TAZ regulates cell proliferation and epithelial–mesenchymal transition of human hepatocellular carcinoma

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The transcriptional coactivator with PDZ binding motif (TAZ) has been reported to be one of the nuclear effectors of Hippo-related pathways. TAZ is expressed in many primary tumors and could regulate many biological processes. However, little is known about the role of TAZ in hepatocellular carcinoma (HCC). In the current study, we show that TAZ regulates cellular proliferation and epithelial–mesenchymal transition (EMT) of HCC. TAZ is overexpressed in HCC tissues and cell lines and upregulation of TAZ correlates with a lower overall survival rate of HCC patients after hepatic resection. TAZ knockdown results in inhibition of cancer cell proliferation through decreases in expression of stem cell markers (OCT4, Nanog, and SOX2). Reduction in HCC cell migration and invasion is also evident through reversal of EMT by increases E-cadherin expression, decreases in N-cadherin, vimentin, Snail, and Slug expression, and suppression of MMP-2 and MMP-9 expression. In a xenograft tumorigenicity model, TAZ knockdown could effectively inhibit tumor growth and metastasis through reversal of the EMT pathway. In conclusion, TAZ is associated with the proliferation and invasiveness of HCC cells, and the *TAZ* gene may contribute to a novel therapeutic approach against HCC.

Hepatocellular carcinoma (HCC), is the third leading cause of cancer death worldwide, and the second in China.[1] Existing therapies, including surgical resection and liver transplantation, are insufficient for tumor eradication and there is a high frequency of tumor recurrence; the prognosis for HCC patients remains pessimistic. It is therefore important to investigate new molecular mechanisms of growth and metastasis of HCC, and to establish the identity of new targets for therapeutic approaches that will improve the prognosis of HCC patients.

The transcriptional coactivator with PDZ binding motif (TAZ), also referred as WWTR1 (WW-domain containing transcriptional regulator 1), was first reported as a 14-3-3 binding protein.[2] TAZ is expressed in many primary tumors, such as breast cancer, thyroid carcinoma, colorectal cancer, and glioma.[3–6] It has been reported that TAZ may regulate many biological processes, including cell proliferation, migration, and invasion.[3,7,8] TAZ has been reported to be one of the nuclear effectors of Hippo-related pathways that regulate organ size control, cancer stem cell (CSC) properties, and epithelial–mesenchymal transition (EMT).[8–12]

However, little is known about the role of TAZ in HCC. In the present study, we show that TAZ is highly expressed in HCC tissues compared to peritumoral tissues. We also show

that TAZ reduction inhibits HCC cell lines' proliferation, migration, and invasion both *in vitro* and *in vivo* in a nude mouse model. These results show that TAZ contributes to the tumorigenesis of HCC by regulating cell proliferation and EMT.

Materials and Methods

Clinical specimen collection. Samples from 90 patients with HCC who underwent hepatic resection at our hospital (First Affiliated Hospital, Chonqing Medical University, Chongqing, China) between 2006 and 2010 were collected in this study. Consent was obtained from all patients, and the experimental protocols were approved by the local ethics committee. Patient charts were reviewed to obtain clinical data regarding age, gender, tumor size, TNM stage (according to the American Joint Committee on Cancer), and tumor differentiation, and death or time of last follow-up. Patient survival was calculated from the day of surgery until death, in months.

Cell culture. Six human HCC cell lines (HepG2, Huh7, Bel-7402, SK-HepG1, SMMC-7721, and HCC-LM3) and one immortalized liver cell line (L02) were purchased from the Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences (Shanghai Institute of Cell Biology,

Shanghai, China) and were cultivated as described by the suppliers.

Antibodies. Rabbit mAbs recognizing TAZ (ab110239, dilution 1:1000), Oct4 (ab109183, dilution 1:1000), Nanog (ab109205, dilution 1:1000), and rabbit polyclonal antibody recognizing SOX2 (ab97959, dilution 1:1000) were obtained from Abcam (Cambridge, MA, USA) and MMP-9 (Cat.#: 1939-1, dilution 1:1000), MMP-2 (Cat.#: 1948-1, dilution 1:1000), and β-actin(Cat.#: 1854-1, dilution 1:1000) were obtained from Epitomics (Abcam). Epithelial–mesenchymal transition-related antibodies (No. 9782S, dilution 1:1000) (E-cadherin, N-cadherin, Slug, Snail, β-catenin, and vimentin) were obtained from Cell Signaling Technology (Danvers, MA, USA).

Immunohistochemistry. Immunohistochemistry was carried out as described previously.[13] Paraffin sections were scored semiquantitatively as: grade 0, 0% immunoreactive cells; grade 1, ≤5% immunoreactive cells; grade 2, >5–50% immunoreactive cells; and grade 3, ≥51% immunoreactive cells. For statistical purposes, cases with grade 0 or 1 were considered as having low expression, and those with grade 2 and 3 were considered as having high expression. The immunoreactive area was assessed by two independent investigators in our team without prior knowledge of clinical pathologic data using the software Image-Pro Plus 6.0. (Media Cybernetics Inc., NewYork, USA) The color of the immunoreactive area was chosen by histogram, and the false-positive area was wiped out by the filter option. The assessed data were used to draw column diagrams.

Transfections of lentiviral vectors with TAZ siRNA. To study the function of TAZ, a TAZ siRNA lentiviral vector (lenti-siRNA/TAZ) was constructed (Shanghai GeneChem, Shanghai, China). A GFP-lentiviral vector (Scr-siRNA/GFP) was used as the negative control (NC). Double-strained oligonucleotides encoding human TAZ siRNA were inserted into the siRNA expression vector Scr-siRNA/GFP (Shanghai GeneChem). The lentiviruses were transected according to the manufacturer's instructions. For stable cell lines, transfected cells were selected using 3 μg/mL puromycin. The siRNA sequences were: siRNA1, GATGAATCAGCCTCTGAAT; siRNA2, CCTTTCTAACCTGGCTGTA; and siRNA3, GCCAAATCTCGTGATGAAT.

