGF involves MEK/ERK inhibition

Previous works demonstrated that the activation of Ets-1 requires its phosphorylation by upstream MAPKs, including p38, MEK/ERK, and JNK [28,29]. So we next tested which MAPK was targeted by SATB1-mediated Ets-1 inhibition. The results show that SATB1 failed to activate p38 and JNK activity (Fig. 5C and D). Remarkably, knocking down of SATB1 resulted in an increase in the phosphorylation of MEK and ERK; and upregulation of SATB1 significantly reduced the phosphorylation of MEK and ERK (Fig. 4A and B). Additionally, we used MEK1/2 inhibitors U0126 and PD98059 in SATB1-knockdown cells. Consistent with the
Fig. 5. SATB1-dependent downregulation of CTGF involved Ras/Raf-1/MEK/ERK/Ets-1. After SATB1 expression was silenced or enhanced in LX-2 cells, (A and B) lysates corresponding to cytosolic and membrane fractions were assessed by western blot. ATP1A1 and GAPDH were used as a loading control. \( n = 3 \). \( * P < 0.05 \) vs. NC or Vec; (C and D) cells were lysated for the Ras activity assay. Total Ras was used as a loading control. \( n = 3 \). \( * P < 0.05 \) vs. NC or Vec. (E and F) The LX-2 cells were transfected with NC or siSATB1-1. After culture for 24 h, the cells were treated with GW5074 (10 \( \mu \)M) and manumycin A (3 \( \mu \)M) for 24 h. The expression of CTGF, p-ERK, t-ERK, p-MEK, t-MEK, and GAPDH were measured by Western blot. \( n = 3 \). \( * P < 0.05 \) vs. NC + DMSO; \( * P < 0.05 \) vs. SATB1 + DMSO. (G and H) The LX-2 cells were transfected with NC or siSATB1-1. After culture for 24 h, the cells were treated with U0126, PD98059, GW5074, and manumycin A for 24 h. The expression of p-Ets-1 and t-Ets-1 were measured by Western blot (G) and immunofluorescent staining of p-Ets-1 was assayed by confocal microscopy (H). Data are shown as mean ± S.E.M.
above results, after SATB1 expression was silenced in LX-2, the elevation of CTGF expression was greatly reduced by treatment with the MEK inhibitors (Fig. 4D and E). At the same time, elevation of the phosphorylation of ERK and Ets-1 caused by knockdown of SATB1 was noticeably reduced (Figs. 4D, E, and 5G); the nuclear level of phospho-Ets-1 was also reduced (Fig. 5H). These results imply that inhibition of CTGF by SATB1 may result from a blockade of the MEK/ERK/Ets-1 cascade.

3.4.3. SATB1-dependent downregulation of CTGF involves Ras/Raf-1 inhibition

MEK/ERK activation is frequently a result of Ras activation, which recruits the Raf-1 to the plasma membrane, and in turn activates MEK and ERK [30]. As shown in Fig. 5A, Raf-1 was clearly enriched in membrane fractions in LX-2 cells after SATB1 was silenced compared with NC. Consistent with this, Ras activity remarkably increased (Fig. 5C). On the contrary, upregulation of SATB1 inhibited the membrane translocation of Raf-1 when compared with the vector control cells (Fig. 5B). In the meantime, the activity of Ras was inhibited (Fig. 5D). A Raf-1 inhibitor (GW5074) and a Ras inhibitor (manumycin A) were used, and both of them effectively reversed the increased levels of CTGF in LX-2-siSATB1 cells (Fig. 5E and F). Furthermore, the elevation of MEK and ERK phosphorylation and the nuclear level of phospho-Ets-1 caused by the knock down of SATB1 was noticeably reduced by treatment with GW5074 and manumycin A (Fig. 5E–H). All in all, these results suggest that the Ras-Raf-1-MEK-ERK-Ets-1 signaling pathway is involved in SATB1-induced CTGF downregulation in LX-2 cells.

3.5. SATB1 inhibits TAA-induced rat liver fibrosis

Finally, we assessed the role of SATB1 in vivo by using the rat model of TAA-induced liver fibrosis. In our pilot study, the efficacy of AdSATB1 delivery to the rat liver was assessed. The SATB1 mRNA and protein began to express at day 1 and peaked at day 3 after being injected with AdSATB1 and then declined when compared with AdGFP injection (Fig. S6A and B). Thus, a tail-vein injection was given twice a week to maintain consistent SATB1 expression.

