Biochemical and Biophysical Research Communications 460 (2015) 793–798

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

MiR-520b suppresses proliferation of hepatoma cells through targeting ten-eleven translocation 1 (TET1) mRNA

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ARTICLE INFO

Article history:
Received 17 March 2015
Available online 28 March 2015

Keywords:
miR-520b
TET1
Proliferation
Hepatocellular carcinoma

ABSTRACT

Accumulating evidence indicates that microRNAs are able to act as oncogenes or tumor suppressor genes in human cancer. We previously reported that miR-520b was down-regulated in hepatocellular carcinoma (HCC) and its deregulation was involved in hepatocarcinogenesis. In the present study, we report that miR-520b suppresses cell proliferation in HCC through targeting the ten-eleven translocation 1 (TET1) mRNA. Notably, we identified that miR-520b was able to target 3′-untranslated region (3′UTR) of TET1 mRNA by luciferase reporter gene assays. Then, we revealed that miR-520b was able to reduce the expression of TET1 at the levels of mRNA and protein using reverse transcription-polymerase chain reaction and Western blotting analysis. In terms of function, 5-ethynyl-2-deoxyuridine (EdU) incorporation and colony formation assays demonstrated that the forced miR-520b expression remarkably inhibited proliferation of hepatoma cells, but TET1 overexpression could rescue the inhibition of cell proliferation mediated by miR-520b. Furthermore, anti-miR-520b enhanced proliferation of hepatoma cells, whereas silencing of TET1 abolished anti-miR-520b-induced acceleration of cell proliferation. Then, we validated that the expression levels of miR-520b were negatively related to those of TET1 mRNA in clinical HCC tissues. Thus, we conclude that miR-520b depresses proliferation of liver cancer cells through targeting 3′UTR of TET1 mRNA. Our finding provides new insights into the mechanism of hepatocarcinogenesis.

1. Introduction

MicroRNAs (miRNAs) contain approximately 19–25 nucleotides that suppress gene expression through RNA-induced silencing complex mediated mRNA cleavage or translational repression in a sequence-dependent manner [1,2]. The expression patterns of miRNAs are often altered in different human tumors [3]. Many studies have shown that miRNAs are involved in variety of cellular processes including proliferation, development, differentiation, or tumorigenesis [4–6]. Emerging evidence reveals that miRNAs may serve as a novel class of oncogenes or tumor suppressor genes [7–11]. Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related mortality worldwide and the 5-year survival rate even after surgery has been only 25%–39% [12,13]. Some reports have demonstrated that miRNAs are essential in HCC progression and directly contribute to cell proliferation and metastasis of HCC by targeting a large number of critical protein-coding genes [14–17]. Despite the growing evidence for identification of miRNAs in hepatocarcinogenesis, limited information is available about their roles and the underlying mechanisms in HCC development. Hepatitis B virus (HBV) infection is one of the major causes of HCC. Among the four proteins encoded by HBV, the HBV X protein (HBx) is a multifunctional regulatory protein and plays a crucial role in hepatocellular carcinogenesis. We have reported that HBx is able to transform liver LO2 cells with partner survivin through tumor

Abbreviations: HCC, hepatocellular carcinoma; TET1, ten-eleven translocation 1; 3′UTR, 3′-untranslated region; EdU, 5-ethynyl-2-deoxyuridine; miRNAs, microRNAs; HBV, hepatitis B virus; HBx, HBV X protein; HBXIP, hepatitis B X-interacting protein; MEKK2, mitogen activated protein kinase kinase kinase 2; qRT-PCR, quantitative real-time polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; 5hmC, 5-hydroxymethylcytosine.

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http://dx.doi.org/10.1016/j.bbrc.2015.03.108
0006-291X/

suppressor miR-520b and oncoprotein hepatitis B X-interacting protein (HBXIP), in which miR-520b was down-regulated in HBx/survivin-stably transfected LO2 cells [18]. MiR-520b can target cyclin D1, mitogen activated protein kinase kinase kinase 2 (MEKK2), HBXIP and interleukin-8 in liver cancer and breast cancer [19,20]. However, the role of miR-520b in hepatocarcinogenesis remains poorly understood.

In this study, we investigated the role of miR-520b down-regulation in hepatocarcinogenesis. We show that miR-520b depresses proliferation of liver cancer cells through targeting 3′UTR of TET1 mRNA. Our finding provides fascinating insights into the mechanisms of hepatocarcinogenesis mediated by miR-520b.

