

Original Article

Down-expression of homeobox A9 promotes cancer cell invasion in human hepatocellular carcinomas

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Abstract: Metastasis is the most important contributor to the mortality of patients with hepatocellular carcinomas (HCCs), but the molecular basis for this is unknown. Previous studies had found that homeobox A9 (HOXA9) was frequently methylated in HCCs; however, little is known about whether HOXA9 can influence the malignant properties of HCC cells and the exhaustive mechanisms in HCC progression. In a screen of 82 patients with HCCs, we found that HOXA9 protein expression was inversely correlated with invasion, lymphnode metastasis, and poor clinical outcomes. Silencing HOXA9 in HCC cells increased their neoplastic properties *in vitro* and *in vivo*, markedly increasing the migratory, invasive, and metastatic capabilities of malignant cells. In contrast, ectopic expression of HOXA9 in HCC cells inhibited these processes. We also found that HOXA9 promoted the expression of E-cadherin and inhibited the expression of N-cadherin. These observations contribute to our understanding of the important roles of HOXA9 in HCC development and progression and HOXA9 could be a promising molecular target for the development of new diagnostic and therapeutic strategies for HCC.

Keywords: HOXA9, hepatocellular carcinomas, migration

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide with poor prognosis [1]. Despite advance in the treatment of HCC, there is currently no curative option for this life-threatening disease and the overall 5-year survival is about 40% for patients treated with major hepatectomy [1, 2]. Metastasis is the most important contributor to the mortality of patients with HCCs [3]. The pathogenesis of HCC metastasis involves increased cell invasion, angiogenesis, cell proliferation, loss of cellular adhesion, survival in the circulation, entry into new tissue, and eventual colonization of distant organs [3, 4]. Furthermore, biomarkers that are currently used clinically to predict the prognosis of HCC patients after curative surgical resection remain unsatisfactory in terms of both accuracy and reproducibility. Therefore, it remains clinically important to identify novel prognostic

biomarkers to improve the diagnosis and treatment of HCC patients.

Homeobox (HOX) genes are transcription factors that regulate the expression of multiple genes that influence cell growth and viability and that mediate stromal-epithelial interactions to drive tissue-specific differentiation [5]. HOX expression is frequently perturbed in tumors, in which they can act as oncogenes by promoting cell growth and invasion or as tumor suppressors [5]. Previous studies revealed that HOXA9 as a gene whose levels were reduced in breast tumors and whose reexpression promoted breast tumor morphogenesis [6]. Although, paradoxically, HOXA9 has been characterized as a leukemic oncogene and angiogenesis promoter [7], HOXA9 also plays an important role in skeletal, urogenital tract, kidney, and mammary gland development [8, 9], and HOXA9 expression can be regulated by micro RNAs that have been implicated in tissue differentiation [10].

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Table 1. HOXA9 staining and clinicopathologic characteristics of 82 hepatocellular carcinoma patients

Variables	HOXA9 staining		Total	P ^a
	Low	High		
Age (y)				
≤50	16 (64%)	9 (36%)	25	0.141
>50	42 (74%)	15 (26%)	57	
Sex				
Male	45 (73%)	17 (27%)	62	0.473
Female	13 (65%)	7 (35%)	20	
HBsAg				
Negative	17 (74%)	6 (26%)	23	0.102
Positive	41 (69%)	18 (31%)	59	
HCV				
Negative	55 (70%)	55 (70%)	79	0.379
Positive	3 (100%)	0 (0%)	3	
AFP				
≤20	28 (74%)	10 (26%)	38	0.468
>20	30 (68%)	14 (32%)	44	
γ-GT				
≤4682% a	26 (67%)	13 (33%)	39	0.2596
>54	32 (74%)	11 (26%)	43	
Liver cirrhosis				
No	9 (69%)	4 (31%)	13	0.416
Yes	49 (71%)	20 (29%)	69	
Tumor diameter (cm)				
≤5	35 (88%)	5 (22%)	40	0.017
>5	23 (55%)	19 (45%)	42	
Microvascular invasion				
Absence	8 (31%)	18 (69%)	26	<0.001
Present	50 (89%)	6 (11%)	56	
Tumor encapsulation				
Complete	26 (70%)	11 (30%)	37	0.196
None	32 (71%)	13 (29%)	45	
Tumor differentiation				
I+II	30 (61%)	19 (39%)	49	<0.001
III+IV	28 (85%)	5 (15%)	33	
TNM stage				
I	33 (66%)	17 (34%)	50	0.038
II+III	25 (78%)	7 (22%)	32	

Abbreviations: HBsAg, hepatitis B surface antigen; AFP, α-fetoprotein; γ-GT, γ-glutamyltransferase; TNM, tumor-nodes-metastasis. ^ap-value < 0.05 was considered statistically significant. p-values were calculated using the Pearson chi-square test.