Total RNA extraction and RT-PCR. The mRNA levels were determined by our previous method.[14] Primers were synthe-
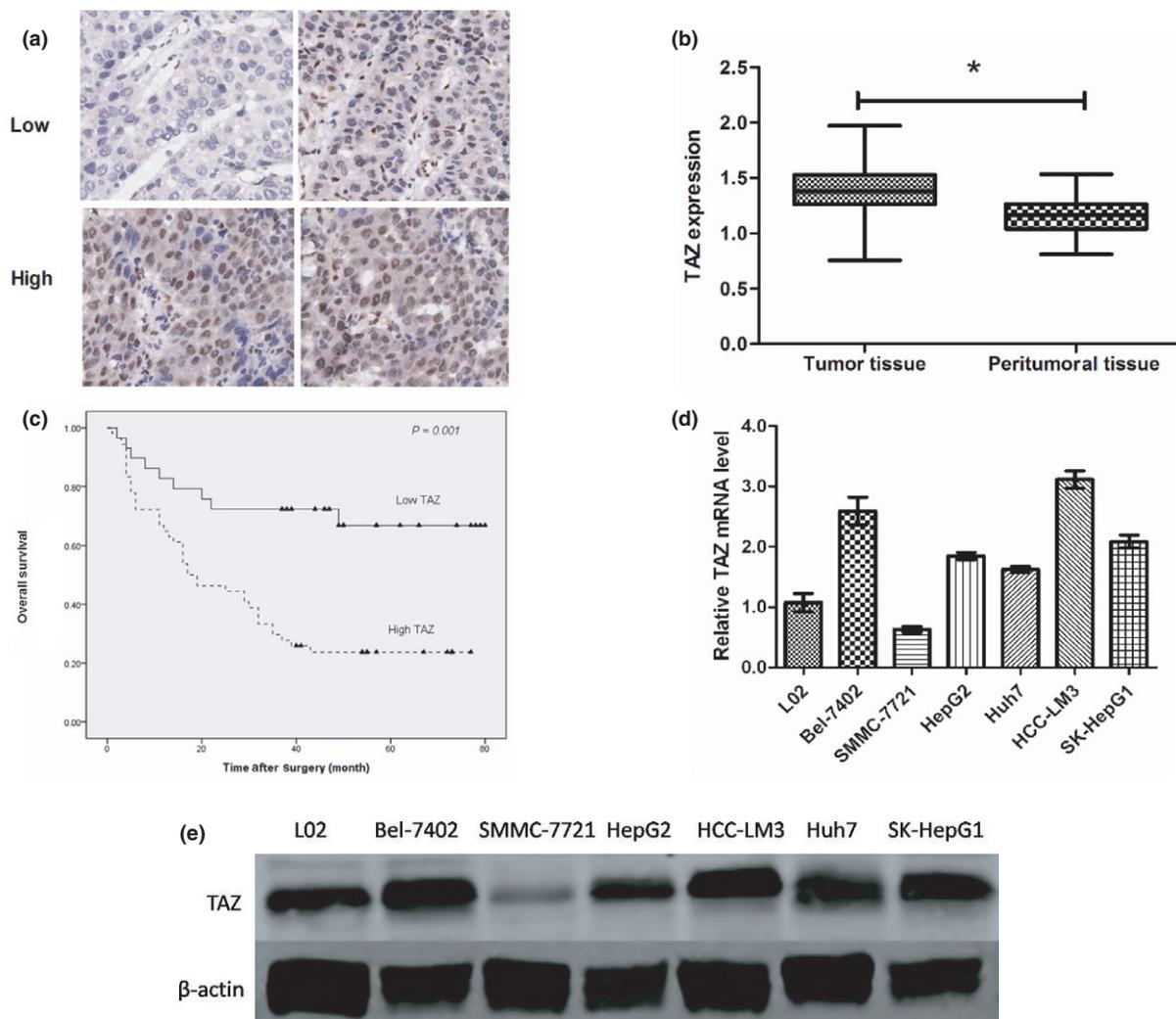
Fig. 1. Transcriptional coactivator with PDZ binding motif (TAZ) is highly expressed in hepatocellular carcinoma (HCC) and increased levels of TAZ indicate worsening prognosis of HCC. (a) HCC samples in a tissue microarray were immunostained with a monoclonal anti-TAZ antibody. Representative low-TAZ and high-TAZ expression samples are shown (×400). (b) Protein levels of TAZ were determined in 83 samples of HCC. (c) Overall survival rates of 83 patients with HCC after hepatic resection were compared between low-TAZ and high-TAZ groups. (d) TAZ mRNA expression was determined by RT-PCR in six HCC cell lines and one immortalized liver cell line. (e) TAZ protein expression was determined by Western blot in seven HCC cell lines. β-actin was used as a positive control. *$P < 0.05$.

sized by Shanghai Sangon Biological Engineering Technology Services (Shanghai, China). The nucleotide sequences of the primers were: GAPDH, 5′-ATGGGGAAGGTGAAGGTCG-3′ and 5′-GGGGTCATTGATGGCAACAATA-3′; TAZ, 5′-GCT GGCTCTGATGGCTCTTA-3′ and 5′-AGACCTGTGACTAA TGCTGCTG-3′; OCT4, 5′-CCTGAAGCAGAAGAGGAT CACC-3′ and 5′-AAAGCGGCAGATGGTCGTTTGG-3′; SOX2, 5′-GCTACAGCATGATGCAGGACCA-3′ and 5′-TC TGCGAGCTGGTCATGGAGTT-3′; Nanog, 5′- CTCCAACA TCCTGAACCTCAGC-3′ and 5′-CGTCACACCATTGCTATT CTTCG-3′; E-cadherin, 5′-CGAGAGCTACACGTTCACGG-3′ and 5′-GGGTGTCGAGGGAAAAATAGG-3′; β-catenin, 5′-CA CAAGCAGAGTGCTGAAGGTG-3′ and 5′-GATTCCTG AGAGTCCAAAGACAG-3′; N-cadherin, 5′-TTTGATGGAGG TCTCCTAACACC-3′ and 5′-ACGTTTAA CACGTTGG AAATGTG-3′; vimentin, 5′-GACGCCATCAACACCGAGTT-3′ and 5′-CTTTGTCGTTGGTTAGCTGGT-3′; Slug, 5′-CGA ACTGGACACACATACAGTG-3′ and 5′-CTGAGGATCTC TGGTTGTGGT-3′; and Snail, 5′- TGCCCTCAAGATGCA-CATCCGA-3′ and 5′- GGGACAGGAGAAGGGCTTCTC-3′. Each sample was taken in triplicate. GAPDH was used as internal reference, and the $2^{-\Delta\Delta C_T}$ method was used to analyze PCR results.

