MicroRNA-125b Attenuates Epithelial-Mesenchymal Transitions and Targets Stem-Like Liver Cancer Cells Through Small Mothers Against Decapentaplegic 2 and 4

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Emerging evidence suggests that epithelial-mesenchymal transitions (EMTs) play important roles in tumor metastasis and recurrence. Understanding molecular mechanisms that regulate the EMT process is crucial for improving treatment of hepatocellular carcinoma (HCC). MicroRNAs (miRNAs) play important roles in HCC; however, the mechanisms by which miRNAs target the EMT and their therapeutic potential remains largely unknown. To better explore the roles of miRNAs in the EMT process, we established an EMT model in HCC cells by transforming growth factor beta 1 treatment and found that several tumor-related miRNAs were significantly decreased. Among these miRNAs, miR-125b expression was most strongly suppressed. We also found down-regulation of miR-125b in most HCC cells and clinical specimens, which correlated with cellular differentiation in HCC patients. We then demonstrated that miR-125b overexpression attenuated EMT phenotype in HCC cancer cells, whereas knockdown of miR-125b promoted the EMT phenotype in vitro and in vivo. Moreover, we found that miR-125b attenuated EMT-associated traits, including chemoresistance, migration, and stemness in HCC cells, and negatively correlated with EMT and cancer stem cell (CSC) marker expressions in HCC specimens. miR-125b overexpression could inhibit CSC generation and decrease tumor incidence in the mouse xenograft model. Mechanistically, our data revealed that miR-125b suppressed EMT and EMT-associated traits of HCC cells by targeting small mothers against decapentaplegic (SMAD)2 and 4. Most importantly, the therapeutic delivery of synthetic miR-125b mimics decreased the target molecule of CSC and inhibited metastasis in the mice model. These findings suggest a potential therapeutic treatment of miR-125b for liver cancer. Conclusion: miR-125b exerts inhibitory effects on EMT and EMT-associated traits in HCC by SMAD2 and 4. Ectopic expression of miR-125b provides a promising strategy to treat HCC. (HEPATOLOGY 2015;62:801-815)

Hepatocellular carcinoma (HCC) is the fifth-most common cancer in the world and has a high mortality because of a lack of effective treatments. Most HCC patients display symptoms of intrahepatic metastases or postsurgical recurrence with a low survival rate. Emerging evidence suggests that the epithelial-mesenchymal transition (EMT) contributes to tumor metastasis and recurrence, including in liver...
cancer. HCC cells undergoing EMT triggered by various stimuli may acquire altered traits, including chemoresistance, migration, and stenness. Although several lines of evidence have demonstrated the significance of EMT in HCC progression, the molecular mechanisms that regulate EMT remain unclear.

Accumulating evidence suggests that microRNAs (miRNAs) play important roles in regulating EMT in cancer cells. miRNAs are a class of conserved, small, noncoding RNA molecules that can negatively control gene expression at the post-transcriptional and/or transcriptional level. miRNAs have become a research focus not only because they play essential roles in human diseases, but also because synthetic miRNAs are similar to small-molecule therapeutics, given that they do not integrate into the patient genome, but do offer a high degree of specificity at the genetic level. It has been reported that almost every type of cancer, including liver cancer, displays a specific profile of aberrantly expressed miRNAs that may be used as potential biomarkers or as therapeutic targets. Because of the essential roles of EMT in cancer metastasis and recurrence, identification of key candidate miRNAs that regulate EMT and exploration of their therapeutic potential in HCC may be helpful for improving treatment.

To better identify miRNAs in the EMT process, we established an EMT model in HCC cells treated with transforming growth factor beta 1 (TGF-β1), one of the major inducers of EMT in the tumor microenvironment. We found that expression levels of several tumor-related miRNAs were altered in this model. Among these miRNAs, miR-125b negatively correlated with EMT phenotypes. We further demonstrated that miR-125b inhibited the EMT process in HCC cells by targeting small mothers against decapentaplegic (SMAD)2 and 4. Overexpression of miR-125b or knockdown of its target genes attenuated EMT-associated traits, including chemoresistance, cancer stem cell (CSC) generation and migration. Most important, delivery of a synthetic miR-125b decreased CSC marker CD13 expression and inhibited metastasis in a mouse model, suggesting a therapeutic potential of miR-125b in liver cancer treatment.

**Materials and Methods**

**HCC EMT Cell Model.** HCC cells were trypsinized and placed in six-well plates at appreciate cell densities. One day later, cells were treated in 1%-2.5% fetal bovine serum–containing medium with 10 ng/mL of TGF-β1 or not (PeproTech EC Ltd., London, UK) for 4-6 days.

