**GATA3 promotes autophagy and activation of hepatic stellate cells through regulating miR-370/HMGB1 pathway**

**Running title:** Role of GATA3 in hepatic fibrosis.

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**Abstract**

**Background** Hepatic stellate cell (HSC) activation is the central link in the occurrence of hepatic fibrosis (HF). This work attempted to determine whether GATA-binding protein 3 (GATA3) can affect HFprogression by regulatingHSC activation.

**Methods**HF mouse model was constructed by administration of CCl4. Enzyme-linked immunosorbent assay and histological analysis were performed to detect the pathological changes of liver tissues. *In vitro*, HSCs were treated with TGF-β1 as HF cell model. Gene and protein expressions were detected by quantitative real-time PCR and western blotting. Autophagy was monitored by observing the GFP-LC3 puncta under fluorescence microscopy. The interaction between miR-370 and high mobility group box 1 protein (HMGB1) was verified by Luciferase reporter assay.

**Results** CCl4-induced HF mice exhibited an increase of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and severe damage and fibrosis of liver tissues. GATA3 and HMGB1 were up-regulated, and miR-370 was down-regulated in CCl4-induced HF mice and TGF-β1-treated HSCs. GATA3 overexpression enhanced the expression of autophagy-related proteins LC3-II/LC3-I, Beclin 1 and HSC activation markers α-SMA and collagen I in TGF-β1-treated HSCs. 3-methyladenine reversed GATA3 overexpression-induced autophagy and activation of HSCs. Moreover, GATA3 overexpression reduced miR-370 expression and enhanced HMGB1 expression in HSCs. The miR-370 inhibited HMGB1 expression by targeting HMGB1. GATA3 overexpression-mediated promotion of autophagy and activation of HSCs was abrogated by miR-370 up-regulation or HMGB1 knockdown.

**Conclusion** GATA3 promotes autophagy and activation of HSCs by regulating regulate miR-370/HMGB1 signaling pathway, suggesting that GATA3 may be a potential target for prevention and treatment of HF.

**Abstract**

Hepatic fibrosis (HF) is a common result of the repair process of various chronic liver diseases. Hepatic stellate cell (HSC) activation is the central link in the occurrence of HF. This work attempted to determine the functional role of GATA-binding protein 3 (GATA3) in HF. HF mouse model was constructed by administration of CCl4. Enzyme-linked immunosorbent assay and histological analysis results showed that CCl4-induced HF mice exhibited an increase of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and severe damage and fibrosis of liver tissues. Furthermore, HSCs were treated with TGF-β1 as HF cell model. GATA3 and high mobility group box 1 protein (HMGB1) were up-regulated, and miR-370 was down-regulated in CCl4-induced HF mice and TGF-β1-treated HSCs. GATA3 overexpression enhanced the expression of autophagy-related proteins LC3-II/LC3-I, Beclin 1 and HSC activation markers α-SMA and collagen I in TGF-β1-treated HSCs. 3-methyladenine (autophagy inhibitor) reversed GATA3 overexpression-induced autophagy and activation of HSCs. Moreover, GATA3 overexpression reduced miR-370 expression and enhanced HMGB1 expression in HSCs. Luciferase reporter assay verified that miR-370 interacted with HMGB1. The miR-370 inhibited HMGB1 expression by directly targeting HMGB1. GATA3 overexpression-mediated promotion of autophagy and activation of HSCs was abrogated by miR-370 up-regulation or HMGB1 knockdown. In conclusion, this work demonstrates that GATA3 promotes autophagy and activation of HSCs by regulating regulate miR-370/HMGB1 signaling pathway, which contributes to accelerate HF. Thus, this work suggests that GATA3 may be a potential target for prevention and treatment of HF.

