Pathology - Research and Practice 215 (2019) 546–554

Contents lists available at ScienceDirect

Pathology - Research and Practice

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

β-catenin-coordinated lncRNA MALAT1 up-regulation of ZEB-1 could enhance the telomerase activity in HGF-mediated differentiation of bone marrow mesenchymal stem cells into hepatocytes

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ARTICLE INFO

Keywords:
BM-MSCs
miRNA-217-3p
MALAT1
β-Catenin
ZEB1
TERT

ABSTRACT

Objective: To investigate role of β-catenin and lncRNA MALAT1/miR-217 axis to converge into the regulation of ZEB-1 in hepatocyte growth factor (HGF)-induced hepatocytes differentiated from bone marrow mesenchymal stem cells (BM-MSCs).

Methods: BM-MSCs were isolated and HGF was used to induce the differentiation of BM-MSCs into hepatocytes. HSC-T6 cells, BRL-3 A cells and differentiated BM-MSCs were treated by lipopolysaccharide(LPS). shRNAs were used to silence β-catenin and recombinant plasmids were used to over-express ZEB1. Measurement of cell viability was conducted using MTT assay and Hoechst 33342 staining. RNA immunoprecipitation (RIP) assay was used to determine binding of miR-217-3p and MALAT1.

Results: BM-MSCs successfully differentiated into hepatocytes by HGF treatment. Expression of β-catenin, ZEB-1 and TERT was up-regulated to a higher level in hepatocytes differentiated from BM-MSCs than HSC-T6 cells and BRL-3 A cells after LPS stimulation. When β-catenin was knocked down in all cell lines, expression of β-catenin, ZEB-1 and TERT was significantly decreased as well as telomerase activity. While when ZEB1 was over-expressed, expression of TERT and telomerase activity was all significantly up-regulated. In hepatocytes differentiated from BM-MSCs, miR-217 was down-regulated and lncRNA MALAT1 was up-regulated. RIP analysis showed MALAT1 was physically associated with miR-217 and might function in the regulation of ZEB-1, further enhancing the expression of TERT so as to augment telomerase activity.

Conclusion: We successfully used HGF to mediate differentiation of BM-MSCs into hepatocytes, and found that β-catenin-coordinated MALAT1/miR-217 axis could up-regulate expression of ZEB-1 and further enhanced the telomerase activity through regulation of TERT in BM-MSCs differentiating into hepatocytes.

1. Introduction

Hepatocyte growth factor (HGF) is a pleiotropic cytokine of mesenchymal origin, mainly produced by hepatic Küpffer cells and hepatic sinusoidal endothelial cells [1]. HGF plays an important role in liver regeneration; it acts as an initiator of liver regeneration and is rapidly elevated from 3 to 48 h at the early stage of liver regeneration [2,3]. Besides, studies show HGF also has various other bioactivities, including promoting motility, proliferation, mitosis, differentiation, invasion and morphogenesis of a wide spectrum of cells [1,4,5]. It is considered HGF can promote differentiation of bone marrow mesenchymal stem cells (BM-MSCs) into hepatocytes [6,7]. Since the application of autologous bone marrow stem cell transplantation is a potential method for treatment of liver diseases such as chronic liver disease and liver failure [8], deeper insights for process of differentiation of BM-MSCs into hepatocytes are necessary.

Telomerase, a cellular ribonucleoprotein complex consisting of RNA and proteins, has shown wide application in studies of cell proliferation, senescence, as well as cancer diagnosis and treatment [9]. Telomerase reverse transcriptase (TERT) is the telomerase catalysis subunit of the telomerase holoenzyme and the telomerase RNA component (TERC). Studies reveal TERT plays an important role in the replication of telomere repeat sequences and the extension of telomeres [10]. TERT, critical for canonical telomerase function, elongating telomere lengths and extend cells' lifespan is largely studied in models of aging and cancer owing to its telomere-lengthening effects in the nucleus, including BM-MSCs [11–12]. Meanwhile, inhibition of TERT significantly suppresses the telomerase activity.

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https://doi.org/10.1016/j.prp.2019.01.002
Received 25 June 2018; Received in revised form 18 December 2018; Accepted 5 January 2019

The β-catenin/ZEB1 signaling has been proven to play important roles in many bioprocesses, such as cancer metastasis and chemo-sensitivity of cancer therapy [13–14]. Recently, a study showed in the process of intestinal regeneration, the up-regulation of β-catenin could significantly enhance the expression of TERT [15]. What's more, ZEB1 is also thought to influence telomerase expression in colorectal cancer [16].

The long non-coding RNAs (lncRNAs), involved in many bioprocesses, are non-coding RNAs of more than 200 nucleotides in length which do not contain any open reading frame. In a recent study, it was reported lncRNA MALAT1 could regulate ZEB1 expression by sponging microRNA (miR)-143-3p [17]. Studies showed microRNAs (miRNAs) could interact with lncRNA in cancer [18]. It was also reported MALAT1 could regulate expression of many miRNAs, such as miR-217 [19] and miR-99a [20], etc. However, no study focused on effects of MALAT1/miR-217/ZEB1 signaling on regulation of telomerase activity in BM-MSCs up to now.

The present study aimed to investigate effects of ZEB1 related signaling on regulation of telomerase activity in process of differentiation of BM-MSCs into hepatocytes and the mechanisms. This study might give deeper insights for role of β-catenin and MALAT1/miR-217 axis to converge into the regulation of ZEB1 in differentiation of BM-MSCs, as well as provide basis for potential application of liver regeneration through differentiation of BM-MSCs into hepatocytes.

2. Material and methods

2.1. Animals

Six-week-old female SD rats were obtained from The Animal Center of Human Children's Hospital. All animals were housed in micro-isolator cages with free access to food and water in a light-controlled room under a 12 h/12 h light/dark cycle and controlled temperature (23–25 °C) according to the Guide for the Care and Use of Laboratory Animals. In particular, any effort was put to avoid unnecessary pain of the animals. The whole study was approved by the Institutional Animal Care Committee of Hunan Children's Hospital.