HOXA9 promoted breast tumor cell differentiation and inhibits cancer progression by directly regulating expression of the BRCA1 [11]. Thus, HOXA9 may play different function in different tissues and organs. Recently, researchers founded that HOXA9 gene was also frequently

methylated in HCC [12], however, whether HOXA9 plays a role in HCC formation and metastasis remains unknown.

In the current study, we show that HOXA9 expression was inversely correlated with invasion, lymphnode metastasis, and poor clinical outcomes. Silencing HOXA9 in aggressive HCC cells increased their neoplastic properties invitro and in vivo, markedly increasing the migratory, invasive, and metastatic capabilities of malignant cells. In contrast, ectopic expression of HOXA9 in HCC cells inhibited these processes. We also found that HOXA9 promoted the expression of E-cadherin and inhibited the expression of N-cadherin. Our findings provide a novel mechanistic role of HOXA9 in HCC metastasis, suggesting that HOXA9 may serve as a potential therapeutic target for advanced HCCs.

Materials and methods

Chemicals and antibodies

Lipofectamine 2000 transfection and TRIZOL LS Reagents were purchased from Invitrogen (Grand Island, NY, USA). Antibodies against HOXA9, E-cadherin, and N-cadherin were purchased from Abcam (Cambridge, MA, USA). β-actin antibody was from Cell Signaling technology (Danvers, MA, USA). Unless otherwise noted, all other chemicals were from Sigma (St. Louis, MO, USA).

Patients and specimens

Thirty-seven normal liver tissues and eighty-two hepatocellular carcinoma tissues which, were used for immunohistochemical analysis, were randomly collected from HCC patients who underwent curative resection with informed consent between 2006 and 2009 at the Department of Laparoscopic Surgery, First Affiliated Hospital of Dalian Medical University. Tumor staging was based on the 6th edition of the tumor-node-metastasis (TNM) classification of the International Union Against Cancer. The clinicopathologic characteristics of the 82 hepatocellular carcinoma tissues are summarized in **Table 1**. Follow-up data were summarized at the end of October 2014, with a median observation time