**Abstract (200字)**

Hepatic stellate cell (HSC) activation is the central link in hepatic fibrosis (HF). This work attempted to determine the functional role of GATA-binding protein 3 (GATA3) in HF. HF mouse model was constructed by administration of CCl4. CCl4-induced HF mice exhibited an increase of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and severe damage and fibrosis of liver tissues. Furthermore, HSCs were treated with TGF-β1 as HF cell model. GATA3 and high mobility group box 1 protein (HMGB1) were up-regulated, and miR-370 was down-regulated in CCl4-induced HF mice and TGF-β1-treated HSCs. GATA3 overexpression enhanced the expression of LC3-II/LC3-I, Beclin 1 and α-SMA and collagen I in TGF-β1-treated HSCs. 3-methyladenine (autophagy inhibitor) reversed GATA3 overexpression-induced autophagy and activation of HSCs. Moreover, GATA3 overexpression reduced miR-370 expression and enhanced HMGB1 expression in HSCs. The miR-370 inhibited HMGB1 expression by directly targeting HMGB1. GATA3 overexpression-mediated promotion of autophagy and activation of HSCs was abrogated by miR-370 up-regulation or HMGB1 knockdown. In conclusion, GATA3 promotes autophagy and activation of HSCs by regulating regulate miR-370/HMGB1 signaling pathway, which contributes to accelerate HF. Thus, this work suggests that GATA3 may be a potential target for prevention and treatment of HF.

**Significance**

This work demonstrates that GATA3 regulates miR-370/HMGB1 signaling pathway to promote autophagy and activation of HSCs, which contributes to accelerate HF. Thus, this work suggests that GATA3 may be a potential target for prevention and treatment of HF.

**Keywords:** GATA3; autophagy; hepatic stellate cell activation; hepatic fibrosis

**Introduction**

Hepatic fibrosis (HF) is a repair response of the organism to chronic damage caused by various factors, which leads to abnormal deposition of extracellular matrix (ECM) in liver tissues and damage to the structure and function of liver tissues [[1](#_ENREF_1)]. HF is a necessary pathological stage for various chronic liver diseases to develop into liver cirrhosis. If the damaging factors cannot be removed for a long time, HF will develop into liver cirrhosis, even liver cancer [[2](#_ENREF_2)]. Liver cirrhosis causes 1.2 million deaths worldwide each year, ranking as the 10th leading cause of death among the most developed countries [[3](#_ENREF_3)]. Therefore, exploring the pathogenesis and therapeutic targets of HF is still an important issue to be solved urgently.

HF is characterized by the activation of hepatic stellate cells (HSCs) and deposition of ECM, and the activation of HSC is the central link in the occurrence of HF [[4](#_ENREF_4)]. In normal liver tissues, HSCs are in a non-proliferative resting state. In the process of liver injury, HSCs are activated and transformed into myofibroblasts, which in turn participate in damage repair and promote the occurrence of HF [[5](#_ENREF_5)]. In addition, the content of lipid droplets in activated HSCs is reduced, the proliferation capacity of activated HSCs is enhanced. The activated HSCs also secrete pro-inflammatory and fibrotic factors, and specifically express α-SMA and collagen I [[6](#_ENREF_6), [7](#_ENREF_7)]. Thus, the continuous activation of HSCs eventually leads to the progression of HF and even liver cirrhosis. Furthermore, the expression of autophagy-related gene LC3 in liver tissues of CCl4-induced HF mouse model is significantly increased, indicating that autophagy is activated in HF [[8](#_ENREF_8)]. Autophagic activity is significantly enhanced in HSCs isolated from fibrotic liver tissues of hepatitis B patients [[9](#_ENREF_9)]. The activation of HSCs is dependent on autophagy, as autophagy-mediated lipid degradation provides energy for HSC activation [[10](#_ENREF_10)]. The treatment of autophagy inhibitors such as 3-methyladenine (3-MA) causes a significant down-regulation of α-SMA and type I Collagen expression, and induces cell cycle arrest in G2 phase, thereby inhibiting the proliferation and activation of HSCs [[11](#_ENREF_11)]. Both autophagy inhibitor papulomycin and suppression of ATG7 inhibit the accumulation of type I collagen in HSCs [[12](#_ENREF_12)]. Therefore, blocking autophagy and HSC activation may be the therapeutic strategies to control or prevent the progression of HF.