**Chemoresistance Assay.** A total of 5 × 10^3 to 1 × 10^4 HCC cells per well were seeded in 96-well plates. Twenty-four hours later, drugs were added and incubated for 48 hours. Cell viability was determined using the CCK-8 (Dojindo Laboratories, Kumamoto, Japan). The data were normalized to cells without drugs in each group.

**Tumor-Sphere Culture.** The tumor-sphere system mainly consisted of serum-free Dulbecco’s modified Eagle’s medium/F-12 supplemented with B27 (Invitrogen, Carlsbad, CA), N2 (Invitrogen), 10 ng/mL of epidermal growth factor (EGF), 5 ng/mL of basic fibroblast growth factor, 4 μg/mL of heparin, and 2 μg/mL of insulin with 2 weeks of culturing as we previously reported.

**Cell Transfections.** The pHRS-1cla-miR125b-CMV-EGFP lentivirus vector construction, lentivirus packaging, and cell transfection have been previously described. Huh7.5.1-pcDNA3.0-miR-125b stable cells were selected using 1,600 μg/mL of G418 24 hours post-transfection. miR-125b mimics and the inhibitor were purchased from GenePharma (Shanghai, China). Small interfering RNAs (siRNAs) targeting human SMAD2 and SMAD4 were purchased from Sigma-Aldrich (St. Louis, MO). For transient transfections, 100-nm mimics, 4 μg of plasmid DNA, 200 nM of inhibitor, and 200 nM of siRNA were used by Lipofectamine 2000 (Invitrogen) following the manufacturer’s protocol. Total RNA and protein were prepared 48 and 72 hours, respectively, after cell transfection. The

Supported by grants from the National High Technology Research and Development Program of China (nos.: 2011AAA020109), the Major State Basic Research Program of China (nos.: 2011CB801011 and 2011CB964804), and the National Nature Science Foundation of China (nos.: 81101812 and 81201611).

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DOI: 10.1002/hep.27887

Potential conflict of interest: Nothing to report.
sequences of these oligonucleotide fragments are listed in Supporting Table 1.

Dual-Luciferase Reporter Assay. The 3′ untranslated region (UTR) sequences were cloned into the pGL3 control vector (Promega, Madison, WI), with the primers summarized in Supporting Table 2. For the luciferase reporter assay, cells were cotransfected with 50 nM of miR-125b mimic (or 800 ng of pcDNA3.0-miR-125b vector) and 100 ng of pGL3-SMAD2−3′ UTR vector or pGL3-SNAIL1−3′ UTR vector, with the pRL Renilla luciferase vector (Promega) as an internal control. Luciferase activities were assayed 48 hours post-transfection according to the manufacturer’s protocol.

Flow Cytometry Experiments. Fluorescence-activated cell sorting was performed as we previously described. Briefly, prepared HCC cells were washed twice with phosphate-buffered saline and then incubated with the antibody for 30 minutes at 4°C. After incubation, cells were washed three times and then sorted on a FACSARia instrument (BD Bioscience, San Jose, CA).

Patient Specimens. Fresh HCC tissues and corresponding adjacent nontumorous (NT) tissues were provided from the General Hospital of PLA (Beijing, China) and the 302 Military Hospital of China (Beijing, China). Tumor specimens were obtained according to protocols approved by the institutional review board of the local ethics committee of Beijing, China. In paired HCC tissues, miR-125b expression levels were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR), and immunohistochemistry (IHC) staining of E-CADHERIN, N-CADHERIN, β-CATENIN, CD13, and SMAD2 was conducted. Data regarding the HCC patients are listed in Supporting Table 3.

miR-125b Therapeutic Studies in Mouse. Mice were manipulated and housed according to protocols approved by the Beijing Medical Experimental Animal Care Commission. Male Nu/Nu nude mice (ages 6-7 weeks) were purchased from Vital River Laboratories (Beijing, China). HCCLM3 (5 × 10⁶) or MHCC97-H cells were first subcutaneously (SC) injected into left flanks of nude mice. Twenty days after cell injection, mice were randomly divided into two groups and received local injections of synthetic miR-125b mimics or negative control (NC) into SC tumors every 3-4 days during 1 month of miR-125b therapy. For each injection, 10 µg of miR-125b mimics or NC mixed with 5 µL of a nonliposome and nanoparticle in vivo RNA transfection reagent (Entranster-in vivo; Engreen Biosystem Co., Ltd., Beijing, China) in a 5% sucrose solution was used according to the manufacturer’s protocol.