2. Materials and methods

2.1. Cell lines and cell culture

The human immortalized liver LO2 were purchased from Nanjing KeyGEN Biotech Co., Ltd (Nanjing, China). Previously, we stably transfected pCMV-HBx and pcDNA3-survivin plasmids into liver LO2 cells and termed it as LO2-X-S cells [18]. LO2 and LO2-X-S cells were cultured in RPMI Medium 1640 (Gibco, Grand Island, NY, USA) containing 100 U/ml penicillin, 100 μg/ml streptomycin and 10% fetal calf serum (FCS, Gibco, Grand Island, NY, USA) in 5% CO_2 at 37 °C.

2.2. Plasmids and construction of the 3′UTR of TET1

FH-TET1-pEF was a gift from Anjana Rao (Addgene plasmid # 49792). The fragment containing the target site of miR-520b in 3′UTR of TET1 mRNA was subcloned into pGL3-control vector (Promega, Madison, WI, USA) immediately downstream of the stop codon of the luciferase gene to generate pGL3-TET1-wt. Mutant construct of TET1 3′UTR, carrying a substitution of 6 nucleotides within the core seed sequence of miR-520b, was named as pGL3-TET1-mut. The primers used in this study for construction were as follows: pGL3-TET1 forward, 5′-GCTCTAGAAAATCAAGACACAC AGTG-3′, reverse, 5′-GTGGCCGGCCGTGAGAGGACACAGTAAA-3′; pGL3-TET1-mut forward, 5′-AATAT ATATTTTAAATCGAGGTACTA TTTTTAAAAGT-3′, reverse, 5′-ACTTTTAAAAATAGTA CCTCGATTTA AAATATATATT-3′.

2.3. Cell transfection

The cells were cultured in a 6-well, 12-well or 24-well plate for 24 h and then were transfected with plasmids, miRNAs or siRNAs. All transfections were performed using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. TET1 siRNA oligonucleotides and a non-specific scrambled control (si-Ctrl), miR-520b (or anti-miR-520b), miRNA control (miRNA Ctrl) and anti-miRNA control (anti-miRNA Ctrl) were synthesized by RiboBio (Guangzhou, China). The siRNA duplexes sequences used were as follows: TET1 siRNA, 5′-GCA-TAUUCCU UUGAAAUAAdTdT-3′.

2.4. Luciferase reporter gene assays

Cells were plated into 24-well plates (3×10^4 cells/well). After 24 h, the cells were co-transfected with the pRL-TK plasmid containing the Renilla luciferase gene (Promega, Madison, WI, USA) and various constructs containing the seed sequence or mutant seed sequence of TET1 3′UTR. At 48 h post-transfection, a standard dual luciferase reporter assay was performed, and the results were normalized using pRL-TK. All experiments were performed at least three times.

2.5. Quantitative real-time polymerase chain reaction (qRT-PCR), reverse transcription- PCR (RT-PCR)

Total RNA was extracted from the cells (or human clinical HCC tissues) using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Reverse transcription was performed using poly (A)-tailed total RNA and reverse transcription primer with ImPro-II Reverse Transcriptase (Promega, Madison, WI, USA) according to the manufacturer's instructions. RT-PCR analysis was performed to detect the expression of TET1 mRNA in the cells treated with miR-520b or anti-miR-520b. The qRT-PCR analysis was explored to test the expression of miR-520b and TET1 in human clinical HCC tissues using Fast Start Universal SYBR Green Master (Rox) (Roche Diagnostics GmbH Mannheim, Germany). The expression of specific genes or miRNAs was tested by the comparative Ct method using $2^{-\Delta\Delta Ct}$. The primers used were as follows: TET1 forward, 5′-GATGACAGAGGTTCTTGCACAT-3′, reverse, 5′-AGGTTGCACGGTCTCAGTGT-3′; GAPDH forward, 5′-CAT CACCATCTTCCAGGAGCG-3′, reverse, 5′-TGACCTTGCCCACAGC CTTG-3′; miR-520b forward, 5′-AAAGTGCTTCCTTTTAGAGGG-3′, reverse, 5′- GCGAGCACAGAATTAATACGAC-3′; U6 forward, 5′-AGAG CCTGTGGTGTCCG-3′, reverse, 5′-CATCTTCAAAGCACTTCCCT-3′.

2.6. Western blotting analysis

Western blotting analysis protocol was described previously [18]. The primary antibodies used were mouse anti-β-actin (Sigma–Aldrich, St Louis, MO, USA), rabbit anti-TET1 (GeneTex, Irvine, CA, USA). All experiments were repeated three times.

2.7. Cell proliferation analysis

Five-ethynyl-2'-deoxyuridine (EdU) incorporation assays were carried out as described previously using the Cell-Light™ EdU imaging detecting kit according to the manufacturer's instructions (RiboBio, Guangzhou, China) [20].