GATA-binding protein 3 (GATA3) is a transcription factor that belongs to the GATA family. GATA3 regulates the downstream molecules through the combination of zinc finger structure and consensus sequence [T/A(GATA)A/G] [[13](#_ENREF_13)]. A previous study has confirmed that GATA3 can regulate the expression of multiple microRNAs (miRNAs). For example, GATA3 regulates the differentiation process of T cells by targeting miR-135 and miR-126 [[14](#_ENREF_14)]. GATA-3 overexpression reduces interferon-gamma levels in the lung by promoting the development of pulmonary fibrosis [[15](#_ENREF_15)]. GATA3 is also expressed in activated HSCs and contributes to HF by down-regulating PPARγ [[16](#_ENREF_16)]. Moreover, high mobility group box 1 protein (HMGB1), which has binding sites in the sequence of the anti-fibrosis molecule miR-370, can promote autophagy and participate in multiple organ fibrosis [[17](#_ENREF_17)]. Therefore, we wondered to known whether GATA3 can regulate miR-370/HMGB1 signaling pathway to promote autophagy and activation of HSCs. Here, we and investigated the mechanism of action of GATA3 in HF through *in vivo* and *in vitro* assays.

**Materials and methods**

**Animals**

Male C57BL/6 mice (6 weeks old, 20-22 g) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, China). All mice were raised in a controlled humidity (20-22°C) and temperature (40%-60%), and a 12 h light/dark cycle. All animal protocols were approved by the Animal care and Use committee of XXX.

**Mouse model of HF**

HF mouse model was induced by CCl4 as previous described with minor modification [[18](#_ENREF_18)]. Mice were randomly divided into 2 groups (n = 6): CCl4 group: mice were intraperitoneally injected with 10 μL/g CCl4 (20% in olive oil) twice a week for 4 weeks. Control group: mice were intraperitoneally injected with the same dosage of olive oil at the same time intervals.

Mice were intraperitoneally injected with 4% chloral hydrate (400 mg/kg), and then blood samples were collected from orbital sinus of mice following removal of eyeball. Next, mice were euthanized by cervical dislocation. Liver tissues were separated from mice, and then fixed in 4% paraformaldehyde for histological analysis, or snap-frozen in liquid nitrogen and stored at -80°C for quantitative real-time PCR (qRT-PCR) and \western blotting.

**Cell culture**

Primary mouse hepatic stellate cells (HSCs) were isolated from liver tissues of C57BL/6 mice following previously described [[19](#_ENREF_19)]. Following perfusion in situ, the liver tissues were separated from mice, and sliced into small pieces and digested with Life Technologies Liver Digestion Media (Invitrogen, Carlsbad, CA, USA). The liver digests were filtered through a cell strainer, and washed with Gey’s balanced salt solution (GBSS; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 2 mg/mL DNase I (Beyotime, Shanghai, China). The homogenate was centrifuged at 2000 × *g* for 5 min to remove the hepatocytes. The cell pellet was resuspended in 15% OptiPrep (Sigma-Aldrich) and subjected to density gradient centrifugation. The final cell pellet was resuspended in Dulbecco's modified eagle medium (DMEM; Gibco, Grand Island, NY, USA) containing 1% penicillin/streptomycin, and 10% fetal bovine serum (FBS, Gibco), and incubated on uncoated plastic at 37°C and 5% CO2 for 24 h. After that, the adherent cells were collected by centrifugation. Cell viability was assessed by trypan blue, and cell viability greater than 90% can be used for subsequent experiments. HSCs were treated with 10 ng/mL TGF-β1 for 72 h as HF cell model. HSCs were treated with 10 mM 3-MA (autophagy inhibitor; Sigma-Aldrich) for 24 h.

**Cell transfection**

The overexpression vectors pcDNA3.1 carrying GATA3 (pcDNA-GATA3), empty pcDNA3.1 (vector), miR-370 mimic and mimic NC were obtained from GeneChem (Shanghai, China). The gene knockdown vectors small interference RNA (siRNA) specifically targeting GATA3 (si-GATA3) or HMGB1 (si-HMGB1), si-NC, miR-370 inhibitor and inhibitor NC were bought from GeneChem. HSCs were transfected with vectors utilizing Lipofectamine 2000 Transfection Reagent (Invitrogen) at room temperature for 20 min.