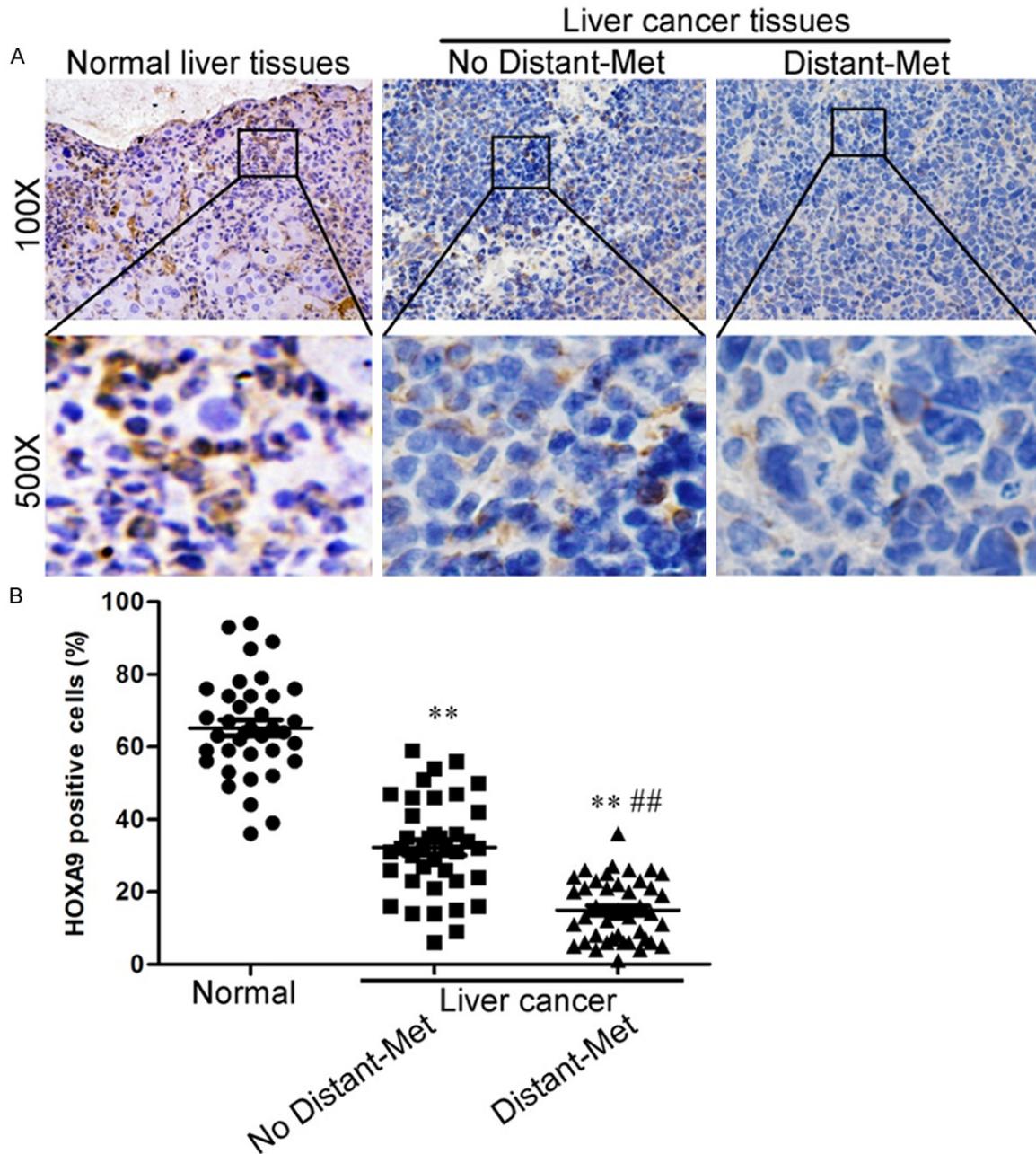


Figure 1. HCCs malignancy is associated with reduced HOXA9 expression. A. HOXA9 protein expression was analyzed by immunohistochemical analysis in 37 normal liver tissues and 82 cases HCC tissues, and the representative results were shown. B. Semi quantification of HOXA9 expression in normal tissues, primary HCC tissues without or with distant metastasis. Normal, normal liver tissues; no distant met, primary cancers without distant metastasis (in situ); distant met, primary cancers with distant metastasis. **, $P < 0.01$ is based on the Student t test. Error bars, SD.

of 65.3 months. Study protocols were approved by the Hospital Ethics Committee of Dalian Medical University, and written informed consent was obtained from patients based on the Declaration of Helsinki.

Histological and immuno histochemical analysis

The normal human liver tissues, human HCC tissues, and lungs dissected from mice were

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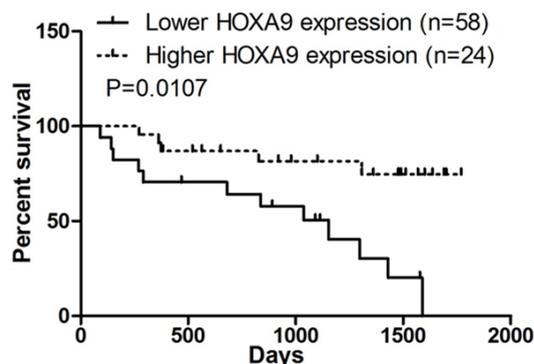


Figure 2. HOXA9 protein expression was inversely correlated with poor clinical outcomes. The association between HOXA9 expression in HCC and the survival time of selected patients was analyzed with Kaplan-Meier survival analysis.

fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight and subsequently embedded in paraffin wax. Sections cut at a thickness of 4 μ m were stained with hematoxylin and eosin for histological analysis. Immunohistochemical analysis was performed for different markers in these arrays as described previously [13]. The proportion of stained cells (lower, <30% staining; higher, \geq 30% staining) was semiquantitatively determined following published protocols [14].