2.8. Colony formation analysis

For colony formation analysis, 48 h after transfection with indicated reagents, 1000 viable transfected cells were placed in 6-well plates and maintained in complete medium for 2 weeks. Once colonies were visible, they were stained with methylene blue and photographed. All assays were repeated at least three times. The colonies were counted using a dissecting microscope.

2.9. Patient samples

Twenty clinical HCC tissues and their corresponding noncancerous liver tissues used in this study were obtained from Tianjin First Center Hospital (Tianjin, China) after surgical resection. Informed consents were obtained from each patient to approve the use of their tissues for research purposes. The study protocol was approved by the Institute Research Ethics Committee at Nankai University.

2.10. Statistical analysis

Each experiment was repeated at least three times. Statistical significance was assessed by comparing mean values (±SD) using a Student's t test for independent groups and was assumed for $^{*}P < 0.05$, $^{**}P < 0.01$, No significant (NS). The expression levels of TET1 in tumor tissues and matched adjacent nontumorous tissues were compared using the Wilcoxon signed rank test. The

correlation between miR-520b levels and TET1 mRNA levels in HCC tissues was determined with Pearson *r*.

3. Result

3.1. MiR-520b directly inhibits the expression of TET1 through targeting its 3′UTR

We have reported that miR-520b is down-regulated in LO2-X-S cells (HBx/survivin-transformed liver LO2 cells) comparing with liver LO2 cells and its target genes are associated with HCC [18]. Here, to better explore the role of miR-520b in the development of liver cancer, we screened other target genes of miR-520b using Targetscan and microRNA.org (http://www.targetscan.org/ and http://www.microrna.org). TET1 was of particular interest, as it is an important enzyme for epigenetic modification of DNA in cancer. We identified the binding site of miR-520b in the 3′UTR of TET1 mRNA (Fig. 1A) and cloned the 3′UTR of TET1 mRNA and its mutants into downstream of pGL3-control luciferase reporter gene vector (named pGL3-TET1-wt or pGL3-TET1-mut), respectively (Fig. 1B). The luciferase reporter gene assays demonstrated that miR-520b significantly suppressed the firefly luciferase activities of pGL3-TET1-wt in a dose-dependent manner, whereas it failed to work when the target site was mutated in LO2-X-S cells (Fig. 1C). Moreover, the inhibition of endogenous miR-520b by anti-miR-520b resulted in increasing firefly luciferase activities of pGL3-TET1-wt in LO2 cells, but the mutant not (Fig. 1D). Thus, our data indicate that TET1 is one of the target genes of miR-520b in HCC.

3.2. MiR-520b is able to down-regulate TET1 in HCC cells

To further investigate the effect of miR-520b on TET1, we performed the transient transfection of miR-520b in LO2-X-S cells. Our data showed that miR-520b was able to decrease the expression of TET1 at the levels of mRNA and protein in a dose-dependent manner in LO2-X-S cells (Fig. 2A). Conversely, the expression of TET1 was elevated in LO2 cells when endogenous miR-520b was inhibited by anti-miR-520b (Fig. 2B), supporting that miR-520b can modulate the expression of TET1 *in vitro*. Meanwhile, the transfection efficiency of miR-520b and anti-miR-520b was validated by qRT-PCR in the cells (Fig. 2A and B). Taken together, we conclude that miR-520b is able to down-regulate TET1 in HCC cells.

3.3. MiR-520b suppresses proliferation of HCC cells via TET1

It has been reported that miR-520b is implicated in tumor growth [18–20]. Therefore, we are interested in whether miR-520b inhibits proliferation of HCC cells through TET1. The EdU assays manifested that cell proliferation was decreased when the cells were treated with miR-520b, but TET1 overexpression could rescue inhibition of cell proliferation mediated by miR-520b in the cells treated with both miR-520b and FH-TET1-pEF [21]. Meanwhile, anti-miR-520b could increase cell proliferation and cotransfection of TET1 siRNA was able to abolish anti-miR-520b-induced acceleration of cell proliferation (Fig. 3A), suggesting that miR-520b inhibits the proliferation of hepatoma cells relying on TET1 in part. Taken a further step to reinforce our conclusion, we performed colony formation assays. Our data revealed that miR-520b could remarkably reduce colony formation ability of LO2-X-S cells and the enforced TET1 expression resulted in the increase of colony numbers of the cells transfected with miR-520b. Furthermore, there was an increase of colony numbers in the anti-miR-520b treated cells. In contrast, the increasing colony formation induced by anti-miR-520b could be blocked by TET1 siRNA (Fig. 3B). The efficiency of TET1 siRNA was validated by Western blotting analysis in the cells (Fig. 3C). Collectively, we conclude that miR-520b is able to suppress cell proliferation through targeting TET1 in hepatoma.