2.2. Isolation and culture of rats BM-MSCs

BM-MSCs were obtained by flushing the femurs and tibias of the rats with D-Hanks' solution. Cells were cultured in low-glucose Dulbecco's modified eagle's medium (DMEM, Gibco, Gaithersburg, MD, USA) supplemented with 10% Gibco® fetal bovine serum (FBS), 10 mm N-2-hydroxy-ethylpiperazine-N'-2'-ethanesulfonic acid (Sigma-Aldrich, St. Louis, MO, USA) and 100 μg/mL penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA) at 37 °C and 5% CO_2. When confluence reached 80%, cells were trypsinized and subcultivated at a density of 1×10^4 cells/cm^2. Cells were characterized by surface markers, positive for CD90, CD105 and negative for CD45 and CD34 by flow cytometry analysis.

2.3. Differentiation of BM-MSCs into hepatocytes

Obtained BM-MSCs were treated with 20 ng/mL HGF (Sigma-Aldrich, St. Louis, MO, USA) for 14 days to perform the differentiation into hepatocytes as described elsewhere [21]. Untreated cells were used as the control. Cell morphology was observed using a phase-contrast microscope. Cells were characterized by surface markers using flow cytometry analysis. Briefly, after cell confluence reached 80%, cells were trypsinized and subcultivated at a density of 2×10^5 cells/cm^2. Then primary antibodies of CD45, CD34, CD90 and CD105 (all purchased from Abcam, Cambridge, MA, USA) were added and cells were incubated for 20 min at 37 °C, followed with incubation of the corresponding secondary antibodies fluorescein isothiocyanate (FITC)-rabbit anti-rat IgG (ab6730, Abcam, Cambridge, MA, USA) or phycoerythrin (PE)-goat anti-rat IgG (ab7010, Abcam, Cambridge, MA, USA). Surface markers were then evaluated by flow cytometry method and negative staining controls were conducted by omitting the primary antibody. At time point of 7 d, 14 d and 21 d after HGF-induction, levels of biomarkers of alpha-fetoprotein (AFP) and albumin (ALB) were determined by Quantitative real time PCR (qRT-PCR) as described below.

2.4. Lipopolysaccharide (LPS) induction

Rat normal hepatocyte cells BRL-3 A and hepatic stellate cells HSC-T6 were purchased from ATCC (Manassas, VA, USA). Briefly, cells were cultured in RPMI-1640 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% Gibco® fetal bovine serum (FBS) and 100 μg/mL penicillin-streptomycin (Sigma-Aldrich Co, USA) at 37 °C and 5% CO_2. Cells including BRL-3 A cells, HSC-T6 cells and above hepatocytes differentiated from BM-MSCs, were further treated with 2 μg/mL LPS for 12 h for further studies.

2.5. Plasmids construction and cell transfection

shRNAs for β-catenin (sh-β-catenin), ZEB1 (sh-ZEB1) and overexpression plasmids for ZEB1 (ZEB1-OE), MALAT1 and corresponding negative controls were all purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). miRNA-217-3p mimics and negative control were all purchased from RiboBio (Guangzhou, China). For cell transfection, cells were cultured to 30–50% confluence and transfected with corresponding shRNAs, miRNA-217-3p mimics or negative controls at a final concentration of 50 nmol/L using Lipofectamine 3000 (Invitrogen) in serum-free Opti-MEM medium (Gibco) according to the manufacturer's instruction.

2.6. Measurement of cell viability

MTT assay and Hoechst 33,342 staining were used to measure the cell viabilities. Briefly, cells were seeded at density of 2×10^4 in 96-well plates. After further culture for 24 h, 48 h, 72 h and 96 h, respectively, 10 μL MTT solution (5 mg/mL) was added followed by further culture for 4 h at 37 °C and 5% CO_2. After removal of MTT, the supernatant was subsequently replaced with 180 μL DMSO and the optical density (OD) value was evaluated under 490 nm. For Hoechst 33,342 staining, samples were washed 2 times with PBS for 5 min and nuclear stain Hoechst33342 (Invitrogen, H3570) at 1:2500 was added for 15 min at room temperature. The cells were then photographed and the cell apoptosis rate was calculated.

2.7. Periodic acid-Schiff (PAS) staining

PAS staining was conducted for BM-MSCs, BRL-3 A and HSC-T6 cells after LPS induction. Briefly, cells were fixed with 4% paraformaldehyde, washed with PBS and then incubated with 0.5% periodic acid solution for 5 min. Then cells were stained with Schiff's reagent for 15 min, followed by counterstaining with hematoxylin solution for 2 min A phase-contrast microscope was used to observe the cell morphology.

2.8. RNA immunoprecipitation (RIP)

Ago2-RIP assay was performed using the EZ-Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit(Millipore, USA). The BM-MSCs and BRL-3 A cells were scraped off and lysed in complete RIP lysis buffer, and 100 μL of whole cell extract were incubated with RIP buffer containing magnetic beads conjugated with anti-Ago2 antibody (Cell Signaling, USA). PCR was used to detect the co-precipitated RNAs. Total RNAs (input controls) and IgG were also assayed to detecte whether the signals resulted from RNAs specifically binding to Ago2.