![Image](http://example.com/image1.jpg)

**Fig. 6.** SATB1 ameliorates TAA-induced rat liver fibrosis. (A) Liver fibrosis was assessed by H&E staining (40×), Masson’s trichrome staining (40×), and Sirius Red staining (100×). (B and C) Semiquantitative analysis of the Masson’s trichrome (B) and Sirius Red staining (C), n = 7. *P < 0.05 vs. AdGFP. (D) The content of hydroxyproline. n = 7. *P < 0.05 vs. AdGFP. Data are shown as mean ± S.E.M.

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<th>Table 1</th>
<th>SATB1 improves hepatic serum biochemical parameters.</th>
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<tr>
<td><strong>Group</strong></td>
<td>ALB (g/L)</td>
</tr>
<tr>
<td>Normal</td>
<td>35.9 ± 3.6</td>
</tr>
<tr>
<td>PBS</td>
<td>13.5 ± 3.6</td>
</tr>
<tr>
<td>AdGFP</td>
<td>15.8 ± 3.5</td>
</tr>
<tr>
<td>AdSATB1</td>
<td>27.4 ± 4.2</td>
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Normal means rats without treatment. * P < 0.05 vs. PBS or AdGFP group.

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We observed that AdSATB1 administration inhibited the development of liver fibrosis as confirmed by H&E, Masson’s trichrome, and Sirius Red staining, compared with AdGFP injection (Fig. 6A–C). Meanwhile, a quantitative analysis revealed significantly lower hydroxyproline content in rats receiving AdSATB1 (Fig. 6D). In addition, AdSATB1 administration significantly improved hepatic serum biochemical parameters (Table 1). In line with the in vitro results, the data indicated that, following AdSATB1 treatment, the expression of α-SMA, CTGF, and COL1A1 decreased when compared with AdGFP group (Fig. 7). These genes were mainly producing by activated HSCs [31], which indicated that SATB1 may inhibit HSCs activation in vivo.

4. Discussion

Our results showed that the expression of SATB1 was detected in liver non-parenchymal cells but not hepatocytes in normal liver tissues. It is consistent with previous studies that the expression of SATB1 could be detected in liver tumor samples but hardly detected in healthy liver tissues [32]. Besides, SATB1 expression...
transcription factor-binding sites including Smad, AP-1, NF-

expression by SATB1. It has been reported that there are many signaling pathway was involved in the inhibition of CTGF SATB1 modulated numerous genes involved in TGF-

MAPK signaling pathways[34]. Therefore, we sought to investigate for 7 days or treated with TGF-

activation. a decrease in SATB1 expression using all of these methods for HSCs can be detected in HSCs. Primary rat HSCs were studied in the quiescent phenotype and after their activation in vivo (isolated from rats with TAA-induced fibrosis) and in vitro (cultured on plastic for 7 days or treated with TGF-β1). The present data demonstrated a decrease in SATB1 expression using all of these methods for HSCs activation.

Next, we investigated the role of SATB1 on HSCs activation. Our results reveal that SATB1 dramatically inhibits expression of fibrosis-related genes (α-SMA, CTGF, and COL1A1) in LX-2 and rat HSCs, providing evidence that SATB1 negatively regulates HSCs activation and collagen synthesis. Moreover, SATB1 inhibits LX-2 cells proliferation and cyclin E1 expression. Additionally, the migration of LX-2 cells is also inhibited by SATB1. These findings imply that SATB1 can inhibit the process of HSCs activation.