Cell culture

HCC cells (ATCC, Manassas, VA, USA) were cultured under the following conditions: HHL-5, and HL7702 cell lines were cultured using 10% fetal bovine serum (Cat#10099-141, Invitrogen, Carlsbad, CA) in either RPMI-1640 (Cat#C11875, Invitrogen). Huh7, HepG2, MHCC97-L, MHCC97-H, SMMC-7721 and SNU423 cell lines were cultured using 10% fetal bovine serum (Invitrogen) in Dulbecco's modified Eagle medium (Cat#C11965, Invitrogen). Cell culture was according to manufacturer's protocol. All the cell lines were grown at 37°C in a 5% CO₂/95% air atmosphere and were revived every 3 to 4 months.

Establishment of HOXA9 stable expression and HOXA9 knockdown cell lines

Retroviral construct containing human pBabe-HOXA9 cDNA, and pSuper. retro.puro with shRNA against human HOXA9 were prepared as described previously [15, 16]. The generation of retrovirus supernatants and transfection of cell lines were conducted as described previously [15, 17]. The expression of HOXA9

was confirmed by qRT-PCR and Western blot analysis.

Cell invasion and motility assay

Invasion of cells was measured in Matrigel (BD, Franklin Lakes, NJ, USA) -coated Transwell inserts (6.5 mm, Costar, Manassas, VA, USA) containing polycarbonate filters with 8- μ m pores as detailed previously [18, 19]. The inserts were coated with 50 μ l of 1 mg/ml Matrigel matrix according to the manufacturer's recommendations. 2×10^5 cells in 200 μ l of serum-free medium were plated in the upper chamber, whereas 600 μ l of medium with 10% fetal bovine serum were added to lower well. After 24 hrs incubation, cells that migrated to the lower surface of the membrane were fixed and stained. For each membrane, five random fields were counted at $\times 10$ magnification. Motility assays were similar to Matrigel invasion assay except that the Transwell insert was not coated with Matrigel.

Western blot

Cells were lysed in lysis buffer and total protein contents were determined by the Bradford method. 30 μ g of lysis were separated by reducing SDS-PAGE and probed with specific antibodies. Blots were washed and probed with respective secondary peroxidase-conjugated antibodies, and the bands visualized by chemoluminescence (Amersham Biosciences).

qRT-PCR

Total RNA was extracted using Trizol reagent and cDNA was synthesized using SuperScriptII Reverse Transcriptase (Invitrogen). qRT-PCR and data collection were performed with an ABI PRISM 7900HT sequence detection system. The primers used for the amplification of the indicated genes are available upon request.

In vivo tumor metastasis

Nude mice were purchased from the Shanghai Slac Laboratory Animal Co. Ltd and maintained in microisolator cages. All animals were used in accordance with institutional guidelines and the current experiments were approved by the Use Committee for Animal Care. Cells were resuspended in PBS at a concentration of 1×10^7 cells ml⁻¹. Cell suspension (0.1 ml) was injected into tail veins of nude mice. All of the mice were killed by CO₂ 60 days after inoculation.