3.4. MiR-520b is negatively associated with TET1 in clinical HCC tissues

Next, we analyzed the TET1 expression in 20 paired clinical HCC and adjacent noncancerous liver tissues using qRT-PCR and normalized against an endogenous control (U6 RNA). When compared with their noncancerous counterparts, significant up-

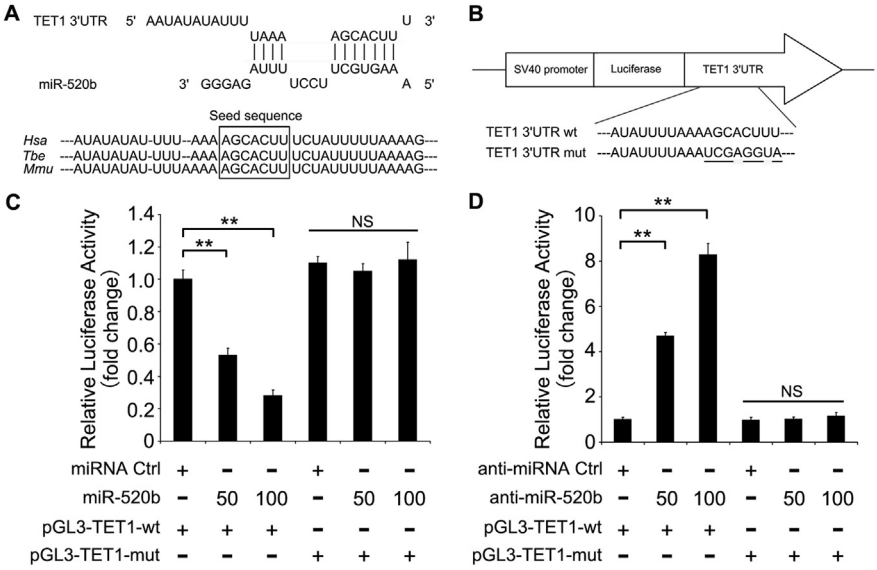

Fig. 1. MiR-520b directly inhibits the expression of TET1 through targeting its 3′UTR. (A, B) The binding site of miR-520b in 3′UTR of TET1 mRNA is shown in a model. Mutant was generated at the TET1 3′UTR as indicated. A TET1 3′UTR fragment containing wild type or mutant (wt or mut) of the miR-520b-binding sequence was cloned into the downstream of the pGL3-control luciferase reporter gene vector. (C, D) The effect of miR-520b (or anti-miR-520b) on reporters of pGL3-TET1-wt and pGL3-TET1-mut in LO2-X-S cells or LO2 cells was measured by luciferase reporter gene assays, respectively. Statistically significant differences are indicated: $^{**}P < 0.01$; NS; Student's *t* test. The experiment was repeated at least three times.

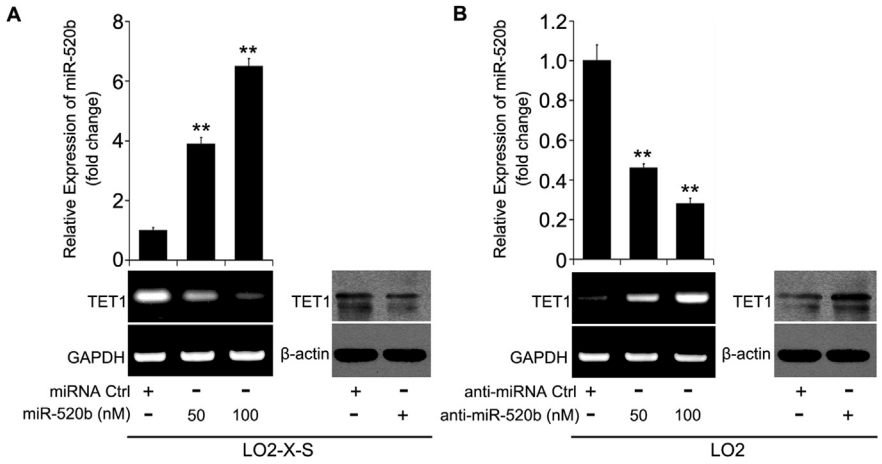

Fig. 2. MiR-520b is able to down-regulate TET1 in HCC cells. (A) The mRNA and protein levels of TET1 were examined in LO2-X-S cells transfected with miR-520b by RT-PCR and Western blotting analysis, respectively. The transfection efficiency of miR-520b was detected by qRT-PCR. (B) The mRNA and protein levels of TET1 were examined in LO2 cells transfected with anti-miR-520b by RT-PCR and Western blotting analysis, respectively. The transfection efficiency of anti-miR-520b was detected by qRT-PCR. Statistically significant differences are indicated: $^{**}P < 0.01$; Student's t test.

regulation of TET1 was observed in all the 20 HCC samples (Fig. 4A). Then we assessed the correlation between miR-520b and TET1. As expected, we found that the levels of miR-520b exhibited a significant negative correlation with the levels of TET1 mRNA (Pearson's correlation coefficient of −0.6737, $p < 0.01$) (Fig. 4B). Overall, our finding indicates that the levels of miR-205 are negatively associated with those of TET1 mRNA in clinical HCC tissues.

