Int J Clin Exp Pathol 2014;7(11):7744-7751
www.ijcep.com /ISSN:1936-2625/IJCEP0002339

Original Article

TET3 mediates the activation of human hepatic stellate cells via modulating the expression of long non-coding RNA HIF1A-AS1

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Received September 5, 2014; Accepted October 31, 2014; Epub October 15, 2014; Published November 1, 2014

Abstract: Activated Hepatic stellate cells (HSCs) play a critical role in liver fibrosis and a lot of efforts have been made to dissect the underlying mechanism involved in activation of HSCs. However, the underlying mechanism remains douteux up to now. In the present study, we found that TET3, one member of ten-eleven translocation (TET) protein family, reduced significantly in HSCs LX-2 activated by TGF-β1. To study the function of TET3 in activation of HSCs, knockdown was performed by RNA interference. Results showed that cell proliferation rise significantly and cell apoptosis reduce obviously after knockdown of TET3. Meanwhile, IHC showed that the expression of α-SMA rise significantly compared to control. These results indicated that TET3 is closely associated with the activation of HSCs. Further studies found that long non-coding RNA HIF1A-AS1 was reduced significantly in LX-2 cell after treatment with siRNA for TET3. The result hinted that TET3 activate HSCs through modulating the expression of HIF1A-AS1. To confirm this hypothesis, RNA interference was performed to silence the HIF1A-AS1. Results showed that HIF1A-AS1 silencing lead to enhancing in cell proliferation and declining apoptosis. Taken together, TET3 can mediate the activation of HSCs via modulating the expression of the long non-coding RNA HIF1A-AS1.

Keywords: Liver fibrosis, HSCs, TET3, long non-coding RNA, HIF1A-AS1

Introduction

Liver fibrosis is a common chronic liver disease attributed to wound-healing and scarring response to liver injury, and it has been increased dramatically all over the world. Various etiologies, such as viral infection, alcohol toxicity, autoimmune hepatitis and *Clonorchis sinensis* infection, will trigger liver fibrosis, and some even result in cirrhosis, liver failure or hepatocellular carcinoma [1, 2]. Although extensive studies on liver fibrosis have been reported, the underlying mechanism involved in live fibrosis remains largely elusive. At present, the association between HSCs, as a key fibrogenic cell population of the liver, and the risk of liver fibrosis is well established [3, 4]. It has been reported that activated HSCs play a critical role in liver fibrosis [5]. For example, activated HSCs have been demonstrated to expression of α-SMA and synthesis of extracellular matrix (ECM), both are critical process in liver fibrosis [6, 7]. However, little is known about the underlying mechanisms for the activation of HSCs.

It is well known that DNA methylation at the carbon-5 position of cytosine (5-mC) often leads to gene silencing, affects chromatin structure and gene expression. Due to 5-mC is a rather stable structure, people used to debate how DNA methylation could be erased and whether required [8]. Recently, studies have demonstrated that TET family proteins could lead to DNA demethylation through catalyzing 5-mC to 5-hydroxymethylcytosines (5-hmCs) [9-11]. It has been reported that DNA demethylation mediated by TETs play an important role in diverse tumors including gliomas, breast cancers, liver cancers and so on [12, 13]. However, whether TETs also play an important role in liver fibrosis is still unclear.

It is clear that protein-coding genes are only a small part of the human genome, most tran-

Table 1. PCR primers and sequence information

Name		Sequences (5'-3')
TET3	F	CTTATGGTCAATGGTGTC
	R	GTTCAGGTTGTTGTTGTA
HIF1A-AS1	F	TCATCATCATCATCATCATC
	R	TGCTTCTGTCTCTTCATA
GAPDH	F	GGACCAATACGACCAAATCCG
	R	AGCCACATCGCTCAGACAC

scripts are non-coding RNA (ncRNAs). NcRNAs include small ncRNA (such as siRNAs, miRNAs and piRNAs) and long ncRNAs (LncRNAs). An increasing number of data have demonstrated that miRNAs play an important role in hepatic fibrotic process [14-16]. Over the past several years, accumulating studies has found that LncRNAs also play essential roles in many biological processes, including cell differentiation, cell cycle and apoptosis through comprehensive mechanisms [17, 18]. However, most LncRNAs are still less well characterized and the role of LncRNAs is still unknown in diseases, liver fibrosis is also no exception.