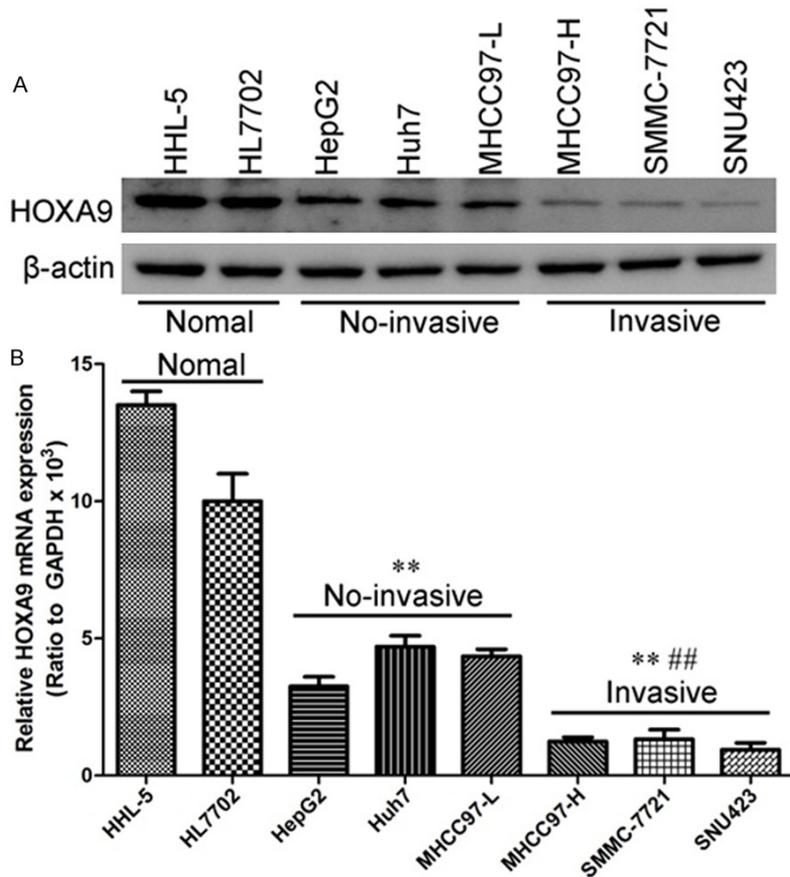


Figure 3. HCC cell lines express HOXA9 protein at various levels. A. The expression of HOXA9 protein was measured by Western blot in HHL-5, HL7702, HepG2, Huh7, MHCC97-L, MHCC97-H, SMMC-7721, and SNU423 cell lines. B. The expression of HOXA9 mRNA was measured by qRT-PCR in HHL-5, HL7702, HepG2, Huh7, MHCC97-L, MHCC97-H, SMMC-7721, and SNU423 cell lines.

HCC tissues. We examined HOXA9 protein expression in HCC samples by IHC (Figure 1A). We observed that the level of HOXA9 positive cells was markedly reduced in HCC tissues than the level in the normal liver tissues (Figure 1B). Most importantly, HOXA9 down expression was consistently significantly correlated to distant metastasis in these HCC samples (Figure 1B). To investigate the relationship between HOXA9 expression and clinicopathological parameters in the 82 cases with HCCs, these cases were first divided into two subgroups: “low HOXA9 expression” and “high HOXA9 expression” as defined in the immunohistochemistry section of “Materials and methods”. Significant correlations were found between HOXA9 expression and tumor diameter, microvascular invasion, and tumor differentiation. There were no statistical connections between HOXA9 expression and the rest clinicopathological parameters, such as patient age, gender, and HBsAg (Table 1). The association between HOXA9 expression in HCC and the survival time of selected patients was analyzed with Kaplan-Meier survival analysis (Figure 2). The median overall survival time of low HOXA9 expression group was significantly shorter than that of high HOXA9 expression group (P < 0.001). These data show that HOXA9 is significantly down regulated in HCC and that its loss correlates with increased disease aggression.

Statistical analysis

Results were analyzed with SPSS13.0 statistical software. Correlation between HOXA9 expression and clinicopathologic parameters was evaluated using the Chi-square (χ^2) test, and quantitative variables were analyzed by the independent t test. The survival probability was estimated by Kaplan-Meier method, and the comparison of survival curves between groups was done with the log-rank test. The statistical significance of the differences between mean values was determined by P < 0.05.

Results

HCCs malignancy is associated with reduced HOXA9 expression

HOXA9 gene was frequently methylated in HCCs, therefore, we firstly investigated the expression of HOXA9 protein in normal liver tissues and

HCC cell lines express HOXA9 protein at various levels

We next analyzed the expression of HOXA9 protein in the immortalized normal human liver cell lines (HHL-5, HL7702) and human HCC cell lines (HepG2, Huh7, MHCC97-L, MHCC97-H, SMMC-7721, and SNU423). Immortalized nor-