Western blot analysis. As described previously,[14] soluble proteins were collected and stored at −80°C after a centrifugation at 12 000 *g* for 15 min. The protein amount was determined by Bradford assay (Bio-Rad, Hercules, CA, USA). For testing, after denaturation, the proteins were separated with gel electrophoresis using 10% SDS-PAGE, and then wet transferred to a PVDF membrane for 2 h of blocking in 5% skim milk. The membrane was washed once with TBST, then incubated for overnight at 4°C with relevant antibodies (1:1000). The membrane was again washed three times with TBST, then incubated with secondary antibody (goat anti-rabbit/mouse IgG 1:2000) for 2 h at room temperature. The membrane was washed a third time with TBST then ECL liquid was added and placed in a darkroom. β-actin (1:2000) was used as a positive control.

Cell proliferation, migration, and invasion assays. Cell proliferation assay and migration and invasion assays were carried out as described previously.[14]

Tumor xenograft experiments. All experimental procedures were carried out in accordance with the National Institute Guide for the Care and Use of Laboratory Animals. Cells (2×10^7) were resuspended in 100 μL PBS and injected s.c. into the lateral flanks of immunodeficient mice (six animals per group). Tumor volumes were measured weekly and calculated using the equation: V (cm^3) = width^2 (cm^2) × length (cm) / 2. After 4 weeks, tumors were harvested for immunostaining after tumor implantation.

Statistical analysis. Statistical analysis was carried out using SPSS 17.0 software (SPSS Inc, Chicago, IL, USA). The experimental data were expressed as mean ± SD, and assessed by a two-tailed Student's *t*-test. The χ^2-test or Fisher's exact test was used to evaluate any potential association between TAZ expression and clinicopathologic parameters. Overall survival rate was calculated with the Kaplan–Meier method, and the statistical difference between survival curves was determined with the log–rank test. Statistical significance was accepted if *P* < 0.05.

Results

High expression of TAZ in HCC and upregulation correlates with low overall survival rate after hepatic resection. We analyzed the expression levels of TAZ in 90 pairs of clinical

Table 1. Relationship between transcriptional coactivator with PDZ binding motif (TAZ) expression and clinicopathologic features in patients with hepatocellular carcinoma

Variable	TAZ density		*P*-value
	Low-TAZ	High-TAZ	
In general			
Adjacent tissue	61	22	<0.000
Tumor tissue	29	54	
Sex			
Male	26	44	0.260
Female	3	10	
Age, years			
≤50	8	15	0.598
>50	21	39	
Tumor size, cm			
≤5	12	11	**0.039**
>5	17	43	
AFP, ng/mL			
≤400	7	16	0.396
>400	22	38	
HBsAg			
Positive	27	49	0.532
Negative	2	5	
Liver cirrhosis			
Yes	13	18	0.213
No	16	36	
Portal vein emboli and metastasis			
Yes	2	12	**0.028**
No	27	32	
TNM stage (AJCC)			
I–II	17	26	0.249
III–IV	12	28	
Tumor differentiation			
I–II	18	34	0.560
III–IV	11	20	

P-values shown in bold are significant. AFP, alpha fetoprotein; AJCC, American Joint Committee on Cancer; HBsAg, hepatitis B surface antigen.

HCC samples by immunohistochemistry. Tissue microarray analysis results showed that TAZ levels (Fig. 1a,b) were higher in tumor tissues of patients than in peritumoral tissues (*P* < 0.001). We also analyzed the relationship between TAZ expression and clinicopathologic features. No detailed clinical data were available for seven patients, so we analyzed clinical data from a total of 83 patients. The results revealed that tumor size and metastasis were significantly different between tumor and peritumoral tissues (*P* = 0.039 for size and *P* = 0.028 for metastasis) (Table 1). To determine whether TAZ expression in HCC was associated with overall survival rate, all 83 patients with HCC after hepatic resection were

Table 2. Relationship between transcriptional coactivator with PDZ binding motif (TAZ) expression and survival rate in patients with hepatocellular carcinoma