For details on other methods, see the Supporting Materials and Methods.

Results

Four miRNAs Are Down-Regulated in a TGF-β1-Induced EMT Model of HCC Cells. After TGF-β1 treatment, HCC cell shape changed from epithelial-like to spindle-like. We detected a significant decrease in E-CADHERIN expression and an increase in N-CADHERIN, SNAIL1, and alpha smooth muscle actin (α-SMA) expression. Nuclear β-CATENIN, associated with a poor HCC prognosis,12 was also observed (Fig. 1A). We also detected the increase expressions of reported CSC-associated markers, including CD13, epithelial cell adhesion molecule (EpCAM), CD90, CD133, and CD44,13 among which CD13, a therapeutic target in human liver CSCs,14 was significantly increased in the model (Fig. 1B and Supporting Fig. 1). These results indicated that our model could be used for further screening of EMT-related miRNAs.

As an initial step toward identifying miRNAs that may inhibit EMT, we focused on the decreased miRNAs, identified from tumor suppressor miRNAs reported in liver cancer.15-20 in our EMT model. qRT-PCR results indicated that four miRNAs (let-7a, miR-99a, miR-100, and miR-125b) were down-regulated in the model (Fig. 1C). Interestingly, among these four miRNAs, miR-125b was clustered with the other miRNAs21 and was most strongly decreased. TGF-β1-induced down-regulation of miR-125b and up-regulation of CD13 could be attenuated by SB431542 (a specific inhibitor of the TGF-β pathway), reinforcing the link between TGF-β1 and miR-125b expression (Supporting Fig. 2). Therefore, we selected miR-125b for further investigation.

miR-125b Is Down-Regulated in HCC Cancer Cells and Correlates With Cellular Differentiation in Patients. To further explore the function of miR-125b, we first examined miR-125b expression in HCC cells and paired HCC specimens. We detected miR-125b down-regulation in 7 of 10 HCC cell lines, compared with L02 cells, a human fetal liver cell line (Fig. 1D), and in HCC tissues, compared with the corresponding NT tissues (P < 0.01; Fig. 1E, left). To exclude individual differences in miR-125b expression, miR-125b expression levels were normalized to those in the corresponding NT tissue in each pair (Fig. 1E, middle). We found miR-125b down-regulation in 22 of 27 HCC cases (Fig. 1E, right). These results are consistent with previous reports.17,22-24 Moreover, we found a correlation between loss of miR-125b expression and cellular differentiation in HCC patients (P < 0.05; Supporting Table 2). These findings led us to hypothesize that miR-125b overexpression may inhibit EMT and its associated traits.
Fig. 1.
**miR-125b Attenuates EMT in HCC In Vitro and In Vivo.** To confirm the function of miR-125b in HCC EMT, we established two stable miR-125b-overexpressing HCC cell lines. miR-125b overexpression induced an epithelial-clone-like appearance, compared to control cells (Fig. 2A), with differential down-regulation of EMT markers in HCC cells (Fig. 2B). miR-125b knockdown partially enhanced EMT phenotypes, including down-regulation of E-CADHERIN and/or up-regulation of N-CADHERIN and TWIST1 in cancer cells (Fig. 2C and Supporting Fig. 3B). Moreover, miR-125b knockdown decreased and miR-125b overexpression increased E-CADHERIN expression in human normal liver cells (Supporting Fig. 4). qRT-PCR results for miR-125b expression in the experiments are shown (Supporting Fig. 5). Furthermore, after TGF-β1 treatment, miR-125b-stable cells remained epithelial-clone-like in shape with SNAIL1 and TWIST1 not induced, whereas control cells exhibited up-regulation of SNAIL1 and TWIST1 (Fig. 2D).

To further investigate the effects of miR-125b on HCC EMT in vivo, we SC injected 5 x 10⁶ of Huh7.5.1-pcDNA3.0-miR-125b or control cells and SMMC-7721 cells transiently transfected with an miR-125b inhibitor or NC in mice. IHC results demonstrated that N-CADHERIN, α-SMA, and TWIST1 expression were down-regulated in Huh7.5.1-pcDNA3.0-miR-125b-derived tumors, compared to control (P < 0.01; Fig. 2E). In SMMC-7721-miR-125b-inhibitor cell-derived tumors, E-CADHERIN expression was downregulated (P < 0.05) and N-CADHERIN and TWIST1 expression was up-regulated, compared to NC cells (P < 0.05; Fig. 2F). Together, these findings suggest that miR-125b attenuates EMT in HCC.