In our preliminary experiment, we found fortunately that the expression of TET3 was significantly down-regulated in hepatic stellate cell line LX-2 activated with TGF-β1, which hinted that TET3 may be involved in the process of the activation of hepatic stellate cell line LX-2. Hence, we designed and conducted this study to dissecting the underlying mechanism of the activation of hepatic stellate cell line LX-2. The study will help to understand the pathogenesis of liver fibrosis disease.

Materials and methods

Cell culture and reagents

Human hepatic stellate cells (HSCs) cell line LX-2 was gift from professor Scott Friedman (Icahn Medical Institute). Cells were cultured in Dulbecco's modified Eagle medium (Gibco; USA) supplemented with 10% fetal bovine serum (Gibco; USA), 100 U/mL penicillin (Gibco; USA) and 100 μg/ml streptomycin (Gibco; USA), and incubated at 37°C in a humidified atmosphere with 5% CO_2. TGF-β1 was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The primary antibodies anti-α-SMA, anti-TET1, anti-TET2, anti-TET3 and anti-Actin were purchased from Abcam, United States. Secondary antibody conjugated horseradish peroxidase were obtained from Beyotime, China.

Activation of cell lines LX-2 by TGF-β1

To obtain the activated HCSs, LX-2 cells were treated with different concentrations of TGF-β1 for 48 h. At the same time, we set up blank control group and PBS control group. Then, the expression of α-SMA, which is a marker of myofibroblast differentiation of HSCs, was analyzed using western blotting.

Cell proliferation assay

We used cell counting Kit-8 (Beyotime, China) to evaluate the proliferation of rat HSCs (HSCs) cell lines LX-2. Briefly, LX-2 cells were transferred into a 96-well cell culture plates at a density of 8000 cells/cm^2, and allowed to attach for 24 h. Then, cells were transfected with siRNAs or si-scramble. All experiments were performed in triplicate. After 48 h, 20 μL CCK-8 was added to each well, and then the plates were incubated for 2 h. Finally, absorbance was measured at 490 nm with a microplate reader (BioRad, USA).

Determination of apoptosis

The extent of apoptosis was detected with AnnexinV-FITC apoptosis detection kit (Beyotime, China). LX-2 cells were planted on 96-well plates and allowed to attach for 24 h. Then, cells were transfected with siRNAs or si-scramble. All experiments were performed in triplicate. After 48 h, cells were harvested and washed twice with cold PBS. Then, cells were stained in 200 μL Annexin Vbinding buffer with 5 μL of Annexin V-FITC and 200 μL Annexin Vbinding buffer with 10 μL PI solution for 10 min at RT. The stained apoptotic cells were analyzed by flow cytometry.

Phalloidin-FITC staining

The cells were fixed in 4% paraformaldehyde at room temperature (RT) for 10 min and washed with PBST for three times. Samples were incubated with 0.25% Triton X-100 at RT for 3 min and then washed with PBST for three times. Then, cells were stained with 1.0 μg/mL phalloidin-FITC (Sigma, United States) for 40 min and washed with PBST for three times. Finally,

Table 2. siRNAs and sequence used in this study

Name		Sequences (5'-3')
SiTET3-1	sense	CCGGCAGUUUGAGGCUGAAUUUGGA
	antisense	UCCAAAUUCAGCCUCAAACUGCCGG
SiTET3-2	sense	CAGUGGCUUCUUGGAGUCACCUCUU
	antisense	AAGAGGUGACUCCAAGAAGCCACUG
SiTET3-3	sense	CCACCUGCGAUUGCGUCGAACAAAU
	antisense	AUUUGUUCGACGCAAUCGCAGGUGG
TETScramble	sense	CCGUGAGUUGGAGUCUAAUUGCGGA
	antisense	UCCGCAAUUAGACUCCAACUCACGG
Si-lncRNA-1	sense	CACCUUAGCCUAUGGUUGUUCAUCU
	antisense	AGAUGAACAACCAUAGGCUAAGGUG
Si-lncRNA-2	sense	CCCUAGCAAGGGCUGUUCCAUGUUU
	antisense	AAACAUGGAACAGCCCUUGCUAGGG
Si-lncRNA-3	sense	CCUAGCAAGGGCUGUUCCAUGUUUA
	antisense	UAAACAUGGAACAGCCCUUGCUAGG
lncRNAScramble	sense	CACAUCGUCUAGGUUUGCUAUCUCU
	antisense	AGAGAUAGCAAACCUAGACGAUGUG