MS2-RIP assay was conducted to use the Stratagene Quik Change

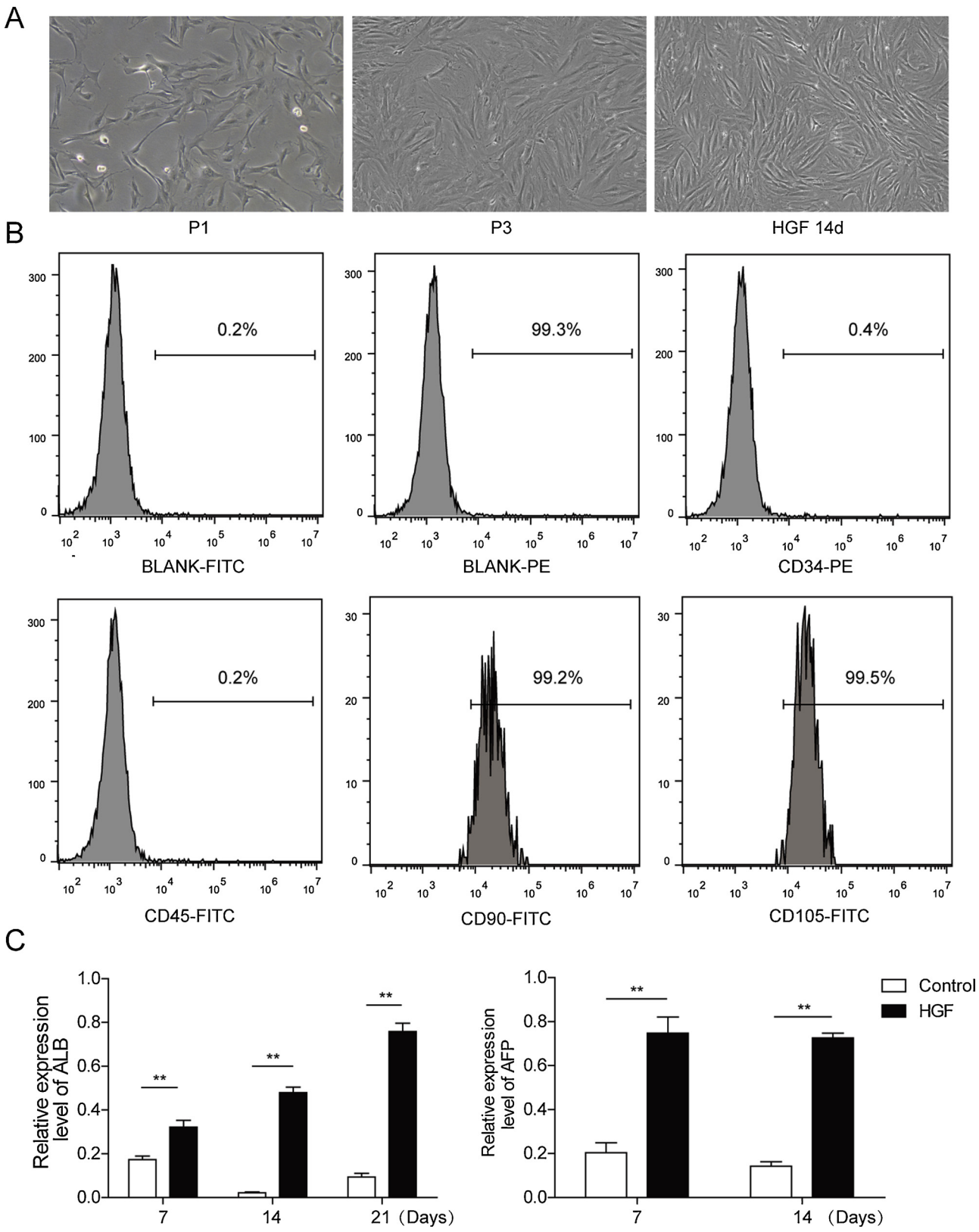


Fig. 1. Successful differentiation of BM-MSCs into hepatocytes. (A) Cell morphology for cells at the first passage, the third passage and cells treated with HGF for 14 d. (B) Surface biomarkers by flow cytometry analysis. (C) Expression of ALB and AFP by qRT-PCR. **P < 0.01. All experiments were conducted in triplicate.

Site-Directed Mutagenesis Kit(Millipore, USA). BM-MSCs and BRL-3 A cell lines were transfected with MS2-tagged MALAT1 constructs. At 48 h following transfection, the cells were subjected to anti-MS2 RIP analysis as described elsewhere [22,23].

2.9. Luciferase reporter assay

The ZEB1-3′-untranslated region (3′-UTR) fragment containing the putative binding sequences of miR-217-3p were cloned into pmirGLO