**miR-125b Regulates EMT-Associated Traits in HCC Cells.** Given the ability of miR-125b to attenuate EMT in HCC, we next examined the capacity of miR-125b to inhibit EMT-associated traits in HCC cells, such as drug resistance, cell migration, and CSC. We found that miR-125b overexpression reduced doxorubicin/sorafenib resistance, whereas miR-125b knockdown promoted doxorubicin/sorafenib resistance in HCC cells (Fig. 3A and Supporting Figs. 3C,D and 6). We also found that miR-125b negatively regulates expressions of multidrug resistance genes, including ABCG1, ABCG2, and PGP (Supporting Fig. 7). miR-125b overexpression was sufficient to abolish cell migration, and miR-125b knockdown facilitated migration compared to controls (Supporting Fig. 8).

For CSCs, we found miR-125b down-regulation in tumor spheres, a CSC cell model we have previously used, compared with parental adherent cells (Fig. 3B). Moreover, miR-125b overexpression reduced tumor sphere diameter, compared to control-transfected cells (28.13% and 33.37% decrease in Huh7.5.1 cells and Li-7 cells, respectively; Fig. 3C). We showed that miR-125b expression was decreased in the EpCAMhigh, CD13high subpopulations or dual high subpopulations, compared to the low/negative subpopulations, respectively (Supporting Fig. 9). Importantly, miR-125b overexpression reduced percentage and expression intensities of EpCAM and CD13 (Fig. 3D). In addition, miR-125b overexpression also reduced the percentage of the side population cells (Fig. 3E).

To evaluate the effect of miR-125b on tumor-initiating capacity in vivo, we compared tumor incidence of Huh7.5.1-pcDNA3.0-miR-125b and control cells. The same number of Huh7.5.1-pcDNA3.0-miR-125b and control cells was SC injected into flanks of nude mice (n = 6) at two dilutions (5 x 10⁴ and 5 x 10⁵). Two months later, tumors seeded at the 5 x 10⁴ dilution had formed only in the Huh7.5.1-pcDNA3.0-con group (2 of 6), whereas no tumors were found in the Huh7.5.1-pcDNA3.0-125b group (0 of 6; Fig. 3F). Regarding the 5 x 10⁵ dilution, tumors formed in all mice of both groups (Fig. 2E). Together, these findings strongly suggest that miR-125b attenuates EMT-associated traits in HCC cells, including chemoresistance, migration, and CSC-like characteristics.

**SMAD2 Is a Novel Direct Target of miR-125b.** Next, we explored the molecular mechanisms by which miR-125b affects EMT. Bioinformatics analysis using TargetScan indicated the potential binding sites in the 3’ UTR of SNAIL1 and SMAD2 (Supporting Fig. 10 and Fig. 4A). We also observed significant down-regulation of SMAD2 and SMAD4 (Fig. 4B) in...
miR-125b-overexpressing HCC cells (Supporting Fig. 5). Lin28B, a previously reported target gene of miR-125b, was also down-regulated in miR-125b-overexpressing MHCC97-H cells.

We detected a direct binding of miR-125b to the 3′ UTR of SMAD2 at position 977-983 (Fig. 4C) and found that miR-125b knockdown up-regulated SMAD2 expression and rescued siRNA-mediated SMAD2 suppression in HCC cells (Fig. 4D and Supporting Fig. 11A). SMAD2 protein was observed to be up-regulated in 24 of 27 HCC cases (Fig. 4E). Importantly, we found that SMAD2 expression inversely correlated with...
miR-125b expression in paired HCC tissues (Fig. 4F). Our laser capture microdissection results further confirmed miR-125 repression correlating with TGF-β pathway activation in HCC specimens (Supporting Fig. 12). Together, these data suggest that SMAD2 is a direct target gene of miR-125b in HCC.
SMAD2 and SMAD4 Are Both Involved in miR-125b Regulating EMT-Associated and Stem-Like Traits. To investigate whether SMAD2 and SMAD4, which have been reported to be targeted by miR-125b, regulate EMT and associated traits in HCC, we silenced their expressions by siRNAs. We found that SMAD2 or SMAD4 knockdown down-regulated expression of mesenchymal markers and/or up-regulated E-CADHERIN expression (Figs. 5A and 6A). Overexpression of SMAD2 or SMAD4 partially rescued down-regulation of α-SMA induced by miR-125b overexpression (Figs. 5B and 6B). CD13+ cells were
decreased by SMAD2 or SMAD4 knockdown (Figs. 5C and 6C) as well as miR-125b overexpression (Fig. 3D). SMAD2 or SMAD4 knockdown reduced HCC cell migration (Supporting Fig. 13) and sensitized HCC cells to doxorubicin (Figs. 5D and 6D). These findings suggest that miR-125b attenuates EMT-associated and stem-like traits, at least partially, by targeting SMAD2 and SMAD4.