CTGF, an important pro-fibrotic mediator, is mainly produced from activated HSCs and plays an important role in HSCs activation and liver fibrosis [22,24,25,33]. Previous studies reported that SATB1 modulated numerous genes involved in TGF-β1, Wnt, and MAPK signaling pathways [34]. Therefore, we sought to investigate which signaling pathway was involved in the inhibition of CTGF expression by SATB1. It has been reported that there are many transcription factor-binding sites including Smad, AP-1, NF-κB, and Ets-1 in the promoter region of the CTGF gene [35–37]; and CTGF expression is modulated in Smad-dependent and Smad-independent pathways [38–40]. Our results showed that SATB1 had no effect on the Smad pathway in LX-2 cells. Surprisingly, SATB1 inhibited the phosphorylation of Ets-1 and its nuclear accumulation. Moreover, Ets-1 siRNA could attenuate elevation of CTGF expression caused by knock down of SATB1. The results were in line with previous studies that found that phosphorylation of Thr38 on Ets-1 was associated with increased transcriptional activity; increased transcriptional activity of Ets-1 led to expression of target genes, including CTGF [41–43]. Numerous studies have also suggested that Ets-1 transcription and activation are induced by upstream kinases MAPKs, including P38, MEK/ERK, and JNK [28,29,44]. Our research showed that SATB1 significantly inhibited the phosphorylation of ERK1/2 and its upstream MEK1/2. Furthermore, the activation of Ets-1 and subsequent induction of CTGF expression caused by knock down of SATB1 were inhibited by MEK1/2 inhibitors (U0126 and PD98059). These results indicate that SATB1 might inhibit MEK/ERK/Ets-1 pathway to repress CTGF expression.

Ras proteins, a member of the superfamily of small GTPase, recruited Raf-1 to the membrane through activation of the Ras molecules via the GTP/GDP exchange, and subsequently activated MEK and ERK [30,45]. In pancreatic cancer cells, activation of the Ras/MEK/ERK pathway was required for CTGF induction [46]. Our study found that SATB1 significantly inhibited activation of Ras and membrane translocation of Raf-1. Furthermore, treatment of SATB1-knockdown cells with manumycin A (a Ras inhibitor) and GW5074 (a Raf-1 inhibitor) attenuated elevation of CTGF expression, as well as the phosphorylation of MEK, ERK, and Ets-1. These results showed that the Ras/Raf-1/MEK/ERK/Ets-1 signaling pathway participated in the inhibition of CTGF expression by SATB1 (Fig. 8).

Eventually, animal experiments were carried out to prove the effect of SATB1 in vivo. Consistent with the in vitro data, adenovirus-mediated SATB1 administration significantly attenuated ECM deposition and improved liver function compared with AdGFP or PBS injection. Additionally, the expression of α-SMA, CTGF, and COL1A1 were also decreased after AdSATB1 injection. These genes were mainly produced by activated HSCs [31], which indicated that SATB1 may attenuate liver fibrosis through deactivating HSCs.

However, contrasting results have been found in some cancer cell lines, where SATB1 overexpression promotes CTGF expression, cell proliferation and migration [12–14]. Notably, the different roles of SATB1 in different cell types have been reported. A recent study revealed that SATB1 had an opposite effect on cell proliferation between mouse embryonic fibroblasts (MEFs) and tumor cells [47]. Coincidentally, different responses to SATB1 overexpression by the different MCF10A cell lines was attributed to their disparate gene expression patterns [48]. One potential interpretation is that the genetic makeup of cells critically determines the function of SATB1. Anyway, the molecular and cellular mechanism underlying such events requires further investigation.

In conclusion, the data reported in this study have shown for the first time to our knowledge that SATB1 was involved in the regulation of HSCs activation and liver fibrosis. SATB1 expression negatively correlated with HSCs activation and, in addition, inhibited HSCs activation, proliferation, and migration. Additionally, the CTGF expression was inhibited by SATB1 through the Ras/Raf-1/MEK/ERK/Ets-1 pathway in LX-2 cells. Furthermore, the in vivo study showed that adenovirus-mediated SATB1 injection can efficaciously inhibit HSCs activation and attenuate rat-liver fibrosis and improve liver function. Collectively, the evidence points to the important role of SATB1 in HSCs activation and fibrosis.

Conflicts of interest

Authors declare no conflict of interest associated with this manuscript.

Acknowledgement

This study is fully supported by National Natural Science Foundation of China (Nos. 81270507 and 81272657).

Fig. 8. Schematic diagram of the signaling pathways involved in inhibition of CTGF expression by SATB1 in LX-2 cells. SATB1 inhibits CTGF expression by inactivation of Ras, Raf, MEK, ERK, and Ets-1, which suppresses DNA binding activity of Ets-1 to the Ets-1 site, resulting in transcriptional repression of CTGF expression.
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.febslet.2015.04.010.

References