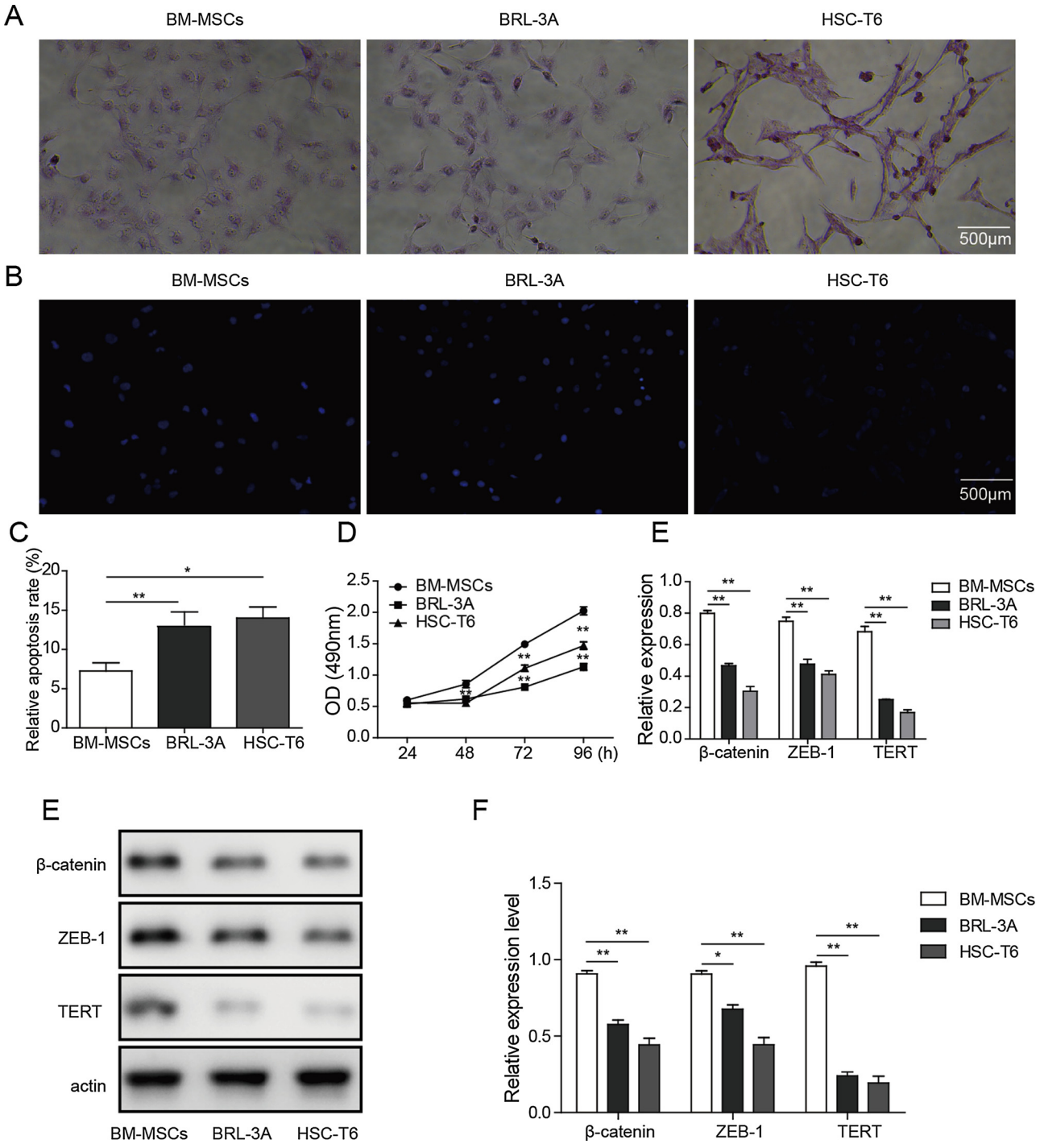


Fig. 2. Expression of β-catenin, ZEB-1 and TERT was up-regulated in hepatocytes differentiated from BM-MSCs after LPS stimulation. (A) PAS staining assay was performed for HSC-T6, BRL-3 A cells and BM-MSCs. (B) Hoechst 33,342 staining assay was performed for HSC-T6, BRL-3 A cells and BM-MSCs. (C) The quantification of apoptotic rate by Hoechst 33,342 staining was showed in HSC-T6, BRL-3 A cells and BM-MSCs. *P < 0.05, **P < 0.01. (D) Cell viability was examined by MTT assay for HSC-T6, BRL-3 A cells and BM-MSCs, **P < 0.05 compared with BM-MSCs. (E)Relative mRNA of β-catenin, ZEB-1 and TERT was determined in HSC-T6, BRL-3 A cells and BM-MSCs by qRT-PCR. (F) Representative Western blot images were shown in HSC-T6, BRL-3 A cells and BM-MSCs. (G) The quantification of relative protein expression was shown in HSC-T6, BRL-3 A cells and BM-MSCs. *P < 0.05, **P < 0.01. All experiments were conducted in triplicate.

vectors, and the fragment of MALAT1 including the binding site was inserted into pmirGLO vectors. The mutated plasmid was used as the control. The BM-MSCs and BRL-3 A cells were co-transfected with miR-217-3p mimic and related reporter constructs. The luciferase activity was detected using Dual Luciferase Reporter Assay System (Promega, USA) after transfection for 48 h.

2.10. Measurement of telomerase activity

Telomerase activity was assayed on cell extracts by using a TRAPeze ELISA Telomerase Detection kit (Chemicon) according to the manufacturer's instructions as described elsewhere [24].