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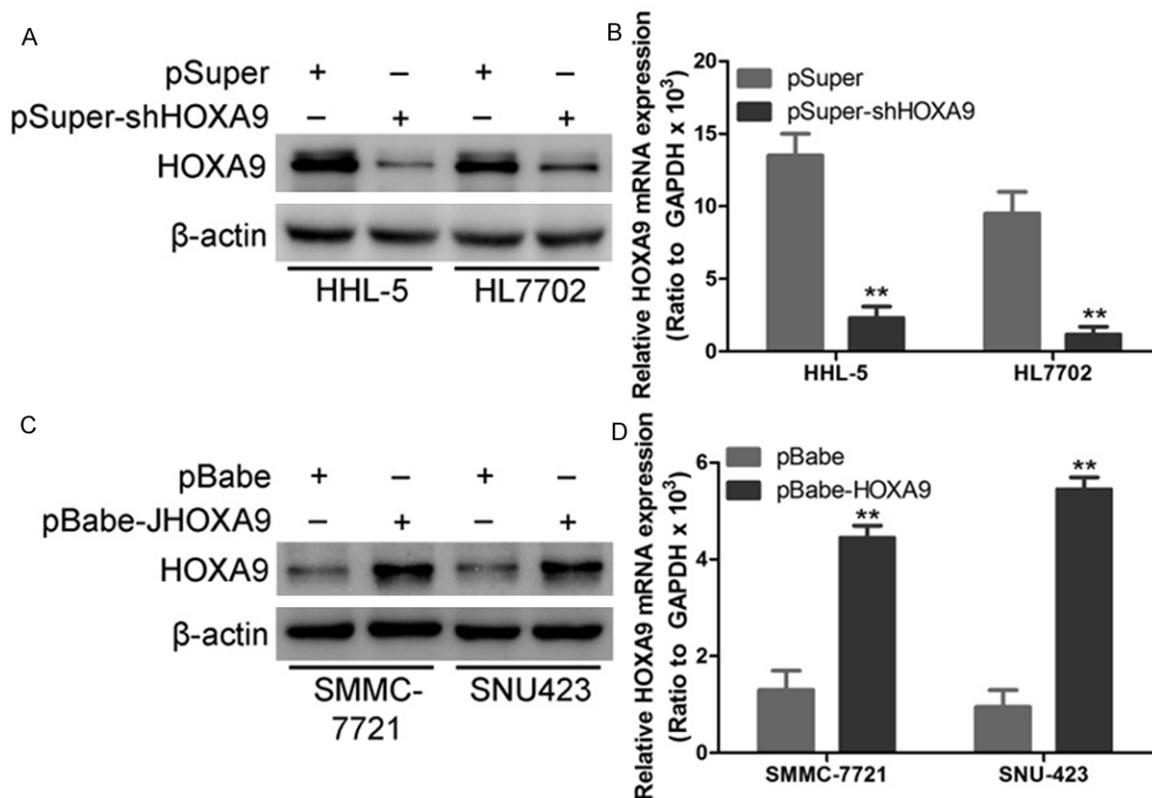


Figure 4. Establishment of stable HOXA9 transfectants in HCC cell lines. A. Expression level of HOXA9 protein was measured by Western blot in stable silencing HOXA9HHL-5 and HL7702 cells. B. Expression level of HOXA9 mRNA was measured by qRT-PCR in stable silencing HOXA9HHL-5 and HL7702 cells. C. Overexpression of HOXA9 in SMMC-7721 and SNU423 cells was measured by Western blot. D. Overexpression of HOXA9 in SMMC-7721 and SNU423 cells was measured by qRT-PCR.

mal human liver cell lines (HHL-5, HL7702) had the highest level of HOXA9 protein expression (Figure 3A). Significantly lower expression of HOXA9 protein was evident in HepG2, Huh7, MHCC97-L, MHCC97-H, SMMC-7721, and SNU423 cell lines. We also found that HOXA9 protein was lowest expressed in invasive cancer cells compared with normal human liver epithelial cell lines and non-invasive cancer cell lines. In addition, we also found that the expression of HOXA9 mRNA were consistent with the protein expression (Figure 3B). These results collectively may indicate a functional role of HOXA9 in aggressive behaviors of HCC cell lines.