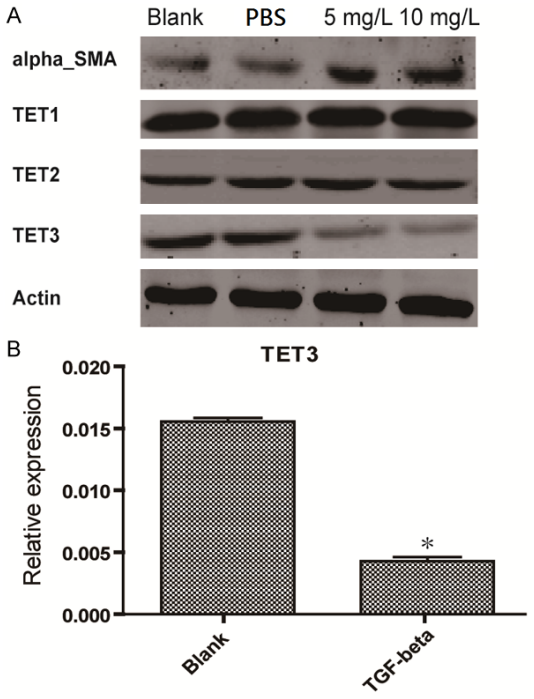

Figure 1. Activation of HSC LX-2 cells induced by TGF-β1 *in vitro*. A. The expression analysis of α-SMA, TET1, TET2 and TET3 proteins by Western blotting in the HCS LX-2 cells activated by TGF-β1. Actin was used as loading control in this experiment; B. The expression analysis of TET3 gene in mRNA level in HCS LX-2 cells activated by TGF-β1 by qRT-PCR assay. Double asterisks represent *P*-value < 0.01.

cells stained with 10 μg/mL DAPI (Beyotime, China) for 10 min. Images were observed and captured with a Nikon fluorescence microscope.

Western blotting analysis

Cells were harvested and homogenized with cell lysis buffer (Beyotime, China). Then, protein concentration amounts were determined by BCA Protein Assay Kit (Beyotime, China). After boiled for 5 min, equal amounts of protein samples were run on denaturing 10% SDS-PAGE and then transferred to nitrocellulose filter (NC) membranes. After being blocked in a 5% skim milk solution, membranes were incubated with specific primary antibodies (anti-α-SMA 1:1000, anti-TET-1 1:1000, anti-TET-2 1:2000, anti-TET3 1:1000 and anti-Actin 1:1000, Abcam, United States), and then incubated with HRP-conjugated secondary antibody (1:2000, Beyotime, China). Finally, protein bands were visualized using an enhanced chemiluminescence (ECL) Western blotting detection system (GE Healthcare, Amersham, UK).

Quantitative real-time PCR

Total RNA was extracted using Trizol reagents (Invitrogen, USA) according to the manufacturer's instructions and diluted to 200 ng/μL. Then, quantitative real-time RT-PCR (QRT-PCR) was performed using One Step SYBR® PrimeScript™ RT-PCR Kit II (TaKaRa, China) according to standard protocol. GAPDH gene was used as an internal control. The QRT-PCR amplification for TET3 was performed as follows: 42°C for 5 min, 95°C for 10 s, followed by 40 cycles of 95°C for 5 s, 60°C for 20 s, and 72°C for 15 s. PCR was followed by a melt curve analysis to determine the reaction specificity. The relative gene expression was calculated by using $2^{-\Delta\Delta ct}$ method. Primers used in qRT-PCR were as shown in **Table 1**.

Small interfering RNA (siRNA) synthesis and transfection

The siRNA oligonucleotides corresponding to the target sequence for TET3 and LncRNA HIF1A-AS1 were synthesized by GenePharma (Shanghai, China). Sequences of si-TET3, si-lncRNA and si-scramble (negative control) were as **Table 2**. The negative control sequence has no homologous to known human genes. All siRNA were transfected into LX-2 cells using