Survival	TAZ density		
	Low-TAZ	High-TAZ	*P*-value
1-year overall survival, %	82.8 ± 7.0	64.8 ± 6.5	0.001
3-year overall survival, %	72.4 ± 8.3	29.6 ± 6.2	
5-year overall survival, %	66.8 ± 9.3	23.8 ± 5.8	
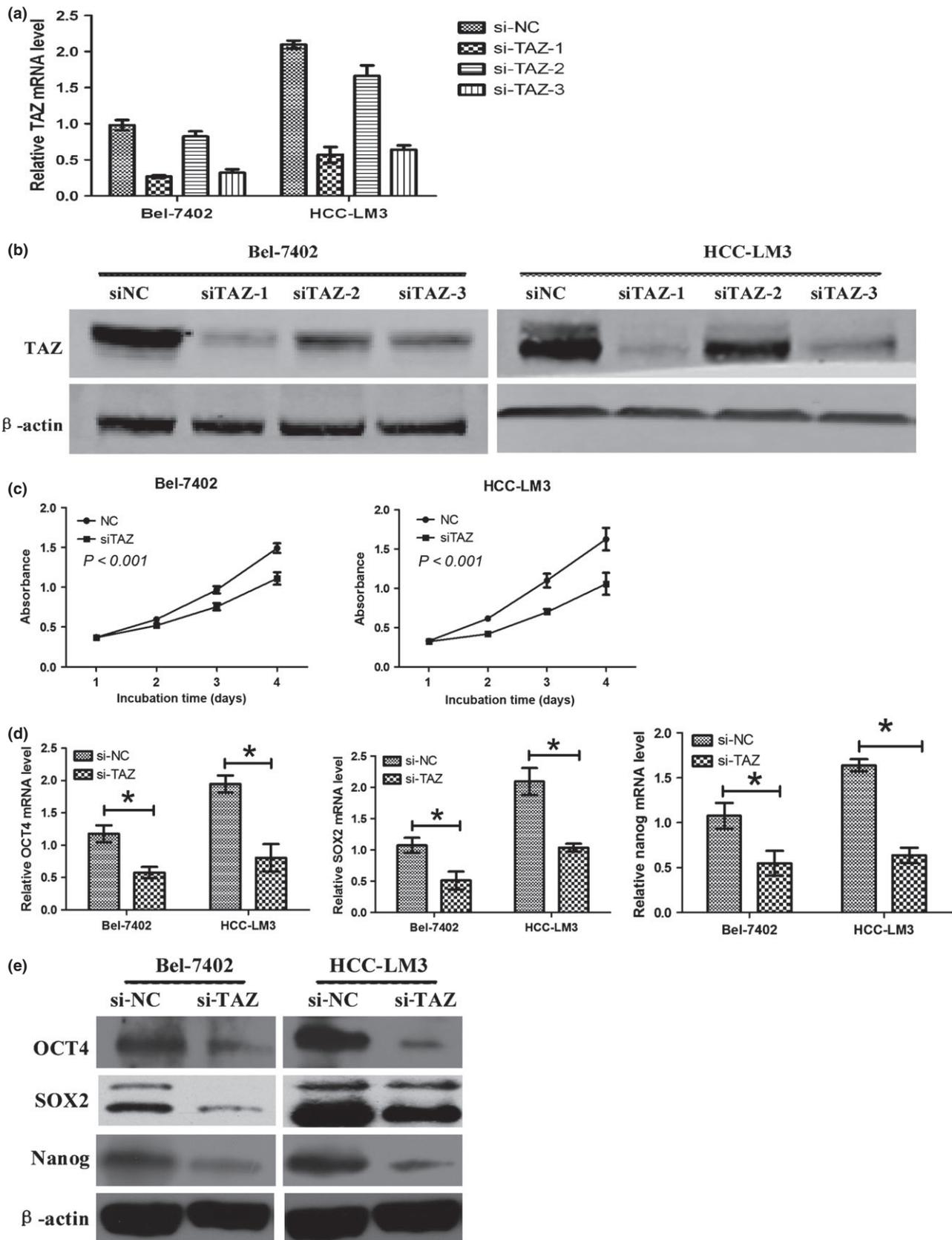
Fig. 2. Knockdown of transcriptional coactivator with PDZ binding motif (TAZ) inhibits cancer cell proliferation. (a, b) Bel-7402 and HCC-LM3 hepatocellular carcinoma cell lines were transfected with non-targeting siRNA (NC) or TAZ siRNA, and the expression of TAZ was measured by real-time PCR and Western blot after stable cell lines were selected. (c) Cell proliferation was measured by CCK-8 assay after cells were incubated for 1, 2, 3, and 4 days (*n* = 8). (d) Effects of TAZ knockdown on stem cell markers (OCT4, Nanog, and SOX2) were measured by RT-PCR and Western blot. β-actin was used as a positive control. Data was expressed as mean ± SD. *P < 0.05.
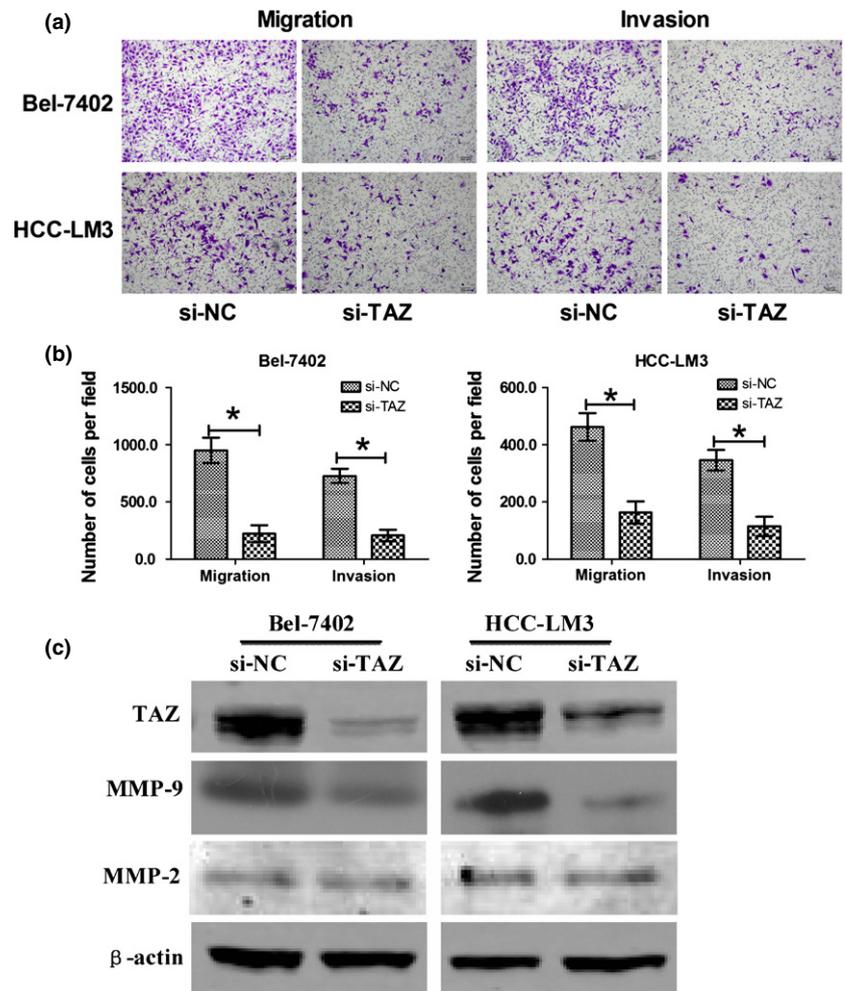
Fig. 3. Knockdown of transcriptional coactivator with PDZ binding motif (TAZ) inhibits migration and invasion of hepatocellular carcinoma cell lines. (a, b) Migration and invasion of stable TAZ knockdown cells were measured by Transwell assay. Results were quantitated by counting migrated and invasive cells in five randomly chosen high-power fields for each replicate ($n = 3$). (c) Effects of TAZ knockdown on MMP-2 and MMP-9 were measured by Western blot. Data was expressed as mean ± SD. *$P < 0.05$. si-NC, non-targeting siRNA; si-TAZ, TAZ siRNA.

divided into two groups: high expression (high-TAZ; $n = 54$) and low expression (low-TAZ; $n = 29$). Kaplan–Meier analysis revealed that HCC tissues with high expression of TAZ had a worse overall survival rate ($P = 0.001$) (Fig. 1c). The 1-, 3-, and 5-year overall survival rates among patients with high-TAZ were 64.8, 29.6, and 23.8%, respectively, whereas the rates in patients with low-TAZ were 82.8, 72.4, and 66.8%, respectively (Table 2). Examination of TAZ mRNA/protein expression in six HCC cell lines (Bel-7402, SMMC-7721, HepG2, HCC-LM3, Huh7, and SK-HepG1) and one immortalized liver cell line (L02) showed that TAZ was expressed in all of these cell lines. Six of these HCC lines except SMMC-7721 showed increased mRNA/protein expression compared to the immortalized liver cell line (L02) (Fig. 1d,e).