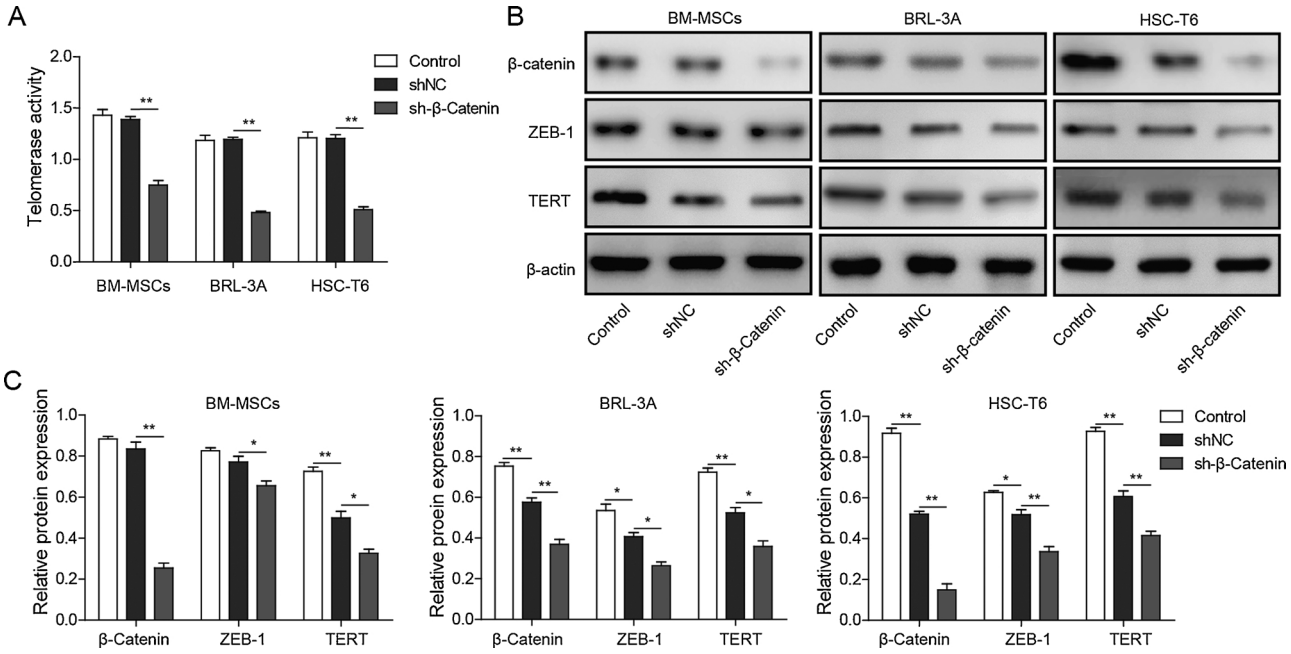


Fig. 3. Down-regulation of β-catenin reduced telomerase activity and expression of ZEB-1 and TERT. (A) Telomerase activity was detected in HSC-T6, BRL-3 A cells and BM-MSCs transfected with sh-β-catenin or control. (B) Representative Western blot images were shown in HSC-T6, BRL-3 A cells and BM-MSCs. transfected with sh-β-catenin or control. (C) The quantification of relative protein expression was shown in HSC-T6, BRL-3 A cells and BM-MSCs transfected with sh-β-catenin or control. $^{**}P < 0.01$, $^{*}P < 0.05$. All experiments were conducted in triplicate.

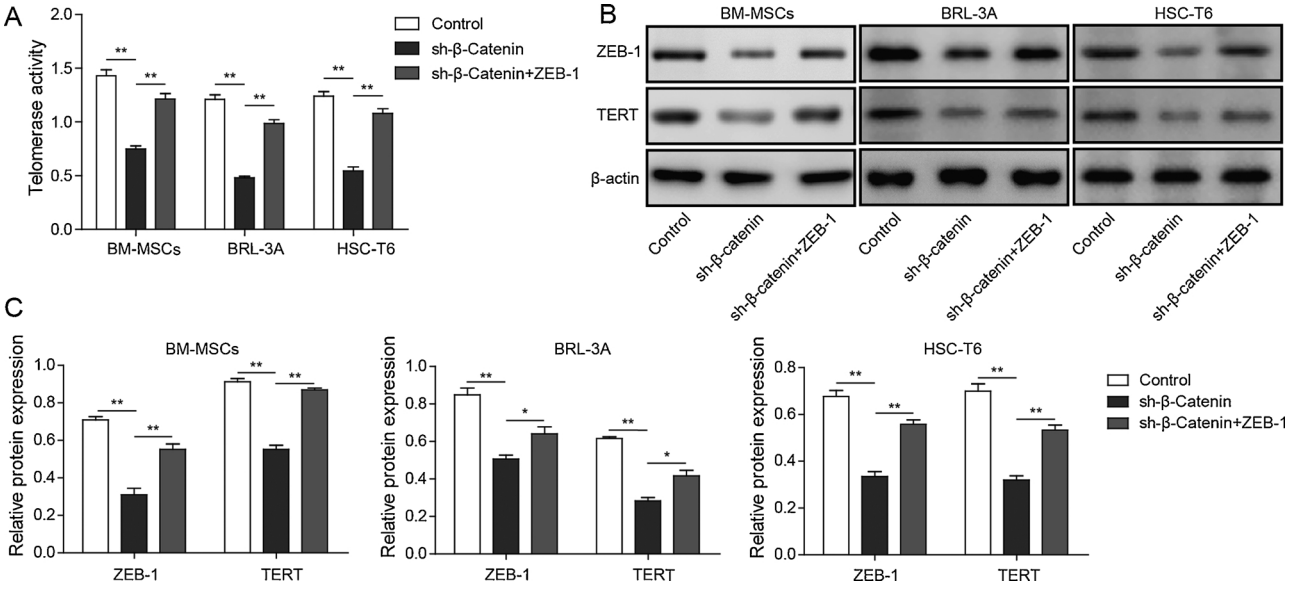


Fig. 4. Overexpression of ZEB1 increased telomerase activity and expression of TERT. (A) Telomerase activity was detected in HSC-T6, BRL-3 A cells and BM-MSCs transfected with sh-β-catenin, sh-β-catenin and ZEB1-OE or control. (B) Representative Western blot images were shown in HSC-T6, BRL-3 A cells and BM-MSCs transfected sh-β-catenin, sh-β-catenin plus ZEB1-OE or control. (C) The quantification of relative protein expression was shown in HSC-T6, BRL-3 A cells and BM-MSCs. transfected with sh-β-catenin, sh-β-catenin plus ZEB1-OE or control. $^{**}P < 0.01$, $^{*}P < 0.05$. All experiments were conducted in triplicate.

2.11. Quantitative real time PCR (qRT-PCR)

Total RNA was extracted from the cells using TRIzol (Invitrogen) according to the manufacturer's instructions. RNA was converted into cDNA using a Prime-ScriptTM one step qRT-PCR kit (TAKARA, Dalian, China). An ABI 7500 Fast RealTime PCR System (Life Technologies) was used to conduct PCR reactions. Primers used in PCR were listed below:

ALB (Genbank ID 158,138,567), F 5′-CTATCTGTCTGCCATCCTGA ACC-3′,

R 5′-TGTCCACGAATTGTGCGAAGT-3′;

AFP (Genbank ID 333,033,772), F 5′-AATTTGCCACGAGACGGA ACT-3′,

R 5′-TGCTGGAACTGCCTTGTCATAC-3′;

β-catenin (Genbank ID 4,731,568), F 5′-GCTGACCTGACGGAGTT GGA-3′,

R 5′-GCTACTTGCTCTTGCGTGAA-3′;

ZEB1 (Genbank ID 815,891,120), F 5′-CTGCCCAGTTACCCACA ATC-3′,

R 5′-CAGGGCTGACCGTAGTTGAG-3′;