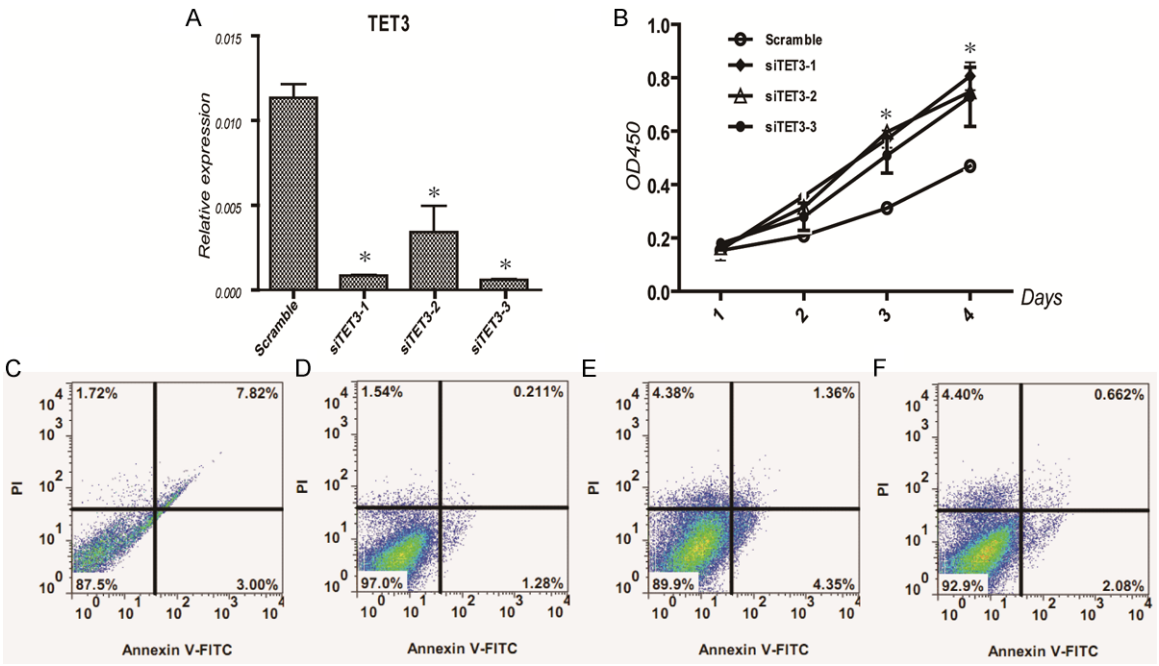

Figure 2. Functional analysis of TET3 by RNA interference in LX-2 cell line. A. The determination of knockdown efficiency of siRNAs for TET3 in LX-2 cells by qRT-PCR. All data are presented as mean ± SD (n=3). Asterisk presents *P*-value < 0.05; B. After RNAi, the proliferation of LX-2 cells was determined by CCK-8 assay. All data are presented as mean ± SD (n=3). Asterisk presents *P*-value < 0.05. C-F. After the knockdown of TET3 by siTET3-3, cell apoptosis was determined by Annexin V-FITC apoptosis detection kit. SiRNA scramble was used as control.

Lipofectamine 2000 according to standard protocol.

Statistical analysis

All data were analyzed with State 7.0 statistical software and are reported as mean ± standard deviation (SD). Statistical significance was determined using Double-sided Student's *t* test. Multiple groups were analyzed using ANOVA. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

Activation of cell lines LX-2 by TGF-β1

In order to activate the human HSCs (HSCs) cell lines LX-2, TGF-β1 was used to treat the cells. alpha-smooth muscle actin (α-SMA) is an important biomarker for the activated HSCs, hence we detected the expression changes of α-SMA in LX-2 treated without/with TGF-β1 to confirm whether the cells have been activated successfully. As shown in **Figure 1A**, compared to control cells, the expression of α-SMA was significantly increased. This result indicated that LX-2 cells were successfully activated by 10 mg/L TGF-β1.

Expression analysis of TET protein family in TGF-β1 treated LX-2 cells

We investigated the expression level of TET1, TET2 and TET3 gene in TGF-β1 treated LX-2 cells using qRT-PCR and Western blotting assay. As shown in **Figure 1A**, after induced with TGF-β1, the protein level of TET-3 significantly decreased. However, the level of TET1 and TET2 has no obvious change. Meanwhile, qRT-PCR results also indicated that cells treated with TGF-β1 result in reduced expression of TET3. All of these results hinted that TET3, which can regulate DNA demethylation, may play a critical role in the activated process of HSCs.

Knockdown TET3 reduces apoptosis, promotes proliferation and induces cell fibrosis in LX-2 cells

To investigate the role of TET3 gene on fibrogenesis of cell lines LX-2, TET3 was silenced using siRNA in LX-2 cells. The interference efficiency was determined using QRT-PCR assay. As shown in **Figure 2A**, the expression level of TET3 in siRNA transfected cells down to 5 or 10-fold compared to scramble group. These result indicated that TET3 was efficiently