Knockdown of TAZ inhibits cancer cell proliferation. For subsequent experiments, low-invasion and high-invasion HCC cell lines (Bel-7402 and HCC-LM3, respectively) were selected due to their TAZ expression characteristics. Knockdown of TAZ in the stably transfected Bel-7402 and HCC-LM3 cells was confirmed by RT-PCR (Fig. 2a) and Western blotting (Fig. 2b). The CCK-8 assay was carried out to evaluate the role of TAZ on proliferation of liver cancer cells Bel-7402 and HCC-LM3. The results of the CCK-8 assay are shown in Figure 2(b). siRNA targeting TAZ significantly inhibited cell growth of both Bel-7402 cells and HCC-LM3 cells compared to its negative group. As TAZ is crucial for the self-renewal of stem cells and cell proliferation, and is implicated in CSC-like properties,[9,15] we examined whether this pathway was inhib-

ited after TAZ knockdown. As shown in Figure 2(c), RT-PCR and Western blot analysis (Fig. 2d and 2e) revealed that transcription factor proteins including OCT4, SOX2, and Nanog were decreased after TAZ knockdown.

Knockdown of TAZ inhibits migration and invasion of HCC cell lines. The effect of TAZ on the migration and invasiveness of HCC cells was analyzed using the Transwell assay (Fig. 3a). The average number of both migratory and invasive cells with si-TAZ was decreased significantly compared to cells with si-NC ($P < 0.001$) (Fig. 3b). To further evaluate the mechanism of these results, we assessed the effect of TAZ on MMP-mediated ECM degradation, which is a crucial step in cell invasion. Western blot results shown that TAZ knockdown significantly decreased the protein levels of MMP-9 in both Bel-7402 cells and HCC-LM3 cell lines, but there was no significant difference in MMP-2 (Fig. 3c).

Knockdown of TAZ reverses EMT pathway. Epithelial–mesenchymal transition is a crucial step in cellular invasion and metastasis. To investigate the effect of TAZ expression on the expression of EMT molecules in HCC cells, quantitative RT-PCR and Western blot analysis were used to analyze EMT marker genes and proteins. The TAZ knockdown resulted in upregulation of the epithelial marker E-cadherin mRNA expression levels, and downregulation of the mesenchymal markers N-cadherin, vimentin, Snail, and Slug mRNA expression, compared with the negative NC growth ($P < 0.001$) (Fig. 4a). Consistent with mRNA expression, Western blot analysis showed that E-cadherin was upregulated in si-TAZ
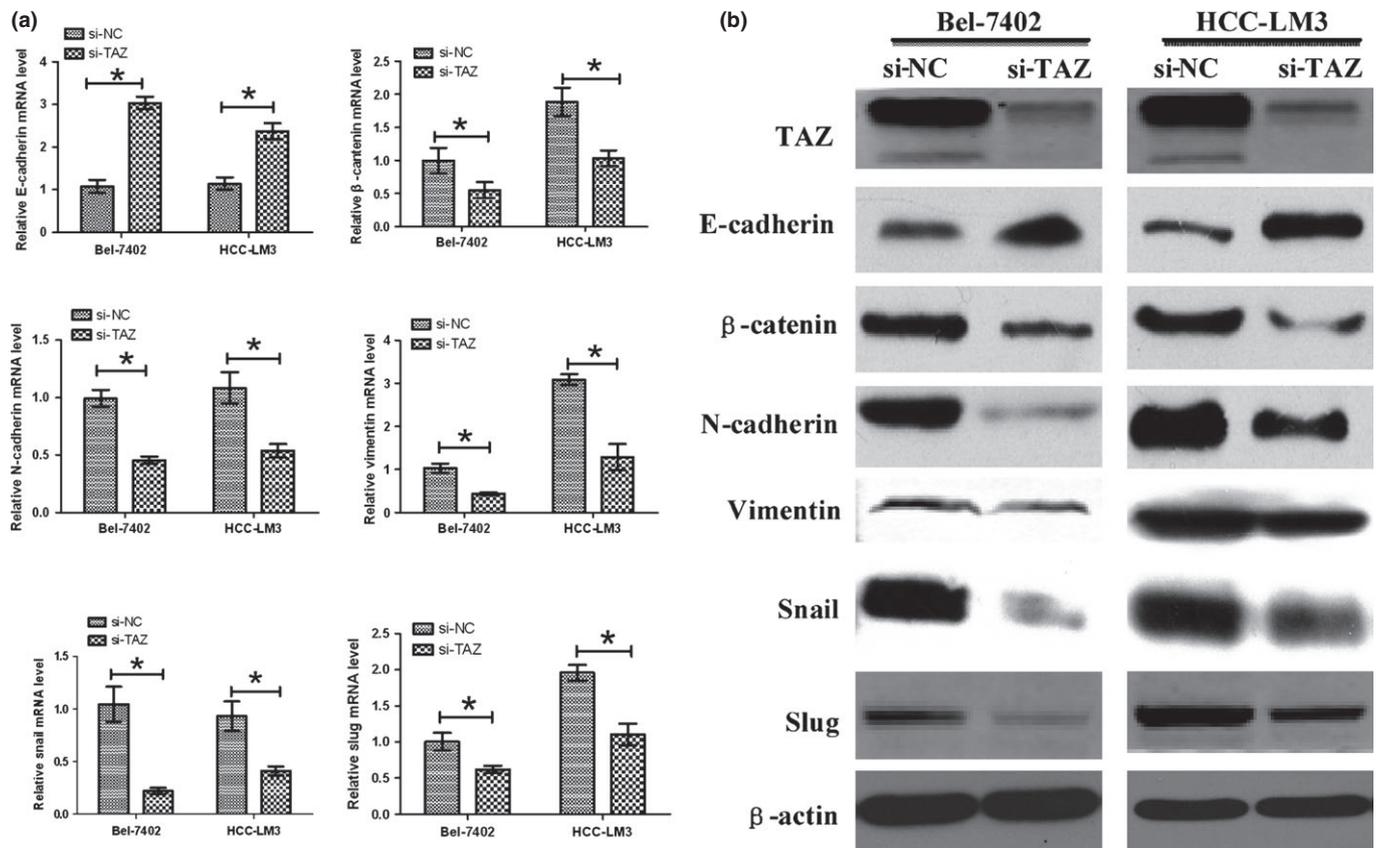
Fig. 4. Transcriptional coactivator with PDZ binding motif (TAZ) knockdown reduces epithelial–mesenchymal transition (EMT) of hepatocellular carcinoma cell lines. (a) mRNA levels of EMT marker genes such as E-cadherin, β-catenin, N-cadherin, Slug, Snail, and vimentin were measured by RT-PCR. Data are expressed as mean ± SD. *$P < 0.05$. (b) EMT marker protein levels were measured by Western blot. β-actin was used as a positive control. Data are expressed as mean ± SD. *$P < 0.05$. si-NC, non-targeting siRNA; si-TAZ, TAZ siRNA.