TERT (Genbank ID 55,741,826), F 5′-AGTGGTGAACTTCCCTG TGG-3′,

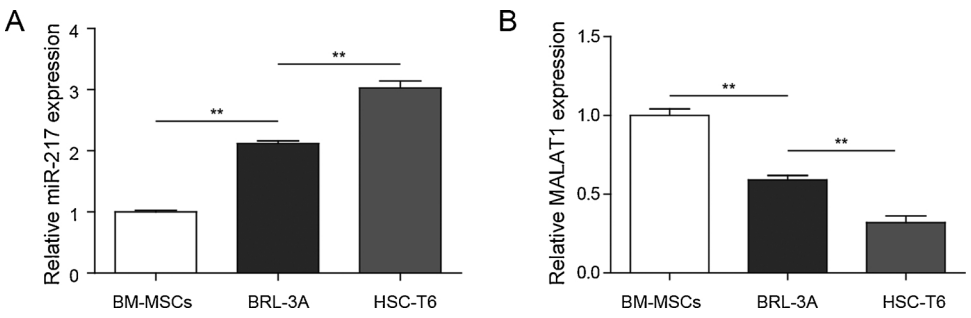


Fig. 5. miR-217 was down-regulated and MALAT-1 was up-regulated in hepatocytes differentiated from BM-MSCs. (A)Relative expression of miR-217-3p in HSC-T6, BRL-3 A cells and BM-MSCs was determined by qRT-PCR.(B) Relative expression of MALAT-1 in HSC-T6, BRL-3 A cells and BM-MSCs was determined by qRT-PCR **P < 0.01. All experiments were conducted in triplicate.

R 5′-CAACCGCAAGACTGACAAGA-3′;

MALAT1 (Genbank ID 209,552,504), F 5′-TGCGAGTTGTTCTCCGTCTA-3′,

R 5′- TATCTGCGGTTTCCTCAAGC-3′;

miR-217-3p, F 5′-GGTCTACAAAGGGAAGC-3′,

R 5′- TTTGGCACTAGCACATT-3′;

GAPDH, F 5′-ACAGCAACAGGGTGGTGGAC-3′,

R 5′-TTTGAGGGTGCAGCGAACTT-3′. All primers were designed by ourselves and synthesized by Genepharma(Shanghai).Relative RNA levels were calculated by the $2^{-\Delta\Delta Ct}$ method. GAPDH was used as an internal control.

2.12. Western blotting analysis

Western blotting was used to determine the expression of β-catenin, ZEB1, and TERT. β-actin was used as a control. Total protein was extracted from the cells, loaded on SDS-PAGE and transferred to PVDF membranes. The membranes were then incubated with primary antibodies after blocked with 5% non-fat milk at room temperature for 1 h. Antibodies (all purchased from Abcam, USA) used were as follows: anti-β-catenin antibody (ab32572, 1/5000), anti-ZEB1 antibody (ab81972, 1/1000), anti-TERT antibody (ab191523, 1/1000). After incubation at 4 °C overnight, samples were subsequently incubated with a corresponding secondary antibody at 37 °C for 45 min. The target bands were then scanned using enhanced chemiluminescence (Bio-Rad).

2.13. Statistical analysis

The measurement data was expressed by mean ± SD. Comparison between two groups was performed using the Student *t*-test. Comparison among three or more groups was conducted using one-way analysis of variance (ANOVA). It was considered to be statistically significant when P-value was less than 0.05. All calculations were made using SPSS 22.0.

3. Results

3.1. Successful differential of BM-MSCs into hepatocytes

After isolation of BM-MSCs, cells were treated with HGF for 14 d. As shown in Fig. 1A-C, apparently cells differentiated into hepatocytes. Further FCM analysis showed surface biomarkers CD34 and CD45 were negative, while CD90 and CD105 were positive. The expression of ALB was significantly up-regulated in HGF treated cells at 7 d, 14 d and 21 d compared with the control (P < 0.05). Expression of AFP was significantly up-regulated at 7 d compared with the control (P < 0.05), but slightly down-regulated at 14 d and was not detected at 21 d in HGF treated cells. All these results indicated the successful differentiation of BM-MSCs into hepatocytes.

3.2. Expression of β-catenin, ZEB-1 and TERT was up-regulated in hepatocytes differentiated from BM-MSCs after LPS stimulation

After LPS treatment, cell morphology was observed by PAS staining and cell viabilities of all three cell lines, HSC-T6 cells, BRL-3 A cells and HGF treated BM-MSCs, were determined by MTT assay. As shown in Fig. 2A, after induction, the cytoplasm of all cells showed red after staining, indicating all cells had the ability of glycogen synthesis. The Hoechst 33,342 staining also showed the apoptosis rate of HSC-T6, BRL-3 A cells and BM-BMCs, and exhibited the lowest level in BM-BMCs than HSC-T6, BRL-3 A cells(P < 0.05, Fig. 2B and C). MTT analysis showed compared with HSC-T6 and BRL-3 A cells, proliferation of HGF treated BM-MSCs was significantly promoted after 72 h(P < 0.05, Fig. 2D) Meanwhile, expression of β-catenin, ZEB-1 and TERT was all up-regulated in HGF treated BM-MSCs compared with HSC-T6 and BRL-3 A cells (P < 0.05, Fig. 2E–G), suggesting HGF treated BM-MSCs might have higher expression of β-catenin, ZEB-1 and TERT, as well as higher cell viability.