Establishment of stable HOXA9 transfectants in HCC cell lines

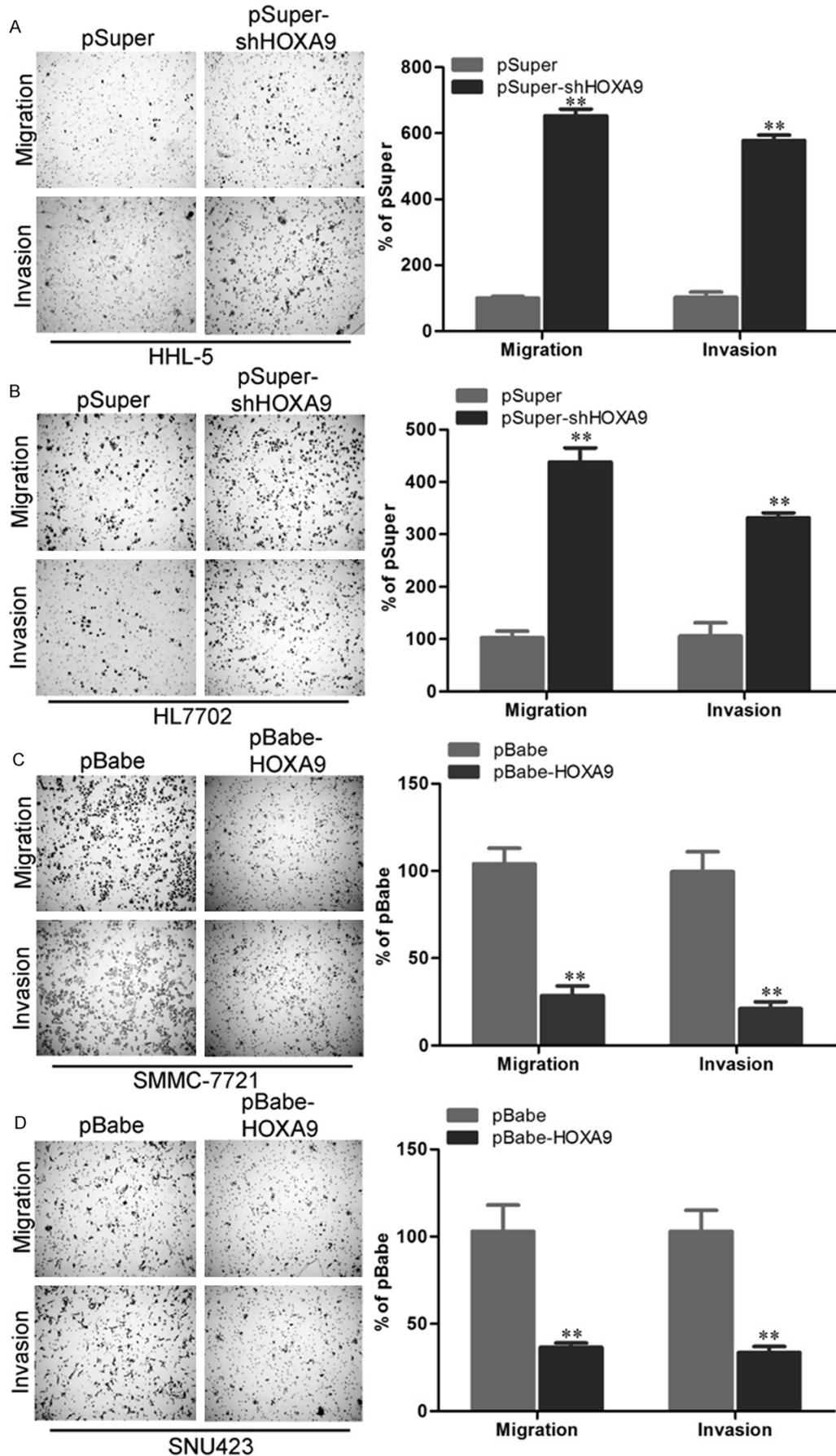
To test the oncogenic activity of HOXA9 in HCCs, we retrovirally established stable silencing HOXA9 in HHL-5 and HL7702 cells (designated as HHL-5-shHOXA9 and HL7702-shHOXA9), and overexpression of HOXA9 in

SMMC-7721 and SNU423 cells (designated as SMMC-7721-HOXA9 and SNU423-HOXA9). The transfection efficiency was confirmed using western blotting and qRT-PCR analyses. As shown in Figure 4A and 4B, the HHL-5 and HL7702 cells that had been transfected with the HOXA9 shRNA plasmid displayed significantly decreased HOXA9 expression at both the mRNA and protein levels compared with the control cells. In addition, the SMMC-7721 and SNU423 cells that had been transfected with the HOXA9 expression plasmid displayed significantly increased HOXA9 expression at both the mRNA and protein levels compared with the vector cell lines (Figure 4C and 4D).