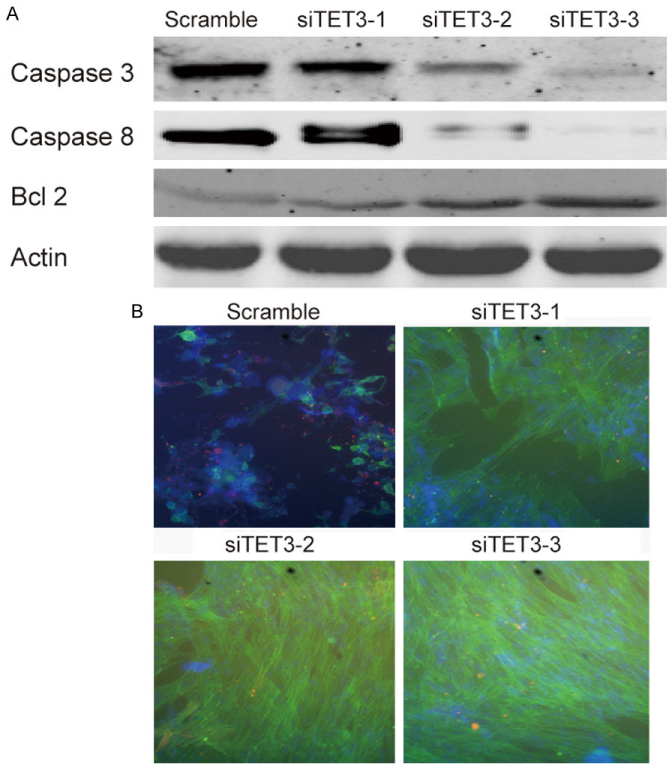

Figure 3. Expression analysis of apoptosis-related protein and α-SMA via Western blotting and immunofluorescence, respectively. A. Apoptosis-related protein analysis by western blotting in LX-2 cell treated with/without siRNAs; B. The expression analysis of α-SMA by immunofluorescencein LX-2 cells treated with/without siRNA. Nucleic acid was marked by DAPI (blue).

silenced in LX-2 cells by siRNAs. Then cell proliferation was measured by a CCK-8 assay. As shown in **Figure 2B**, there was a drastic promotion in the proliferation of cells transfected siTET3. Meanwhile, LX-2 cells apoptosis was detected by Annexin- V FITC/PI double staining assay. As shown in **Figure 2C**, the percentage of apoptotic cells in siScramble group is about 10.8%. However, after cells transfected with siTET3, the percentage of apoptotic cells was down to 1.5%, 5.7% and 2.7% respectively. To further confirm the result, the expression level of Caspase8, Caspase3 and Bcl2 were analyzed using Western blot assay. Compared to scramble group, the expression of Caspase8 and Caspase3 were down-regulated and the expression of Bcl2 was up-regulated (**Figure 3A**).

We also used phalloidin-FITC to mark α-SMA of LX-2 cells and nucleic acid was marked by DAPI. As shown in **Figure 3B**, the expression level of α-SMA was increased in siTET3 groups, and cells morphology also changed. Taken together, these findings indicate that TET3 silencing promotes the activation of LX-2 cell lines. However, it is unknown how does the TET3 activate the HSCs. Therefore, PCR array was performed to explore the underlying mechanism involved in this process.

PCR array

In order to further explore TET3 how to activate the HSCs, the expression profile of long non-coding RNAs was performed after TET3 silence by siRNA. In this experiment, 36 lncRNAs were chose and detected by PCR array. However, 35 lncRNAs were not found obvious changes (data not shown), just 1 lncRNA named HIF1A-AS1 down-regulated significantly after TET3 silence (**Figure 4A**). This result suggested that TET3 can activate the HSCs through modulating the expression of HIF1A-AS1.

LncRNA HIF1A-AS1 silencing reduces apoptosis and promotes proliferation of LX-2 cells

To elucidate the functional role of LncRNA HIF1A-AS1 during activation of LX-2 cells, HIF1A-AS1 was silenced using siRNA. The interference efficiency was determined using qRT-PCR assay (data not shown). After RNA interference, Cells proliferation was measured by a CCK-8 assay. As shown in **Figure 4B**, the proliferation of cells transfected with si-HIF1A-AS1 was increased significantly. Then, LX-2 cells apoptosis was measured by Annexin-V FITC/PI double staining assay. As shown in **Figure 4C**, the percentage of apoptotic cells in si-Scramble group is about 7.9%. However, after cells transfected with si-HIF1A-AS1, the percentage of apoptotic cells was down to 2.4%, 3.1% and 5.6% respectively.