3.3. Down-regulation of β-catenin reduced telomerase activity and expression of ZEB-1 and TERT

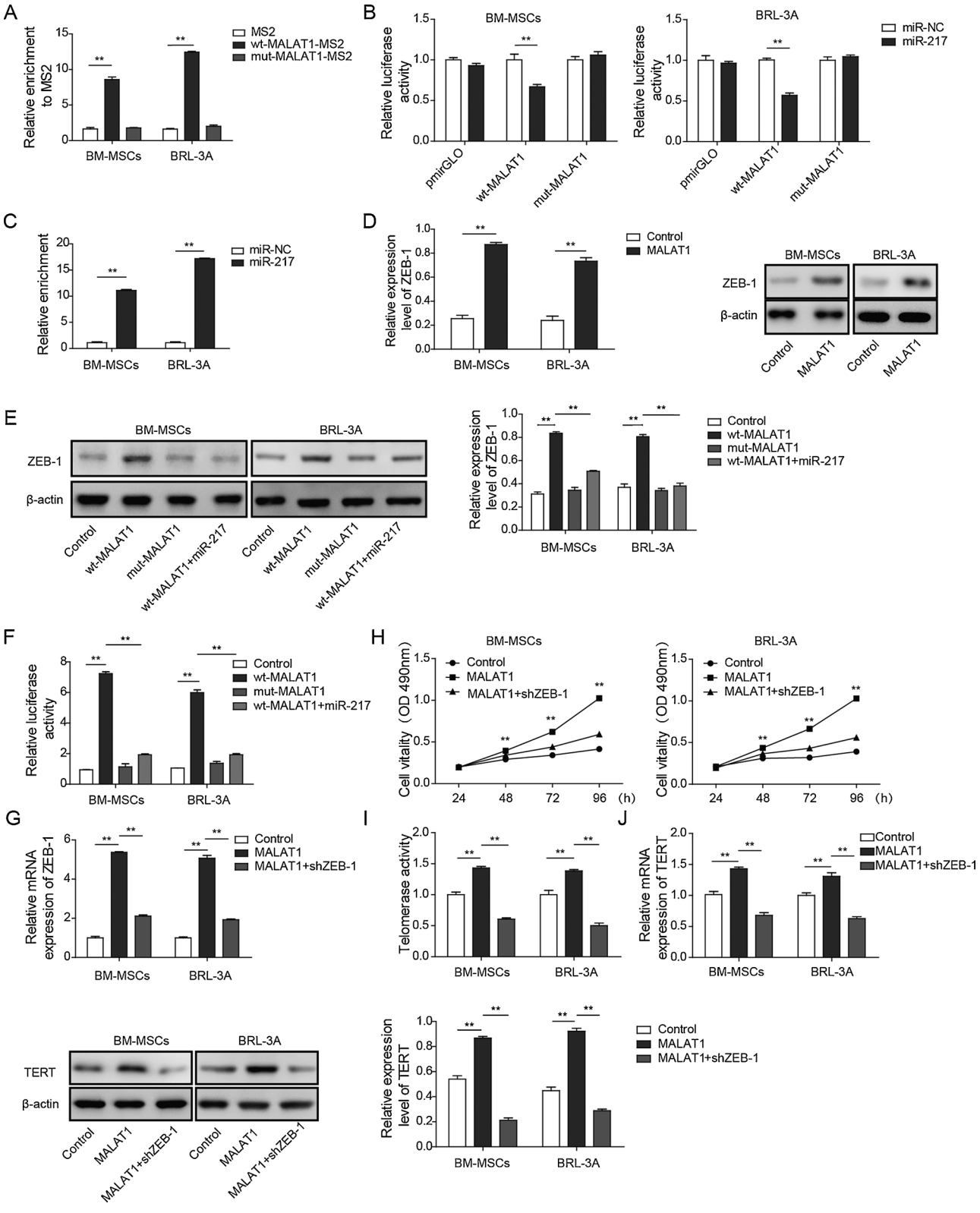
To further investigate role of β-catenin in LPS-induced cell injury of hepatocyte, telomerase activity was determined for different cells. As shown in Fig. 3, telomerase activity was decreased when β-catenin was knocked down in all three cell lines compared with the control (P < 0.05). Meanwhile, expression of β-catenin, ZEB-1 and TERT was all down-regulated in cells transfected with sh-β-catenin compared with the control (P < 0.05). These results indicated β-catenin played an important role in telomerase activity through regulation of ZEB-1 and TERT.

3.4. Overexpression of ZEB1 increased telomerase activity and expression of TERT

ZEB1 was over-expressed to further study mechanisms for β-catenin on telomerase activity. Results showed when cells were transfected with both sh-β-catenin and ZEB1-OE(ZEB1 over-expression), the reduced telomerase activity by sh-β-catenin was significantly recovered (P < 0.05, Fig. 4A). What's more, expression of both ZEB1 and TERT was up-regulated in cells transfected with both sh-β-catenin and ZEB1-OE compared with cells transfected with only sh-β-catenin (P < 0.05, Fig. 4B,C), further suggesting that ZEB1/β-catenin signaling influenced telomerase activity through regulation of TERT.

3.5. miR-217 was down-regulated and lncRNA MALAT1 was up-regulated in hepatocytes differentiated from BM-MSCs

To further investigate deeper insights for ZEB1 in hepatocytes differentiated from BM-MSCs, expression of miR-217 and lncRNA MALAT1 was determined in different cells. As shown in Fig. 5, expression of miR-217 was significantly down-regulated and MALAT1 was significantly up-regulated in hepatocytes differentiated from BM-



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MSCs compared with those of HSC-T6 and BRL-3 A cells ($P < 0.05$).

3.6. LncRNA MALAT1 regulated telomerase activity by targeting miR-217 through regulation of TERT

At last we investigated effect of lncRNA MALAT-1 on telomerase activity and the possible mechanisms. First we chose two of the three cell lines, BRL-3 A cells and HGF treated BM-MSCs to perform further experiments. MS2-RIP analysis showed MALAT-1 was physically associated with miR-217 (Fig. 6A–C). The relative luciferase activity, relative enrichment to MS2 by MS2-RIP were all significantly decreased in wt-MALAT-1 transfected with miR-217 mimic compared with the