cells compared to NC cells. The results also revealed that the expression levels of mesenchymal markers such as vimentin and Snail were downregulated in si-TAZ cells (Fig. 4b). Interestingly, expression of epithelial marker E-cadherin–catenin complex factor–β-catenin was decreased both in mRNA and protein levels (Fig. 4b).

Knockdown of TAZ inhibits tumorigenicity of transfected HCC cell lines *in vivo*. We further investigated the effect of TAZ on HCC growth in nude mice. The TAZ-siRNA-transfected Bel-7402 cells and NC group cells were injected s.c. into nude mice (six animals per group). All mice were killed and the tumors were dissected at end of the experiment. In the NC group, all six mice had tumor formation; in the si-TAZ group, five of six mice formed tumors *in vivo* (Fig. 5a). Compared to the NC group, TAZ knockdown resulted in significant decrease of tumor size and weight ($P < 0.05$) (Fig. 5b). The tumors were stained with TAZ, MMP-9, MMP-2, E-cadherin, β-catenin, N-cadherin, vimentin, and Snail. The immunoreactivity and expression of TAZ, MMP-9, MMP-2, N-cadherin, vimentin, β-catenin, and Snail were decreased in TAZ knockdown-injected tumors as compared with NC-injected mice, and the expression of E-cadherin was increased (Fig. 5c,d).

Discussion

The TAZ protein has been identified as one of the nuclear effectors of the Hippo-related pathways that regulate the switch between CSC properties and EMT.[7,9] The findings of our

study also indicate a role for TAZ in HCC cell proliferation and EMT. In addition, clinical data suggest that TAZ is significantly connected with tumor size, and HCC tissues with high expression of TAZ had worse overall survival rate than those with low expression. These results provide the first evidence supporting the pro-proliferation and pro-metastatic function of TAZ in HCC.

In this study, peritumoral tissues expressed TAZ highly in 22 of 83 (26%) of cases compared to 54 of 83 tumor tissues (65%). The *TAZ* gene distribution varied in different regions and populations, particularly between healthy people and tumor patients. Furthermore, the high TAZ expression in peritumoral tissues (26%) could not be equated with normal liver tissues; this might indicate that TAZ was increased in preneoplastic lesions of HCC and might be a useful biomarker for predicting the risk of HCC.