miR-125b Expression Correlates With E-CADHERIN and Negatively Correlates With N-CADHERIN, 𝛽-CATENIN, and CD13 Expression. To confirm whether miR-125b expression correlates with EMT
markers, CSC markers in patients, we collected fresh paired HCC tissues and analyzed their expressions by IHC. IHC results showed E-CADHERIN down-regulation in 17 of 27 patients (Fig. 7A), N-CADHERIN up-regulation in 21 of 27 patients (Fig. 7B), β-CATENIN up-regulation in 20 of 27 patients (Fig. 7C), and CD13 up-regulation in 21 of 27 patients (Fig. 7D).

Next, we performed a correlation analysis between miR-125b and the markers examined above. A positive correlation between E-CADHERIN and miR-125b was observed in 23 paired HCC and NT tissues. A negative correlation was observed in N-CADHERIN versus miR-125b in 16 paired tissues, in β-CATENIN versus miR-125b in 15 paired HCC tissues, and in CD13 versus miR-125b in 15 paired HCC tissues (Fig. 7E). Taken together, the data above from the HCC cells, mouse model, and specimens demonstrate that miR-125b suppresses EMT and EMT-associated traits in HCC, suggesting a potential therapeutic role in HCC treatment.

**miR-125b Mimics Decreases Target Molecules in CSCs and Inhibits Metastasis in a Mouse Model.** We then established an HCC xenograft model by SC injection of HCCLM3 cells (Fig. 8A and Supporting Fig. 14A) or MHCC97-H cells (Supporting Fig. 15) into...
nude mice. The gross observations of tumors from both groups post-therapy are shown in Fig. 8B. As expected, a significantly higher expression of miR-125b was found in the miR-125b-mimic group, compared to control, suggesting a successful overexpression of miR-125b in vivo (Fig. 8B; \( P < 0.05 \)).

We next examined CD13 expression in tumor tissues and performed histopathological examinations of metastatic foci in lung and liver. CD13 expression intensity was substantially weaker in the miR-125b-mimic group than in control (\( P < 0.01 \); Fig. 8C). Strikingly, lung metastases were substantially fewer in the miR-125b group than in control (103.77 ± 52.46 vs. 250.42 ± 67.68; \( P < 0.01 \)), of which tumors were visible on the surface (Fig. 8D). Metastatic liver nodules were also visible on the liver surface in the NC group. Size of
A. Diagram showing the experimental setup for injecting cells into mice, grouping them, administering therapy, and sacrificing them at different time points.

B. Images showing tumors from different groups: NC group (left) and miR-125b group (right).

C. Graphs showing CD13 expression in injected tumors, with comparisons between NC and miR-125b groups.

D. Micrographs illustrating histological sections of lungs from NC and miR-125b groups, with quantification of metastatic foci.

E. Immunohistochemical analysis of CD13 expression in lungs from NC and miR-125b groups.

F. Scatter plot showing the correlation between CD13 expression and metastatic foci in lungs, with a linear regression line and statistical significance.

Fig. 8.
metastatic liver foci was smaller in the miR-125b group (Supporting Fig. 14B). IHC staining of carcinoembryonic antigen (CEA) confirmed the existence of HCC metastatic foci in lungs and livers (Fig. 8E and Supporting Fig. 14C). Finally, a positive correlation between CD13 expression and the number of metastatic lung foci was observed (Fig. 8F). Similar results were also obtained in MHCC97-H cells (Supporting Fig. 15). Taken together, these data suggest that local injection of miR-125b mimic in vivo can inhibit CSC population and HCC metastasis.

Discussion

There are many reports that demonstrate miRNA dysregulation in HCC. However, miRNAs that modulate the EMT and their therapeutic potentials have not been thoroughly addressed. In this study, for the first time, we provide evidence that miR-125b, identified in our HCC EMT model, attenuates EMT and EMT-associated traits in HCC cells.