4. Discussion

The development and progression of HCC involves the activation of multiple pathways and molecular alterations of multiple genes that are essential to cell biological processes [22,23]. MiRNAs play crucial roles in HCC pathogenesis and their expression profiles can be used for classification of cancers [3]. Identifying the miRNAs and

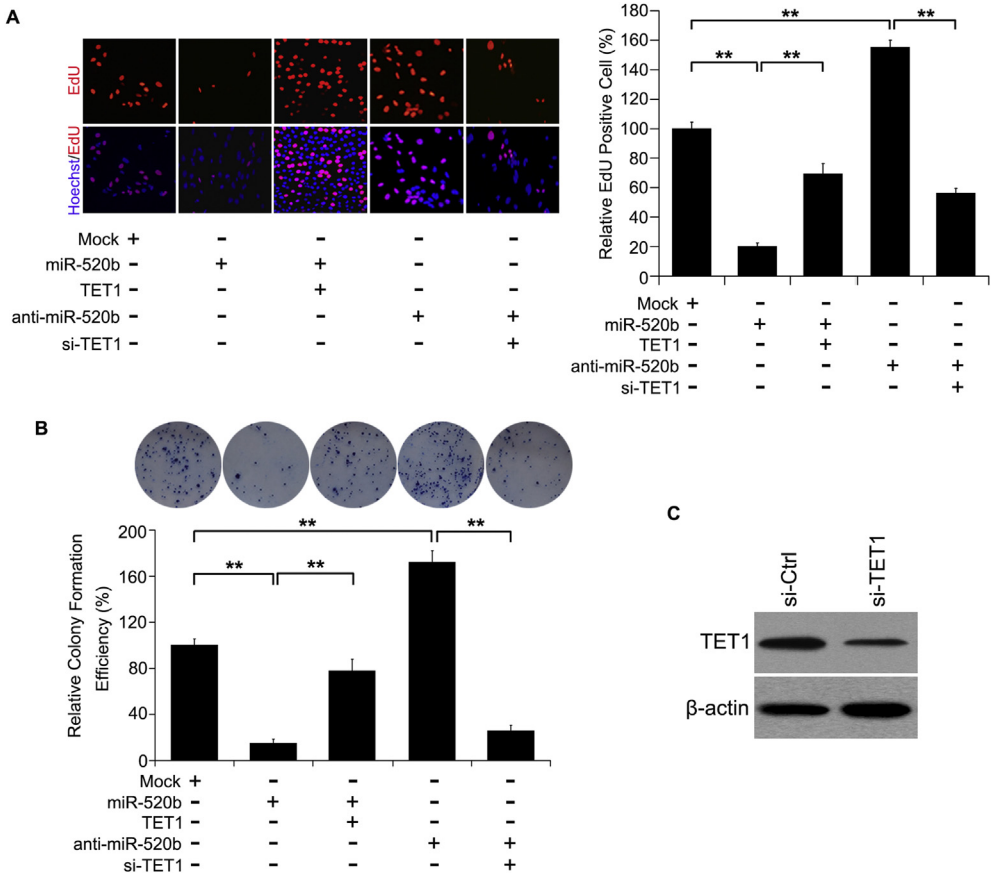

Fig. 3. MiR-520b suppresses proliferation of HCC cells via TET1. (A, B) Effect of miR-520b (or miR-520b/TET1, anti-miR-520b, anti-miR-520b/TET1 siRNA) on cell proliferation was determined by EdU and colony formation assays in LO2-X-S cells, respectively. (C) Interference efficiency of TET1 siRNA was detected by Western blotting analysis in LO2-X-S cells. Statistically significant differences are indicated: $^{**}P < 0.01$; Student's t test.