Discussion

It has been reported that activated HSCs play a key role in liver fibrosis. Several studies have explained the mechanism of HSC activation. Zhao, et al reported that platelet-derived growth factor (PDGF) induce HSCs activation through stimulate mitogen-activated protein kinase (MAPK) signaling pathways [19]. Cheng, et al

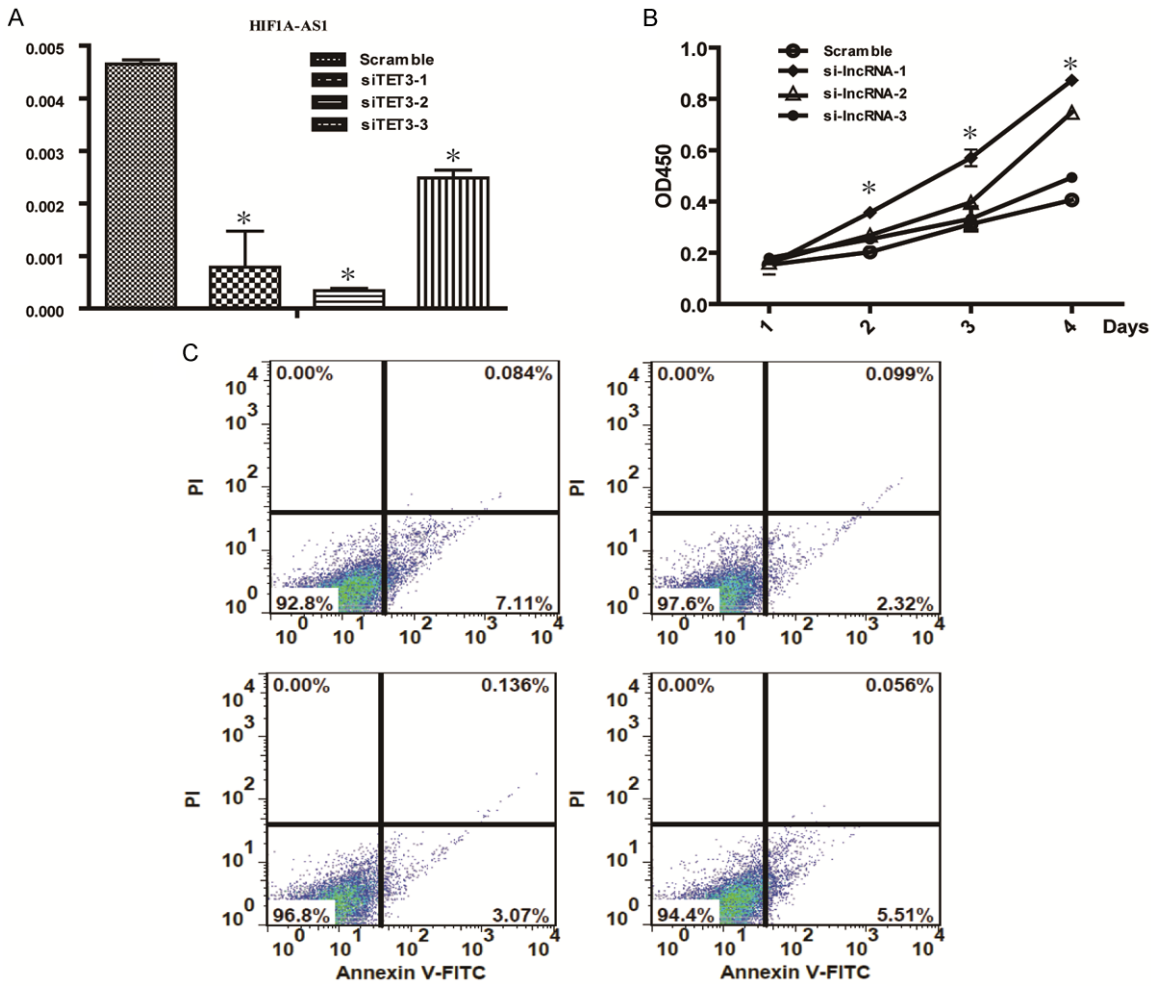

Figure 4. Expression analysis of HIF1A-AS1 in LX-2 cell treated with siTET3 and function of HIF1A-AS1. A. The expression analysis of HIF1A-AS1 by qRT-PCR in LX-2 cell line treated with/without siTET3. All data are presented as mean ± SD (n=3). Asterisk presents *P*-value < 0.05; B. The changes of LX-2 cell proliferation after transfection with si-HIF1A-AS1. All data are presented as mean ± SD (n=3). Asterisk presents *P*-value < 0.05. C. Cell apoptosis analysis by annexin V-FITC/PI double staining assay after cells transfected with si-lncRNA-1.

demonstrated that Wnt pathway is crucial of HSC activation [20]. Although numerous studies have explained the mechanism of HSCs activation, the detailed molecular mechanism is still unknown.