Interestingly, we observed that several HCC cell lines have different responses to miR-125b or TGFβ1, as measured by EMT marker expressions. The possible reasons include: (1) differences in miR-125b expression levels; (2) a different EMT state of the cells; and (3) hepatitis B virus infection. These possibilities reflect the complexity of HCC progression and developing therapeutic strategies.

Tumor cells undergoing EMT have increased migratory and invasive capabilities and chemoresistance. Indeed, we demonstrated the negative regulation of miR-125b on chemoresistance and migration in HCC cells. EMT has also been reported to play an essential role in regulating CSCs, which may represent a source of therapeutic resistance in a wide range of solid tumors. Important associations between EMT and CSC generation have been implicated in breast cancer. Here, we found that miR-125b overexpression reduced CD13+ and/or EpCAM+ populations and the side population cells, which have been used to enrich liver CSCs. Notably, CD13 has been reported to be a therapeutic target in human liver CSCs. Moreover, we demonstrated that miR-125b inhibited tumor incidence in vivo. We also observed that miR-125b negatively correlated with mesenchymal markers, β-CATENIN, and CD13 expressions in HCC patients. Together, these findings support the hypothesis that miR-125b attenuates EMT-associated traits, including chemoresistance, migration, and CSC generation.

We then sought to identify functional target genes of miR-125b attenuating EMT. Given the ability of miR-125b to antagonize up-regulation of SNAIL1 and TWIST1 induced by TGF-β1 (Fig. 2D), we hypothesized that miR-125b may affect EMT by targeting downstream molecules of TGFβ1, of which the SMADs are key candidates. Our data suggest, for the first time, that SMAD2 is a direct target of miR-125b attenuating EMT and associated traits. We also found a functional role of SMAD4, recently identified as a novel target of miR-125b, in miR-125b-attenuating EMT in HCC.

Most important, we demonstrated the therapeutic potential of miR-125b for metastatic HCC. Except for sorafenib, there are almost no drugs for advanced liver cancer. miRNAs are promising candidates for liver cancer gene therapy. We evaluated the therapeutic effects of synthetic miR-125b in an HCC metastasis mouse model by SC injection of highly metastatic HCCLM3 cells into athymic mice. HCCLM3 cells, derived from MHCC97-H cells, are reflective of HCC development in humans, with spontaneous lung metastasis in all animals. In an in vitro study, we observed that different HCC cell lines have almost the same responses to miR-125b overexpression or TGF-β1 treatment with respect to CD13-decreased expression. In vivo, we found that miR-125b significantly reduced CD13+ CSCs, lung, and liver metastasis in tumor-bearing mice. A positive correlation was also observed between CD13 expression in primary tumors and the number of metastatic lung foci in mice, suggesting a definite therapeutic effect of miR-125b. A recent report demonstrated that miR-125b further decreased in recurrent tumor tissues from HCC patients after resection. This supports the clinical significance of miR-125b replacement therapy to...
prevent HCC recurrence, though miRNA-based therapeutic studies are still in the early stages.\(^6\)

Whereas miR-125b is reported to be a tumor suppressor gene in HCC,\(^22-24\) ovarian cancer, bladder cancer, and breast cancer, it has been also reported to act as an oncogene in other cancers, such as prostate, thyroid, neuroblastoma, and breast cancer.\(^25\) In fact, the function duality in various types of cancer has been reported in many miRNAs besides miR-125b. In breast cancer, the functional duality of miR-125b may be correlated with specific breast cancer biopathologic features, including estrogen receptor and progesterone receptor expression.\(^26\) Therefore, the therapeutic potential of miR-125b in HCC should be carefully examined in the other cancers.

Together, these data indicate the potential of miRNA therapy, including miR-125b, for patients with poor prognoses. It is possible that combinatorial treatments, including miRNAs, and currently approved anti-HCC agents, such as doxorubicin and sorafenib,\(^29\) could decrease metastasis and increase survival rate or time after surgical treatment. In summary, to our knowledge, this is the first study to reveal the inhibitory role of miR-125b on EMT and CSC generation in HCC, thus shedding light on therapeutics targeting EMT and CSCs through an miRNA paradigm in HCC therapy.

**Acknowledgment:** The authors thank Prof. Xiao-Fei Zheng (Beijing Institute of Radiation Medicine, China) for the pcDNA3.0-miR-125b vector and Prof. Ye-Guang Chen (Tsinghua University School of Medicine, China) for the pcCMV-Flag-SMAD4 vector and Lin Chen, Jing Li, Zeng Fan, and Jinglei Zhai in the lab for assistance in the study.

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