**Histological analysis**

Liver tissues were fixed in 4% paraformaldehyde and embedded in paraffin. The paraffin sections were dewaxed with xylene and hydrated with concentration gradient of ethanol. The sections were stained with Hematoxylin and Eosin (HE) Staining Kit (Beyotime) to examine the histopathologic changes of liver tissues. Szapiel scoring system was used to evaluate the degree of inflammatory response [[20](#_ENREF_20)]. The sections were stained with Masson Trichrome Stain Kit (Solarbio, Beijing, China) to assess hepatic fibrosis. Ashcroft scoring system was utilized to assess the degree of hepatic fibrosis [[21](#_ENREF_21)].

**Enzyme-linked immunosorbent assay (ELISA)**

After standing for 1-2 h, the blood samples were centrifuged at 3000 rpm for 15 min, and the serum was collected. The serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in mice were detected using Mouse ALT ELISA Kit and Mouse AST ELISA Kit. The absorbance of samples was detected on a Multiskan FC Automatic microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). A series of concentration gradient standards were used to draw a standard curve. The absorbance value of the sample was plugged into formula of the corresponding standard curve to calculate the concentration of samples.

**Quantitative real-time PCR (qRT-PCR)**

The qRT-PCR was utilized to measure the gene expression in liver tissues and HSCs. Total RNA was extracted from cells or tissues using Total RNA Extraction Kit (Solarbio), followed by examination of RNA integrity on 1.5% agarose gel electrophoresis. The cDNA was generated using PrimeScript™ RT reagent Kit (Takara, Tokyo, Japan). PCR reactions were performed applying TB Green® Premix Ex Taq™ II (Takara). The amplification protocol was shown as follow: preheating at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing for 30 s and extension at 72 °C for 30 s. GAPDH served as a loading control for GATA3 and HMGB1. U6 served as a loading control for miR-370. The primers used in qRT-PCR were listed in Table 1. The data were analyzed using 2-∆∆CT method for quantification.

**Western blotting**

Total protein was extracted from liver tissues and HSCs utilizing Total Protein Extraction Kit (Solarbio). Protein concentration was detected using BCA Protein Assay Kit (Solarbio), followed by separation on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis. The separated proteins were transferred onto polyvinylidene fluoride membranes. The membranes were incubated with the primary antibodies, GATA3 (#ab199428, 1:1000), HMGB1 (#ab18256; 1:1000), LC3 (#ab192890; 1:2000), Beclin 1 (#ab210498; 1:1000), α-SMA (#ab5694; 1:1000), collagen I (#ab270993; 1:1000) or GAPDH (#ab9485; 1:2500) at 4°C overnight, and then incubated with goat anti-rabbit horseradish peroxidase-IgG (#ab6721; 1:2000) at room temperature for 1 h. All antibodies were obtained from Abcam (Cambridge, MA, USA). The gray levels of bands were analyzed by Image J software.

**Autophagy**

Autophagy was examined by quantification of fluorescent autophagosomes in HSCs following transfection of GFP-LC3 according to the previous described [[22](#_ENREF_22)]. HSCs were cultured in DMEM at 37°C for 24 h. HSCs were transfected with lentiviral-mediated GFP-LC3 plasmids utilizing 5 mg/mL Polybrene for 6 h. The GFP fluorescent puncta was observed under fluorescence microscopy. Five visual fields were randomly selected, ten cells were selected in each field, and the number of GFP fluorescent puncta in each cell.

**Luciferase reporter assay**

The wild-type (WT)/mutant type (Mut) of pGL3 vector carrying 3’ untranslated regions (UTR) of HMGB1 containing the predicted miR-370 binding sites (pGL3-HMGB1) were synthesized by GeneChem. 293T cells were transfected with the WT/Mut pGL3-HMGB1 and miR-370 mimic or mimic NC. The luciferase activity of the cells was detected using the luciferase assay system (Ambion, Austin, TX, USA).

**Statistical analysis**

Each assay was carried out for 3 times. All data reported as mean ± standard deviation. GraphPad Software (San Diego, CA, USA) was used for statistical analysis. Two-tailed Student’s *t* test and one-way ANOVA were used to analyze the statistical difference. *P* < 0.05 was considered as a significant difference.