In the present study, we used TGF-β1 to activate the HSCs cell LX-2 *in vitro* and confirmed by detected the expression of α-SMA, which is a biomarker for the activated HSCs because the high expression of α-SMA was found in activated HSCs. In our experiment, the expression of α-SMA significantly increased in LX-2 treated with 10 mg/LTGF-β1. This indicated that HSCs were activated successfully. In order to explore the underlying mechanism involved in the process, we determined the expression of TET protein family in LX-2 treated with 10 mg/LTGF-β1. Results showed that the expression of TET3 was significantly down-regulated after activation, but TET1 and TET2 are not found obvious changes in protein level. So we speculated that TET3 may be associated with the activation of HSCs.

The ten-eleven translocation (TET) family including TET1, TET2 and TET3, have been identified capability of catalyzing 5 mC to 5-hydroxymethylcytosines (5 hmCs). It is well known that DNA methylation at the carbon-5 position of cytosine (5-mC) of tumor suppressor gene often contributes to cancer formation. Recent works have indicated that demethylation catalyzing by TETs lead to tumor suppres-

sion [21, 22]. However, whether TETs also play an important role in liver fibrosis is still unreported. Therefore, we knockdown the TET3 through siRNA experiment to analyze the activation of HSCs. Previous studies showed that the cell proliferation ability increased and cell apoptosis decreased in activated HCSs. So we analyzed the cell viability and apoptosis through CCK-8 and Annexin- V FITC/PI double staining assay. As expected, the cell proliferation rose significantly and cell apoptosis reduced slightly. For further confirm LX-2 cells were activated, we detected the expression of α-SMA using IHC. Compared to control group, the expression of α-SMA rose obviously. All of these indicated that TET3 plays an important role in the process of liver fibrosis. However, it is unknown how does the TET3 activate the HSCs. Recently, LncRNAs have been shown to play important roles in a variety of human disease, such as cancer and pulmonary fibrosis [23, 24]. However, the relationship between LncRNA and liver fibrosis is still unknown. Therefore, PCR array was performed to explore the underlying mechanism involved in this process. Results showed that expression of LncRNA HIF1A-AS1 increased significantly in LX-2 treated with siTET3-1, siTET3-2 and siTET3-3, respectively. Based on those results, we suspected that decreased TET3 can reduce the expression of HIF1A-AS1 which possibly involved in the activated process of LX-2. Hence, we performed RNA interference experiment to knockdown the expression of HIF1A-AS1. Results showed that cell proliferation rose significantly and cell apoptosis declined slightly. All results described above indicated that TET3 can induce the activation of LX-2 by modulating the expression of HIF1A-AS1.

In summary, our current study in an activated model of LX-2 cell lines, induced by TGF-β1, demonstrate that decreased TET3 can promote HSCs activation via suppression of the long non-coding RNA HIF1A-AS1. This finding would be helpful to understand the molecular mechanism of liver fibrosis. Our future analysis will focus on whether TET3 is a potential diagnostic even a therapeutic target for liver fibrosis.

Acknowledgements

This study was supported by the Natural Science Foundation of China (Grant No. 8127-0518), Shanghai Municipal Health Bureau (Grant No. XBR2011012).

Disclosure of conflict of interest

None.

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References

[1] Bataller R and Brenner DA. Liver fibrosis. J Clin Invest 2005; 115: 209-218.

[2] Wang X, Hu F, Hu X, Chen W, Huang Y and Yu X. Proteomic identification of potential Clonorchis sinensis excretory/secretory products capable of binding and activating human hepatic stellate cells. Parasitol Res 2014; 113: 3063-3071.

[3] Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 2008; 88: 125-172.

[4] Lee UE and Friedman SL. Mechanisms of hepatic fibrogenesis. Best Pract Res Clin Gastroenterol 2011; 25: 195-206.

[5] Gressner OA and Gressner AM. Connective tissue growth factor: a fibrogenic master switch in fibrotic liver diseases. Liver Int 2008; 28: 1065-1079.

[6] Olaso E and Friedman SL. Molecular regulation of hepatic fibrogenesis. J Hepatol 1998; 29: 836-847.

[7] Moles A, Sanchez AM, Banks PS, Murphy LB, Luli S, Borthwick L, Fisher A, O'Reilly S, van Laar JM, White SA, Perkins ND, Burt AD, Mann DA, Oakley F. Inhibition of RelA-Ser536 phosphorylation by a competing peptide reduces mouse liver fibrosis without blocking the innate immune response. Hepatology 2013; 57: 817-828.