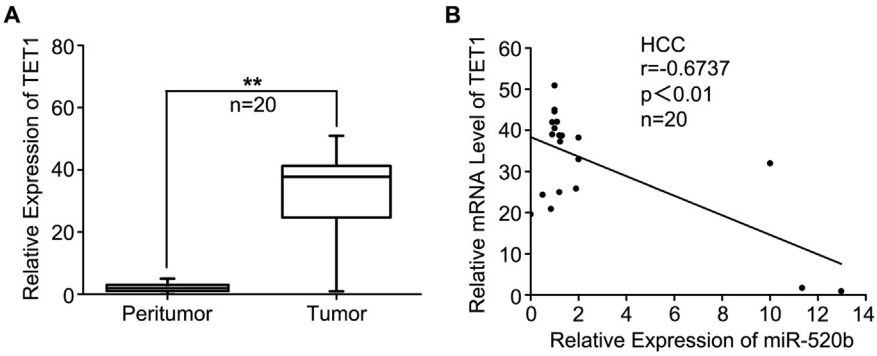

Fig. 4. MiR-520b is negatively associated with TET1 in clinical HCC tissues. (A) TET1 mRNA levels was examined by qRT-PCR in 20 cases of clinical HCC tissues and paired non-tumorous tissues. (B) Correlation of miR-520b levels with TET1 mRNA levels was examined by qRT-PCR in 20 cases of clinical HCC tissues (Pearson's correlation coefficient, r = −0.6737). Statistically significant differences are indicated: $^{**}P < 0.01$; Student's *t* test.

their targets that are essential for HCC progression may provide promising therapeutic opportunities. Our evidence has indicated that the deregulation of miR-520b is involved in the hepatocarcinogenesis induced by HBx and survivin in LO2-X-S cells [18]. In addition, our groups have observed that miR-520b is down-regulated in HCC and may suppress the development of HCC through targeting cyclin D1 and MEKK2 [20]. In the present study, we are interested in the significance of miR-520b down-regulation in hepatocarcinogenesis.

We first predicted the target genes of miR-520b by bioinformatics' method. Strikingly, TET1 was of our particular interest for its particular functions in epigenetic modification of DNA during the development of cancer. As expected, our data demonstrated that miR-520b was able to directly bind to the 3′UTR of TET1 mRNA. Then, we further investigated the effect of miR-520b on TET1 expression in HCC cells. RT-PCR and Western blotting analysis showed that the over-expression of miR-520b was able to down-regulate the expression of TET1 at the levels of mRNA and protein. Furthermore, we uncovered that miR-520b targeted TET1 could contribute to proliferation of hepatoma cells. EdU and colony formation assays indicated that low levels of miR-520b led to acceleration of cell proliferation in HCC relying on its target gene TET1. Then, we used HCC clinical samples to validate the above conclusions as well. Interestingly, we observed that low levels of miR-520b were correlated with high levels of TET1 in clinical HCC samples. Methylcytosine dioxygenase TET1 as one member of TET family of enzymes (TET1/2/3) participates in DNA demethylation by catalyzing the conversion of 5-methylcytosine to 5-hydroxymethylcytosine (5hmC), resulting in active or passive DNA demethylation [24,25]. Previous study reported that TET1 was partially down-regulated in 20 HCC samples [26]. Recently, another report has shown that miR-29 family decreases protein levels of TET1 through directly targeting its 3′UTR. Paradoxically, their result also demonstrated that over-expression of miR-29 were correlated with the increase of TET1 in HCC cells and HCC tissues [27]. Nevertheless, TET1 is found to be over-expressed in diffuse intrinsic pontine glioma, which is a malignant pediatric brain tumor with dismal outcome [28]. It has been shown that TET1 silencing reduce 5-hmC levels and decrease cell proliferation in leiomyoma [29]. TET1 plays an indispensable oncogenic role in the development of MLL-rearranged leukemia through coordination with MLL-fusion proteins in regulating their critical co-targets, including homeobox A9/myeloid ecotropic viral integration 1/pre-B-cell leukemia homeobox 3 genes [30]. However, TET1 also exerts tumor suppressor function in lung, prostate, breast and gastric cancer [31–33]. The controversial function remains unclear, which may be partially explained by multifactorial etiology of cancer and the complexity of clinical tissues.

Taken together, we validate that miR-520b is down-regulated in HCC tissues. We further find that TET1 is one of target genes of miR-520b, which is up-regulated in HCC tissues. MiR-520b is able to suppress proliferation of hepatoma cells through targeting 3′UTR of TET1 mRNA. Thus, our finding provides new insights into the roles of miR-520b in hepatocarcinogenesis.

Conflict of interests

Xiaodong Zhang, Tianqiang Song and Lihong Ye conceived the projects, designed the experiments and drafted the manuscript. Weiying Zhang designed the experiments, performed the experiments and drafted the manuscript. Zhanping Lu performed the experiments and revised the manuscript. Yuen Gao performed the experiments. The authors declare no competing financial interests.

Acknowledgments

This work was supported by the grants of the National Basic Research Program of China (973 Program, No. 2015CB553703), the National High Technology Research and Development Program of China (863 Program, 2014AA020903), the National Natural Scientific Foundation of China (No. 81272218, 81372186, 31470756) and Tianjin Natural Scientific Foundation (No. 14JCZDJC32800).