**Results**

**GATA3 and HMGB1 were up-regulated, miR-370 was down-regulated in CCl4-induced HF mice.**

In order to investigate the functional role of GATA3 in HF, we constructed HF mouse model by administration of CCl4. The serum levels of ALT and AST in CCl4-induced HF mice were examined by ELISA. ALT and AST are biochemical markers of liver damage [[23](#_ENREF_23)]. The serum levels of ALT and AST were increased in CCl4-induced HF mice with respect to normal mice. Then, HE staining and Masson staining were carried out to examine the histopathologic changes of liver tissues. CCl4-induced HF mice had a higher szapiel score than normal mice (Figure 1C). Normal mice exhibited a normal structure of liver tissues. Severe inflammatory cell infiltration and hepatocyte necrosis occurred in the liver tissues of CCl4-induced HF mice (Figure 1D). Compared with normal mice, the levels of collagenous fiber in CCl4-induced HF mice were observed increased (Figure 1E). The ashcroft score of CCl4-induced HF mice also higher than that in normal mice (Figure 1F). Additionally, the results of qRT-PCR and western blotting revealed that miR-370 expression was decreased, GATA3 and HMGB1 were up-regulated in CCl4-induced HF mice (Figure 1G-J). Thus, miR-370, GATA3 and HMGB may be associated with the development of HF.

**GATA3 overexpression promoted autophagy and HSC activation.**

Next, we treated HSCs with TGF-β1 to mimic HF *in vitro*, and GATA3 expression was significantly elevated in the TGF-β1-treated HSCs (Figure 2A). Then, we overexpressed or knocked down GATA3 in HSCs. The mRNA and protein expression of GATA3 was enhanced in HSCs following transfection of GATA3 overexpression vector, while GATA3 mRNA and protein expression was reduced in HSCs in the presence of si-GATA3 (Figure 2B-D). Moreover, we examined the impact of GATA3 on autophagy and HSC activation by western blotting. The expression of autophagy markers LC3-II/LC3-I and Beclin 1, and the expression of HSC activation markers α-SMA and collagen I were obviously increased in the TGF-β1-treated HSCs. Nevertheless, these proteins in the TGF-β1-treated HSCs were repressed by GATA3 deficiency (Figure 2E-H). In addition, we used 3-MA to inhibit autophagy in the TGF-β1-treated HSCs. GATA3 overexpression elevated the number of punctate GFP-LC3 in the TGF-β1-treated HSCs, which was rescued by 3-MA treatment (Figure 3A-B). 3-MA treatment reversed GATA3 overexpression-mediated promotion of α-SMA and collagen I in the TGF-β1-treated HSCs (Figure 3C-D). Thus, these findings indicated that GATA3 overexpression activated HSCs by promoting autophagy.

**GATA3 inhibited miR-370 expression and enhanced HMGB1 expression in TGF-β1-treated HSCs.**

The regulatory mechanism among GATA3, miR-370 and HMGB1 in HSCs was determined. The results obtained from qRT-PCR and western blotting uncovered that GATA3 overexpressed caused a decrease of miR-370 expression, GATA3 up-regulation enhanced HMGB1 mRNA and protein expression in the TGF-β1-treated HSCs. However, GATA3 deficiency led to an opposite result (Figure 4A-C). Luciferase reporter assay demonstrated that the luciferase activity was severely decreased in the 293T cells in the presence of miR-370 mimic and WT pGL3-HMGB1, indicating that miR-370 interacted with HMGB1 (Figure 4D-E). Furthermore, HMGB1 mRNA and protein expression was obviously decreased in TGF-β1-treated HSCs in the presence of miR-370 mimic, whereas miR-370 suppression increased HMGB1 mRNA and protein expression in TGF-β1-treated HSCs (Figure 4F-H). GATA3 overexpression-induced up-regulation of HMGB1 in TGF-β1-treated HSCs was abolished by transfection of miR-370 mimic (Figure 4I-K). In short, these results revealed that GATA3 repressed miR-370 expression and elevated HMGB1 expression in TGF-β1-treated HSCs.

**GATA3 overexpression promoted autophagy and HSC activation by regulating miR-370/HMGB1 axis.**

Finally, we determined the influence of GATA3 on autophagy and HSC activation. The number of punctate GFP-LC3 in TGF-β1-treated HSCs was increased by GATA3 overexpression. GATA3 up-regulation-mediated promotion of autophagy was reversed by miR-370 overexpression or HMGB1 knockdown (Figure 5A-B). The expression of LC3-II/LC3-I, Beclin 1, α-SMA and collagen I was elevated in TGF-β1-treated HSCs following transfection of pcDNA-GATA3. Both miR-370 up-regulation and HMGB1 deficiency reversed GATA3 overexpression-mediation up-regulation of LC3-II/LC3-I, Beclin 1, α-SMA and collagen I in TGF-β1-treated HSCs (Figure 5C-F). To sum up,GATA3 overexpression promoted autophagy and HSC activation by regulating miR-370/HMGB1 axis.