We further investigated the molecular mechanism by which TAZ promotes proliferation activity in HCC. Here, we showed that the protein levels of OCT4, SOX2, and Nanog were decreased after TAZ knockdown. Some studies have reported that these three proteins could be activated in pluripotent stem cells and CSCs.[16,17] Transcription of many genes regulated by OCT4, SOX2, and Nanog could be controlled by transforming growth factor-β/SMADs.[15,17,18] Moreover, TAZ has been identified as one of the critical mediators of the aggressive tumor stem cell properties of TGF-β/SMADs.[19,20] In breast tumor, TAZ has also been found to promote CSC populations.[10] Collectively, these studies and our data suggest the pro-proliferation effect of TAZ may be
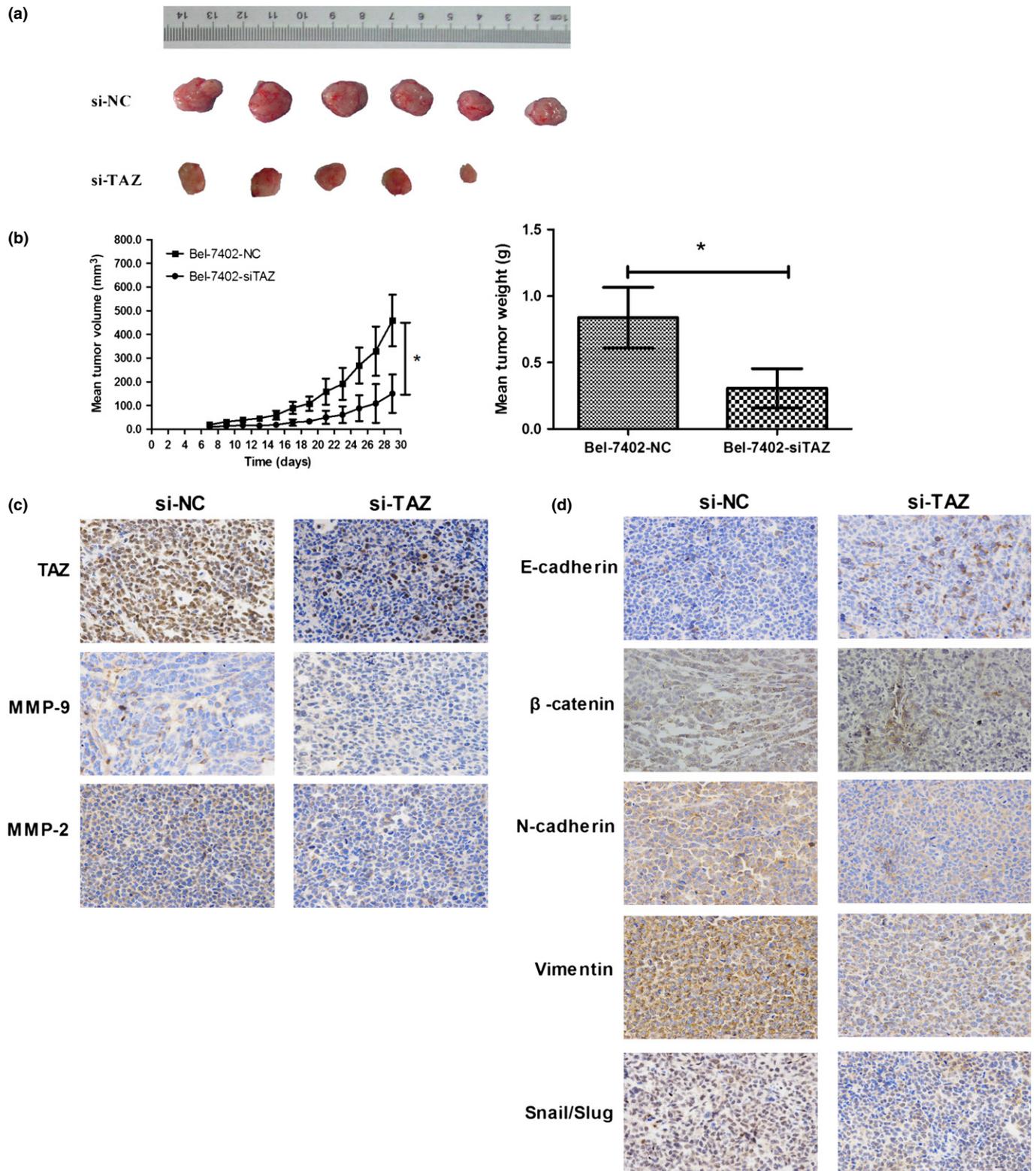
Fig. 5. Transcriptional coactivator with PDZ binding motif (TAZ) knockdown inhibits tumorigenicity and epithelial–mesenchymal transition of hepatocellular carcinoma cells *in vivo*. (a, b) *In vivo* tumorigenesis assay of tumor size and weight after transfected hepatocellular carcinoma cells were injected s.c. into nude mice. (c, d) Immunohistochemistry analysis of TAZ, MMP-2, MMP-9, E-cadherin, N-cadherin, Snail, vimentin, and β-catenin. Data are presented as mean ± SD. *$P < 0.05$. si-NC, non-targeting siRNA; si-TAZ, TAZ siRNA.

related to the regulation of the expression of stem cell markers OCT4, SOX2, and Nanog.

We then studied the molecular role of TAZ in the migration and invasion of HCC cells. One possible mechanism is through MMP-mediated ECM degradation.[21–23] Some studies have reported that TAZ may interact with MMP-2, MMP-9, and MMP-14 to regulate invasion of breast carcinoma cells and skeletal stem cells.[8,24,25] In our study, TAZ significantly

decreased the protein levels of MMP-9 and MMP-2 in the animal experiment after TAZ knockdown, although in the cell line experiment we only found a decreased protein level of MMP-9. This might be because the microvessel was formed *in vivo* or because differences in microenvironments between *in vitro* and *in vivo* experiments might impact the expression of MMP-2.

Another possible mechanism of the role of TAZ in inducing invasion of HCC is that EMT is involved in tumor progress. Epithelial–mesenchymal transition is a transcriptional program that downregulates epithelial gene expression, such as E-cadherin, and upregulates mesenchymal gene expression, such as N-cadherin, vimentin, Snail, and Slug.[26–28] The YAP protein has been reported to induce EMT and TAZ shares almost 50% sequence identity with YAP;[7] our study showed that TAZ knockdown reverses EMT by increasing E-cadherin expression and decreasing N-cadherin, vimentin, Snail, and Slug expression. In addition, CSC-like properties have been linked to the EMT process,[29] and both biological processes are associated with Snail expression.[10] Consistent with these reports, Snail expression significantly decreased after TAZ knockdown in our study at both the mRNA and protein levels.

Interestingly, the expression of β-catenin (an epithelial marker, E-cadherin–catenin complex factor) was decreased in our study. This result contrasts with a previous study that showed knockdown endogenous TAZ could increase the β-catenin protein level.[30] However, it also reported that β-catenin protein accumulation in the nucleus could promote tumor metastasis and downregulate E-cadherin expression.[31] In HCC, nuclear accumulation of β-catenin was associated with increased cell proliferation and tumor recurrence.[32,33] In addition, downregulation of β-catenin expression could effectively inhibit the properties of liver CSCs and the growth of HCC.[34,35] These results further explain the role of TAZ in cell proliferation and invasiveness.

In conclusion, we showed that TAZ is associated with the overall survival rate of patients after hepatic resection and that TAZ knockdown is connected with the proliferation and invasiveness of HCC cells, potentially by inhibiting CSC properties and EMT process. Knockdown of TAZ was also found to effectively suppress the tumorigenicity of HCC cell lines. We suggest that control of proliferation and metastasis through inhibition of the *TAZ* gene may contribute to a novel therapeutic approach against HCC.

Disclosure Statement

The authors have no conflict of interest.