References

[1] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[2] D.P. Bartel, C.Z. Chen, Micromanagers of gene expression: the potentially widespread influence of metazoan microRNAs, Nat. Rev. Genet. 5 (2004) 396–400.
[3] J. Lu, G. Getz, E.A. Miska, E. Alvarez-Saavedra, J. Lamb, D. Peck, A. Sweet-Cordero, B.L. Ebert, R.H. Mak, A.A. Ferrando, J.R. Downing, T. Jacks, H.R. Horvitz, T.R. Golub, MicroRNA expression profiles classify human cancers, Nature 435 (2005) 834–838.
[4] C.Z. Chen, MicroRNAs as oncogenes and tumor suppressors, N. Engl. J. Med. 353 (2005) 1768–1771.
[5] C.Z. Chen, L. Li, H.F. Lodish, D.P. Bartel, MicroRNAs modulate hematopoietic lineage differentiation, Science 303 (2004) 83–86.
[6] V.N. Kim, MicroRNA biogenesis: coordinated cropping and dicing, Nat. Rev. Mol. Cell. Biol. 6 (2005) 376–385.
[7] A. Budhu, H.L. Jia, M. Forgues, C.G. Liu, D. Goldstein, A. Lam, K.A. Zanetti, Q.H. Ye, L.X. Qin, C.M. Croce, Z.Y. Tang, X.W. Wang, Identification of metastasis-related microRNAs in hepatocellular carcinoma, Hepatology 47 (2008) 897–907.
[8] Q. Dong, P. Meng, T. Wang, W. Qin, W. Qin, F. Wang, J. Yuan, Z. Chen, A. Yang, H. Wang, MicroRNA let-7a inhibits proliferation of human prostate cancer cells in vitro and in vivo by targeting E2F2 and CCND2, PLoS One 5 (2010) e10147.
[9] G.A. Calin, C.M. Croce, MicroRNA signatures in human cancers, Nat. Rev. Cancer 6 (2006) 857–866.
[10] G.A. Calin, C.M. Croce, Chromosomal rearrangements and microRNAs: a new cancer link with clinical implications, J. Clin. Invest. 117 (2007) 2059–2066.