**Discussion**

GATA3 has been reported to promote proliferation and differentiation of various tissues and cells, such as lymphocytes, thymocytes, sympathetic nervous system and hair follicles [[24](#_ENREF_24)]. GATA3 also participates in the progression of various diseases. For instance, Gata3 deficiency activates epithelial-mesenchymal transition to induce poorly-differentiated mammary tumors in mice, and thus promotes the initiating and metastatic potential of human breast cancer [[25](#_ENREF_25)]. PM2.5 exposure upsets the balance between Th1 and Th2 by promoting GATA3 expression and inhibiting Runx3 expression, thereby evoking the allergic airway inflammation response in the asthmatic mice [[26](#_ENREF_26)]. GATA3 expression is decreased in hepatocellular carcinoma, and closely associated with the tumor size, tumor node metastasis stage and lymph node metastasis. GATA3 represses the malignant phenotypes of hepatocellular carcinoma by regulating slug expression [[27](#_ENREF_27)]. In the present study, we constructed a HF mouse model by administration of CCl4, and determined the functional role of GATA3 in HF. CCl4-induced HF mice displayed an increase of ALT and AST levels, and severe damage in liver tissues and hepatic fibrosis. GATA3 was highly expressed in the liver tissues of CCl4-induced HF mice. GATA3 expression was also increased in the TGF-β1-treated HSCs. These data suggested that GATA3 may take part in HF development. A previous study has confirmed that adipocyte-derived hormone leptin enhances GATA3 expression to play a unique role in accelerating liver fibrosis [[16](#_ENREF_16)], which is consistent with our results. Moreover, GATA3 overexpression activated autophagy in TGF-β1-treated HSCs by elevating LC3-II/LC3-I ratio and Beclin 1 expression. The expression of α-SMA and collagen I in TGF-β1-treated HSCs were enhanced by GATA3 up-regulation. α-SMA and collagen I are biomarkers for the activated HSCs [[6](#_ENREF_6)]. 3-MA treatment reversed GATA3 overexpression-mediated activation of HSCs by inhibiting autophagy, indicating that GATA3 overexpression activated HSCs by accelerating autophagy.

HMGB1 takes part in the development of various liver diseases. HMGB1 has an irreplaceable role in ductular reaction, and promotes tumor progression in autophagy-deficient livers [[28](#_ENREF_28)]. HMGB1 is highly expressed in the liver tissues of non-alcoholic fatty liver disease mouse model and patients, and HMGB1 is associated with non-alcoholic fatty liver disease-related hepatic fibrogenesis [[29](#_ENREF_29)]. Li et al. have confirmed that HMGB1 induces autophagy and activation of HSCs through regulating ERK/JNK/MAPK and mTOR/STAT3 signaling pathways [[30](#_ENREF_30)]. The present study showed that GATA3 increased HMGB1 expression in HSCs. Moreover, miR-370 inhibited HMGB1 expression in HSCs by interacting with HMGB1. GATA3 overexpression-induced autophagy and activation of HSCs was abrogated by HMGB1 knockdown.

The miR-370 has been reported to exert an anti-fibrotic effect by maintaining the quiescent phenotype of normal HSCs, inhibiting proliferation and activation of HSCs [[31](#_ENREF_31)]. Previous study has confirmed that miR-370 expression is decreased in fibrotic liver tissues of rats and TGF-β1-treated HSCs, and miR-370 up-regulation inhibits activation of HSCs and attenuates liver fibrosis in rats by inhibiting SMO expression [[32](#_ENREF_32)]. Our data also found that miR-370 was down-regulated in the liver tissues of CCl4-induced HF mice. Furthermore, miR-370 expression was repressed by GATA3. Overexpression of miR-370 reversed GATA3-induced autophagy and activation of HSCs.