Fig. 6. MALAT-1 regulated telomerase activity by targeting miR-217 through regulation of TERT.
(A) MS2-RIP followed by qRT-PCR to detect endogenous miR-217 associated with MALAT1.
(B) Luciferase activity in BM-MSCs and BRL-3 A cells co-transfected with miR-217 and luciferase reporters containing nothing (pmirGLO), wt-MALAT1 or Mut-MALAT1.
(C) Anti-AGO2 RIP was performed in BM-MSCs and BRL-3 A cells transfected with miR-217, followed by qRT-PCR to detect MALAT1 associated with AGO2.
(D) The mRNA and protein levels of ZEB-1 in control (Con) and MALAT-1
(MALAT-1) cells were determined by qRT-PCR and western blot, respectively.
(E) The relative protein levels of ZEB-1 in Wt or Mut MALAT-1 overexpressed cells with or without overexpression of miR-217.
(F) The relative luciferase activity of ZEB-1 3′UTR in Wt or Mut MALAT-1 overexpressed cells with or without overexpression of miR-217.
(G) The relative expression of ZEB-1 in MALAT-1 overexpression cells with or without shRNA against ZEB-1 (sh-ZEB-1).
(H) Decreased expression of ZEB-1 abolished the promotion of proliferation.
(I) Decreased expression of ZEB-1 lowered telomerase activity.
(J) Decreased expression of ZEB-1 reduced level of TERT mRNA and protein.
*P < 0.05, **P < 0.01. All experiments were conducted in triplicate.

control (P < 0.05), but showed no significant difference in mut-MALAT-1.

Then, when MALAT-1 was over-expressed in both BRL-3 A cells and HGF treated BM-MSCs, the expression of ZEB1 was significantly up-regulated compared with the control (P < 0.05, Fig. 6D). Further experiments showed ZEB1 was up-regulated when cells were transfected with wt-MALAT-1 compared with the mut-MALAT-1 and the control (P < 0.05, Fig. 6E). Meanwhile transfection of miR-217 could significantly reduce the expression of ZEB1 which was enhanced by wt-MALAT-1 (P < 0.05). The relative luciferase activity also showed similar results (Fig. 6F). All these results showed MALAT-1 might function in the regulation of ZEB1.

Finally we found MALAT-1 could significantly enhance the cell viability, expression of ZEB1, as well as telomerase activity and expression of TERT in both BRL-3 A cells and HGF treated BM-MSCs, while transfection of sh-ZEB1 significantly reduced the effects (P < 0.05, Fig. 6G–J). All these results indicated MALAT-1 might act as a ceRNA and could regulate ZEB1 level, and further take effect in telomerase activity through regulation of TERT.

4. Discussion

Telomerase is a key factor in cell proliferation, metastasis and senescence [9]. Several studies found the β-catenin/ZEB1 signaling was involved in regulation of telomerase activity. Suh et al. showed the up-regulation of β-catenin could significantly enhance the expression of TERT and thus might also influence telomerase activity in the process of intestinal regeneration [15]. Qin et al. demonstrated ZEB1 might be involved in regulation of telomerase expression in colorectal cancer [16]. The lncRNA MALAT1 is considered to play an important role in many diseases and process such as cancer and endothelial cell function [25]. Li et al. showed MALAT1 could promote proliferation and angiogenesis of BM-MSCs [26]. However, up to now, no study focused on effects of β-catenin/ZEB1 signaling on regulation of telomerase activity in BM-MSCs and the role of MALAT1 in this process. In the present study, we for the first time demonstrated that β-catenin-coordinated MALAT1/miR-217 axis could up-regulate expression of ZEB-1 and further enhanced the telomerase activity through regulation of TERT.

Several studies have reported roles of both β-catenin signaling and TERT in MSCs. Li et al. demonstrated miR-9-5p could promote MSCs migration by activating β-catenin signaling pathway [27]. Bermeo et al. found regulation of β-catenin pathway was associated MSCs differentiation [28]. Liang et al. found TERT gene transfection could contribute to differentiation of human umbilical cord mesenchymal stem cells into hepatocyte-like cells [24]. All the previous studies were in consistent with our findings. However, few study reported role of ZEB1 in MSCs. In the present study, we found β-catenin, TERT as well as ZEB1 was up-regulated in HGF-induced hepatocytes differentiated from BM-MSCs through LPS stimulation.

Then we investigated effects of β-catenin/ZEB1 on telomerase activity. Relationship among β-catenin/ZEB1 and TERT, and their roles in telomerase activity have been demonstrated in some related researches. Hoffmeyer et al. found Wnt/β-catenin signaling could regulate telomerase in stem cells and cancer cells [29]. In an early study, it was demonstrated β-catenin driven expression of TERT in colorectal adenocarcinomas [30]. Yu et al. showed ZEB1 could stimulate breast cancer growth by up-regulating TERT expression [31]. In the present study, we for the first time demonstrated β-catenin/ZEB1 signaling could influence telomerase activity through regulation of TERT in BM-MSCs and further influence the cell viabilities.

At last we investigated effects of MALAT1/miR-217 axis on ZEB1/TERT regulated telomerase activity. Results showed MALAT1 might function as a ceRNA and could regulate the ZEB1 level, and affect telomerase activity through regulation of TERT. It was reported MALAT1 could regulate ZEB1 expression and promoted hepatocellular carcinoma progression [17]. Several studies have shown the relationship between MALAT1 and miR-217. Wang et al. demonstrated inhibition of MALAT1 by miR-217 could impede proliferation, migration, and invasion of esophageal squamous cell carcinoma cells [32]. Liu et al. showed MALAT1 acted as a ceRNA by sponging miR-217 pancreatic ductal adenocarcinoma [19]. However few studies focused on relationship between MALAT1 and TERT. In the present study, we for the first time found the MALAT1 might target on miR-217, and further influenced telomerase activity in BM-MSCs. What's more, we also found MALAT1 function as a ceRNA in the regulation of ZEB1 by binding miR-17. All that needs us more studies to confirm.

5. Conclusions

In conclusion, we successfully used HGF to mediate differentiation of BM-MSCs into hepatocytes, and found β-catenin-coordinated MALAT1/miR-217 axis could up-regulate expression of ZEB-1 and further enhanced the telomerase activity through regulation of TERT in BM-MSCs differentiating hepatocytes. This study might give deeper insights for role of β-catenin/ZEB1 as well as MALAT1/miR-217 in differential of BM-MSCs, and could provide basis for potential application of liver regeneration through differential of BM-MSCs into hepatocytes.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work was supported by Key Project of Provincial Health Department (No. 2015-218).

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