References

1 He J, Gu D, Wu X *et al.* Major causes of death among men and women in China. *N Engl J Med* 2005; **353**: 1124–34.
2 Kanai F, Marignani PA, Sarbassova D *et al.* TAZ: a novel transcriptional co-activator regulated by interactions with 14-3-3 and PDZ domain proteins. *EMBO J* 2000; **19**: 6778–91.
3 Chan SW, Lim CJ, Guo K *et al.* A role for TAZ in migration, invasion, and tumorigenesis of breast cancer cells. *Cancer Res* 2008; **68**: 2592–8.
4 de Cristofaro T, Di Palma T, Ferraro A *et al.* TAZ/WWTR1 is overexpressed in papillary thyroid carcinoma. *Eur J Cancer* 2011; **47**: 926–33.
5 Yuen HF, McCrudden CM, Huang YH *et al.* TAZ expression as a prognostic indicator in colorectal cancer. *PLoS ONE* 2013; **8**(1): e54211.
6 Bhat KPL, Salazar KL, Balasubramaniyan V *et al.* The transcriptional coactivator TAZ regulates mesenchymal differentiation in malignant glioma. *Genes Dev* 2011; **25**: 2594–609.
7 Lei Q-Y, Zhang H, Zhao B *et al.* TAZ promotes cell proliferation and epithelial-mesenchymal transition and is inhibited by the hippo pathway. *Mol Cell Biol* 2008; **28**: 2426–36.
8 Matteucci E, Maroni P, Luzzati A, Perrucchini G, Bendinelli P, Desiderio MA. Bone metastatic process of breast cancer involves methylation state affecting E-cadherin expression through TAZ and WWOX nuclear effectors. *Eur J Cancer* 2013; **49**(1): 231–44.
9 Hong JH, Hwang ES, McManus MT *et al.* TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. *Science* 2005; **309**(5737): 1074–8.
10 Cordenonsi M, Zanconato F, Azzolin Luca *et al.* The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell* 2011; **147**: 759–72.
11 Hong W, Guan K-L. The YAP and TAZ transcription co-activators: key downstream effectors of the mammalian Hippo pathway. *Semin Cell Dev Biol* 2012; **23**: 785–93.
12 Yang N, Morrison C, Liu P *et al.* TAZ induces growth factor-independent proliferation through activation of EGFR ligand amphiregulin. *Cell Cycle* 2012; **11**: 2922–30.
13 Xiao H, Tong R, Cheng S *et al.* BAG3 and HIF-1 α coexpression detected by immunohistochemistry correlated with prognosis in hepatocellular carcinoma after liver transplantation. *Biomed Res Int* 2014; **2014**: 9.
14 Xiao H, Cheng S, Tong R *et al.* BAG3 regulates epithelial-mesenchymal transition and angiogenesis in human hepatocellular carcinoma. *Lab Invest* 2014; **94**: 252–61.
15 Hiemer SE, Varelas X. Stem cell regulation by the Hippo pathway. *Biochim Biophys Acta*, 2013; **1830**: 2323–34.
16 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663–76.
17 Mullen AC, Orlando DA, Newman JJ *et al.* Master transcription factors determine cell-type-specific responses to TGF-β signaling. *Cell* 2011; **147**: 565–76.
18 Xu RH, Sampsell-Barron TL, Gu F *et al.* NANOG is a direct target of TGFβ/activin-mediated SMAD signaling in human ESCs. *Cell Stem Cell* 2008; **3**: 196–206.
19 Varelas X, Samavarchi-Tehrani P, Narimatsu M *et al.* The Crumbs complex couples cell density sensing to Hippo-dependent control of the TGF-β-SMAD pathway. *Dev Cell* 2010; **19**: 831–44.
20 Varelas X, Sakuma R, Samavarchi-Tehrani P *et al.* TAZ controls Smad nucleocytoplasmic shuttling and regulates human embryonic stem-cell self-renewal. *Nat Cell Biol* 2008; **10**: 837–48.
21 Stetler-Stevenson WG, Aznavoorian S, Liotta LA. Tumor cell interactions with the extracellular matrix during invasion and metastasis. *Annu Rev Cell Biol* 1993; **9**: 541–73.
22 Curran S, Murray GI. Matrix metalloproteinases: molecular aspects of their roles in tumour invasion and metastasis. *Eur J Cancer* 2000; **36**(13 Spec No): 1621–30.
23 Polette M, Nawrocki-Raby B, Gilles C, Clavel C, Birembaut P. Tumour invasion and matrix metalloproteinases. *Crit Rev Oncol Hematol* 2004; **49**: 179–86.
24 Bendinelli P, Maroni P, Matteucci E, Luzzati A, Perrucchini G, Desiderio MA. Hypoxia inducible factor-1 is activated by transcriptional co-activator with PDZ-binding motif (TAZ) versus WWdomain-containing oxidoreductase (WWOX) in hypoxic microenvironment of bone metastasis from breast cancer. *Eur J Cancer* 2013; **49**: 2608–18.
25 Tang Y, Rowe RG, Botvinick EL *et al.* MT1-MMP-dependent control of skeletal stem cell commitment via a β1-integrin/YAP/TAZ signaling axis. *Dev Cell* 2013; **25**: 402–16.
26 Huber MA, Kraut N, Beug H. Molecular requirements for epithelial–mesenchymal transition during tumor progression. *Curr Opin Cell Biol* 2005; **17**: 548–58.
27 Mimeault M, Batra SK. Interplay of distinct growth factors during epithelial mesenchymal transition of cancer progenitor cells and molecular targeting as novel cancer therapies. *Ann Oncol* 2007; **18**: 1605–19.
28 Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* 2009; **139**: 871–90.
29 Mani SA, Guo W, Liao MJ *et al.* The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008; **133**: 704–15.
30 Varelas X, Miller BW, Sopko R *et al.* The Hippo pathway regulates Wnt/β-catenin signaling. *Dev Cell* 2010; **18**: 579–91.
31 Brabletz T, Jung A, Reu S *et al.* Variable β-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. *Proc Natl Acad Sci* 2001; **98**: 10356–61.

32 Nhieu JT, Renard CA, Wei Y, Cherqui D, Zafrani ES, Buendia MA. Nuclear accumulation of mutated β-catenin in hepatocellular carcinoma is associated with increased cell proliferation. *Am J Pathol* 1999; **155**: 703–10.

33 Zulehner G, Mikula M, Schneller D *et al.* Nuclear β-catenin induces an early liver progenitor phenotype in hepatocellular carcinoma and promotes tumor recurrence. *Am J Pathol* 2010; **176**: 472–81.

34 Quan MF, Xiao LH, Liu ZH *et al.* 8-bromo-7-methoxychrysin inhibits properties of liver cancer stem cells via downregulation of beta-catenin. *World J Gastroenterol* 2013; **19**: 7680–95.

35 Jin X-T, Song L, Zhao J-Y, Li Z-Y, Zhao M-R, Liu W-P. Dichlorodiphenyl-trichloroethane exposure induces the growth of hepatocellular carcinoma via Wnt∕β-catenin pathway. *Toxicol Lett* 2014; **225**(1): 158–66.