[8] Wu SC and Zhang Y. Active DNA demethylation: many roads lead to Rome. Nat Rev Mol Cell Biol 2010; 11: 607-620.

[9] Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 2009; 324: 930-935.

[10] Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC and Zhang Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature 2010; 466: 1129-1133.

[11] Pastor WA, Aravind L and Rao A. TETonic shift: biological roles of TET proteins in DNA demethylation and transcription. Nat Rev Mol Cell Biol 2013; 14: 341-356.

[12] Müller T, Gessi M, Waha A, Isselstein LJ, Luxen D, Freihoff D, Freihoff J, Becker A, Simon M, Hammes J, Denkhaus D, zur Mühlen A, Pietsch T, Waha A. Nuclear Exclusion of TET1 Is Associated with Loss of 5-Hydroxymethylcytosine in IDH1 Wild-Type Gliomas. Am J Pathol 2012; 181: 675-683.

[13] Yang H, Liu Y, Bai F, Zhang JY, Ma SH, Liu J, Xu ZD, Zhu HG, Ling ZQ, Ye D, Guan KL, Xiong Y. Tumor development is associated with decrease of TET gene expression and 5-methylcytosine hydroxylation. Oncogene 2012; 32: 663-669.

[14] Ji J, Zhang J, Huang G, Qian J, Wang X and Mei S. Over-expressed microRNA-27a and 27b influence fat accumulation and cell proliferation during rat hepatic stellate cell activation. FEBS Lett 2009; 583: 759-766.

[15] Guo CJ, Pan Q, Li DG, Sun H and Liu BW. miR-15b and miR-16 are implicated in activation of the rat hepatic stellate cell: An essential role for apoptosis. J Hepatol 2009; 50: 766-778.

[16] Zhang Y, Ghazwani M, Li J, Sun M, Stolz DB, He F, Fan J, Xie W, Li S. MiR-29b inhibits collagen maturation in hepatic stellate cells through down-regulating the expression of HSP47 and lysyl oxidase. Biochem Biophysical Res Commun 2014; 446: 940-944.

[17] Gao L, Mai A, Li X, Lai Y, Zheng J, Yang Q, Wu J, Nan A, Ye S, Jiang Y. LncRNA-DQ786227-mediated cell malignant transformation induced by benzo (a) pyrene. Toxicol Lett 2013; 223: 205-210.

[18] Zhu J, Liu S, Ye F, Shen Y, Tie Y, Zhu J, Jin Y, Zheng X, Wu Y, Fu H. The long noncoding rna expression profile of hepatocellular carcinoma identified by microarray analysis. PLoS One 2014; 9: e101707.

[19] Zhao Y, Wang Y, Wang Q, Liu Z, Liu Q and Deng X. Hepatic stellate cells produce vascular endothelial growth factor via phospho-p44/42 mitogen-activated protein kinase/cyclooxygenase-2 pathway. Mol Cell Biochem 2012; 359: 217-223.

[20] Cheng JH, She H, Han YP, Wang J, Xiong S, Asahina K, Tsukamoto H. Wnt antagonism inhibits hepatic stellate cell activation and liver fibrosis. Am J Physiol Gastrointest Liver Physiol 2008; 294: G39-G49.

[21] Bian EB, Zong G, Xie YS, Meng XM, Huang C, Li J, Zhao B. TET family proteins: new players in gliomas. J Neurooncol 2014; 116: 429-435.

[22] Hsu CH, Peng KL, Kang ML, Chen YR, Yang YC, Tsai CH, Chu CS, Jeng YM, Chen YT, Lin FM, Huang HD, Lu YY, Teng YC, Lin ST, Lin RK, Tang FM, Lee SB, Hsu HM, Yu JC, Hsiao PW, Juan LJ. TET1 suppresses cancer invasion by activating the tissue inhibitors of metalloproteinases. Cell Rep 2012; 2: 568-579.

[23] Yang Y, Li H, Hou S, Hu B, Liu J and Wang J. The noncoding RNA expression profile and the effect of lncRNA AK126698 on cisplatin resistance in non-small-cell lung cancer cell. PLoS One 2013; 8: e65309.

[24] Song X, Cao G, Jing L, Lin S, Wang X, Zhang J, Wang M, Liu W, Lv C. Analysing the relationship between lncRNA and protein - coding gene and the role of lncRNA as ceRNA in pulmonary fibrosis. J Cell Mol Med2014; 18: 991-1003.