In conclusion, this work demonstrates that GATA3 regulates miR-370/HMGB1 signaling pathway to promote autophagy and activation of HSCs, which contributes to accelerate HF. Thus, this work suggests that GATA3 may be a potential target for prevention and treatment of HF.

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**Figure legends**

**Figure 1 The expression of miR-370, GATA3 and HMGB1 in CCl4-induced HF mice.**

The serum levels of ALT (A) and AST (B) in CCl4-induced HF mice and normal mice were detected by ELISA. (C-D) The histopathologic changes of liver tissues in CCl4-induced HF mice and normal mice were assessed by HE staining. (E-F) The hepatic fibrosis in CCl4-induced HF mice and normal mice were examined by Masson staining. (G) The mRNA expression of miR-370 in liver tissues of CCl4-induced HF mice and normal mice was assessed by qRT-PCR. (H-J) The protein expression of GATA3 and HMGB1 in liver tissues of CCl4-induced HF mice and normal mice was assessed by western blotting analysis. \**P* < 0.05, \*\**P* < 0.01 vs. Control.

**Figure 2 GATA3 overexpression promoted autophagy and HSC activation.**

(A) Western blotting was used to assess the protein expression of GATA3 in HSCs following TGF-β1 treatment. HSCs were transfected with pcDNA-GATA3, vector, si-GATA3 or si-NC, followed by TGF-β1 treatment. The qRT-PCR (B) and western blotting analysis (C-D) were performed to detect the mRNA and protein expression of GATA3 in the HSCs. (E-H) The protein expression of LC3-II, LC3-I, Beclin 1, α-SMA and collagen I in the HSCs was examined by western blotting analysis. \**P* < 0.05, \*\**P* < 0.01 vs. Control or vector; #*P* < 0.05, ##*P* < 0.01 vs. si-NC.

**Figure 3 3-MA treatment inhibited autophagy and HSC activation.**

HSCs were transfected with pcDNA-GATA3 or vector, followed by TGF-β1 or combined with 3-MA treatment. (A-B) Autophagy in HSCs was examined under fluorescence microscopy. (C-D) The protein expression of α-SMA and collagen I in the HSCs was examined by western blotting analysis. \*\**P* < 0.01 vs. vector; #*P* < 0.05 vs. pcDNA-GATA3.

**Figure 4 GATA3 inhibited miR-370 expression and enhanced HMGB1 expression in TGF-β1-treated HSCs.**

HSCs were transfected with pcDNA-GATA3, vector, si-GATA3 or si-NC, followed by TGF-β1 treatment. (A) The qRT-PCR was used to assess the mRNA expression of miR-370 in HSCs. The qRT-PCR (B) and western blotting analysis (C) were performed to detect the mRNA and protein expression of HMGB1 in the HSCs. (D-E) The interaction between miR-370 and HMGB1 was verified by Luciferase reporter assay. HSCs were transfected with miR-370 mimic, mimic NC, miR-370 inhibitor or inhibitor NC, followed by TGF-β1 treatment. The qRT-PCR (F) and western blotting analysis (G-H) were performed to detect the mRNA and protein expression of HMGB1 in the HSCs. HSCs were transfected with pcDNA-GATA3 or vector, and miR-370 mimic or mimic NC, followed by TGF-β1 treatment. The qRT-PCR (I) and western blotting analysis (J-K) were performed to detect the mRNA and protein expression of HMGB1 in the HSCs. \**P* < 0.05, \*\**P* < 0.01 vs. vector, HMGB1 3’-UTR-wt or mimic NC; #*P* < 0.05, ##*P* < 0.01 vs. si-NC, inhibitor NC or pcDNA-GATA3 + mimic NC.

**Figure 5** **GATA3 overexpression promoted autophagy and HSC activation by regulating miR-370/HMGB1.**

HSCs were transfected with pcDNA-GATA3 or vector, and miR-370 mimic or si-HMGB1, followed by TGF-β1 treatment. (A-B) Autophagy in HSCs was examined under fluorescence microscopy. (C-F) The protein expression of LC3-II, LC3-I, Beclin 1, α-SMA and collagen I in the HSCs was examined by western blotting analysis. \*\**P* < 0.01 vs. vector; #*P* < 0.05 vs. pcDNA-GATA3.