[11] O.A. Kent, J.T. Mendell, A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes, Oncogene 25 (2006) 6188–6196.
[12] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, Global cancer statistics, CA Cancer J. Clin. 61 (2011) 69–90.
[13] M.B. Thomas, A.X. Zhu, Hepatocellular carcinoma: the need for progress, J. Clin. Oncol. 23 (2005) 2892–2899.
[14] J. Kota, R.R. Chivukula, K.A. O'Donnell, E.A. Wentzel, C.L. Montgomery, H.W. Hwang, T.C. Chang, P. Vivekanandan, M. Torbenson, K.R. Clark, J.R. Mendell, J.T. Mendell, Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model, Cell 137 (2009) 1005–1017.
[15] F. Meng, R. Henson, H. Wehbe-Janek, K. Ghoshal, S.T. Jacob, T. Patel, MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer, Gastroenterology 133 (2007) 647–658.
[16] M. Garofalo, G. Di Leva, G. Romano, G. Nuovo, S.S. Suh, A. Ngankeu, C. Taccioli, F. Pichiorri, H. Alder, P. Secchiero, P. Gasparini, A. Gonelli, S. Costinean, M. Acunzo, G. Condorelli, C.M. Croce, miR-221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation, Cancer Cell 16 (2009) 498–509.
[17] W.C. Tsai, P.W. Hsu, T.C. Lai, G.Y. Chau, C.W. Lin, C.M. Chen, C.D. Lin, Y.L. Liao, J.L. Wang, Y.P. Chau, M.T. Hsu, M. Hsiao, H.D. Huang, A.P. Tsou, MicroRNA-122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma, Hepatology 49 (2009) 1571–1582.
[18] W. Zhang, Z. Lu, G. Kong, Y. Gao, T. Wang, Q. Wang, N. Cai, H. Wang, F. Liu, L. Ye, X. Zhang, Hepatitis B virus X protein accelerates hepatocarcinogenesis with partner survivin through modulating miR-520b and HBXIP, Mol. Cancer 13 (2014) 128.
[19] N. Hu, J. Zhang, W. Cui, G. Kong, S. Zhang, L. Yue, X. Bai, Z. Zhang, W. Zhang, X. Zhang, L. Ye, miR-520b regulates migration of breast cancer cells by targeting hepatitis B X-interacting protein and interleukin-8, J. Biol. Chem. 286 (2011) 13714–13722.
[20] W. Zhang, G. Kong, J. Zhang, T. Wang, L. Ye, X. Zhang, MicroRNA-520b inhibits growth of hepatoma cells by targeting MEKK2 and cyclin D1, PLoS One 7 (2012) e31450.
[21] M. Tahiliani, K.P. Koh, Y. Shen, W.A. Pastor, H. Bandukwala, Y. Brudno, S. Agarwal, L.M. Iyer, D.R. Liu, L. Aravind, A. Rao, Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1, Science 324 (2009) 930–935.
[22] X. You, F. Liu, T. Zhang, N. Lv, Q. Liu, C. Shan, Y. Du, G. Kong, T. Wang, L. Ye, X. Zhang, Hepatitis B virus X protein upregulates Lin28A/Lin28B through Sp-1/c-Myc to enhance the proliferation of hepatoma cells, Oncogene 33 (2014) 449–460.
[23] T. Zhang, J. Zhang, X. You, Q. Liu, Y. Du, Y. Gao, C. Shan, G. Kong, Y. Wang, X. Yang, L. Ye, X. Zhang, Hepatitis B virus X protein modulates oncogene yes-associated protein by CREB to promote growth of hepatoma cells, Hepatology 56 (2012) 2051–2059.
[24] J.U. Guo, Y. Su, C. Zhong, G.L. Ming, H. Song, Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain, Cell 145 (2011) 423–434.
[25] H. Zhang, X. Zhang, E. Clark, M. Mulcahey, S. Huang, Y.G. Shi, TET1 is a DNA-binding protein that modulates DNA methylation and gene transcription via hydroxylation of 5-methylcytosine, Cell. Res. 20 (2010) 1390–1393.
[26] C. Liu, L. Liu, X. Chen, J. Shen, J. Shan, Y. Xu, Z. Yang, L. Wu, F. Xia, P. Bie, Y. Cui, X.W. Bian, C. Qian, Decrease of 5-hydroxymethylcytosine is associated with progression of hepatocellular carcinoma through downregulation of TET1, PLoS One 8 (2013) e62828.
[27] L.L. Lin, W. Wang, Z. Hu, L.W. Wang, J. Chang, H. Qian, Negative feedback of miR-29 family TET1 involves in hepatocellular cancer, Med. Oncol. 31 (2014) 291.
[28] S. Ahsan, E.H. Raabe, M.C. Haffner, A. Vaghasia, K.E. Warren, M. Quezado, L.Y. Ballester, J. Nazarian, C.G. Eberhart, F.J. Rodriguez, Increased 5-hydroxymethylcytosine and decreased 5-methylcytosine are indicators of global epigenetic dysregulation in diffuse intrinsic pontine glioma, Acta Neuropathol. Commun. 2 (2014) 59.
[29] A. Navarro, P. Yin, M. Ono, D. Monsivais, M.B. Moravek, J.S.t. Coon, M.T. Dyson, J.J. Wei, S.E. Bulun, 5-Hydroxymethylcytosine promotes proliferation of human uterine leiomyoma: a biological link to a new epigenetic modification in Benign tumors, J. Clin. Endocrinol. Metab. 99 (11) (2014) E2437–E2445 jc20142264.
[30] H. Huang, X. Jiang, Z. Li, Y. Li, C.X. Song, C. He, M. Sun, P. Chen, S. Gurbuxani, J. Wang, G.M. Hong, A.G. Elkahloun, S. Arnovitz, J. Wang, K. Szulwach, L. Lin, C. Street, M. Wunderlich, M. Dawlaty, M.B. Neilly, R. Jaenisch, F.C. Yang, J.C. Mulloy, P. Jin, P.P. Liu, J.D. Rowley, M. Xu, C. He, J. Chen, TET1 plays an essential oncogenic role in MLL-rearranged leukemia, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 11994–11999.
[31] B.K. Wu, C. Brenner, Suppression of TET1-dependent DNA demethylation is essential for KRAS-mediated transformation, Cell Rep. 9 (2014) 1827–1840.
[32] H.L. Fu, Y. Ma, L.G. Lu, P. Hou, B.J. Li, W.L. Jin, D.X. Cui, TET1 exerts its tumor suppressor function by interacting with p53-EZH2 pathway in gastric cancer, J. Biomed. Nanotechnol. 10 (2014) 1217–1230.
[33] C.H. Hsu, K.L. Peng, M.L. Kang, Y.R. Chen, Y.C. Yang, C.H. Tsai, C.S. Chu, Y.M. Jeng, Y.T. Chen, F.M. Lin, H.D. Huang, Y.Y. Lu, Y.C. Teng, S.T. Lin, R.K. Lin, F.M. Tang, S.B. Lee, H.M. Hsu, J.C. Yu, P.W. Hsiao, L.J. Juan, TET1 suppresses cancer invasion by activating the tissue inhibitors of metalloproteinases, Cell. Rep. 2 (2012) 568–579.