HOXA9 inhibits migratory and invasive capacities of HCC cells in vitro

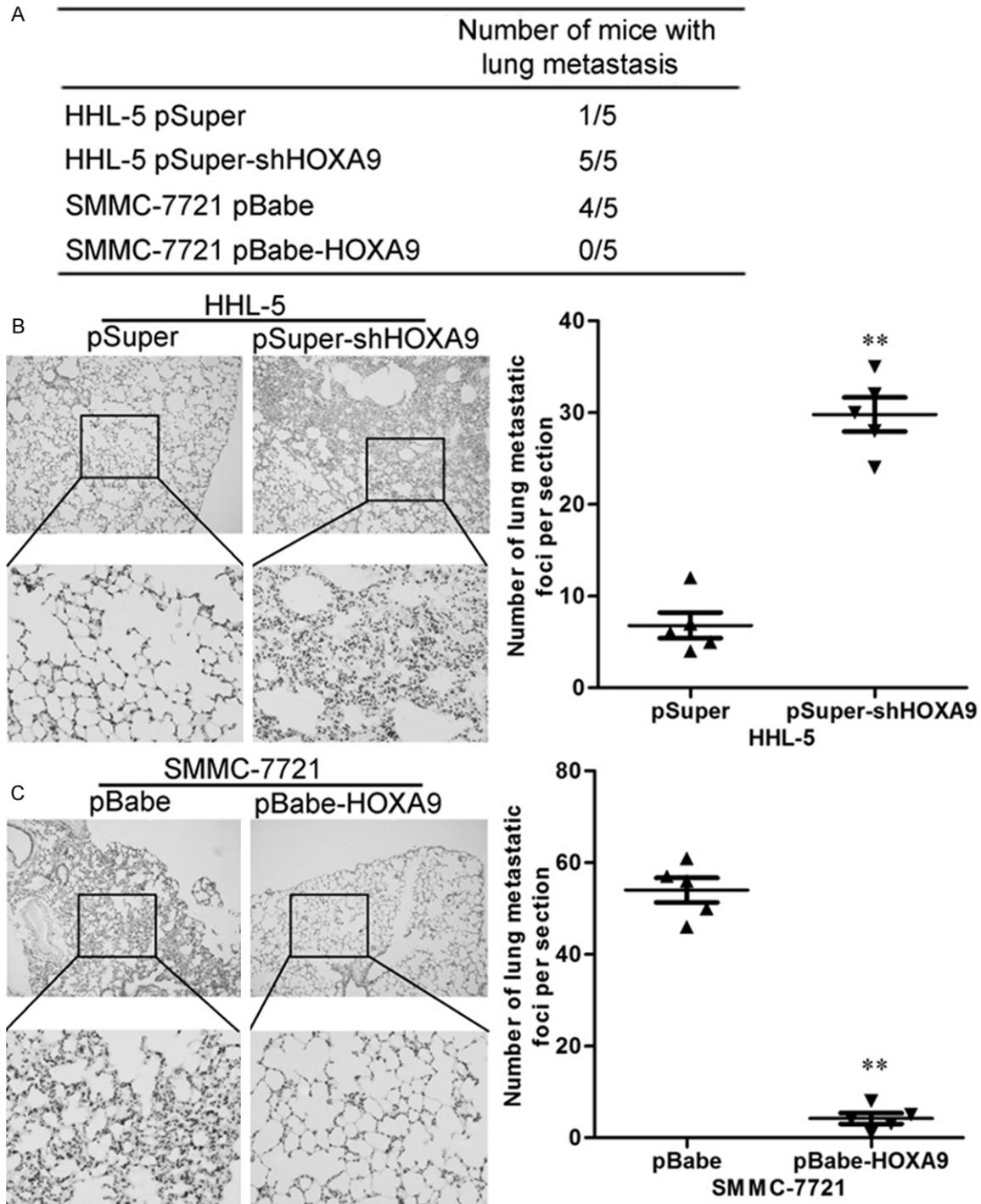
The effect of HOXA9 on cell migration was first assessed by Boyden's chamber assay. Both HHL-5-shHOXA9 and HL7702-shHOXA9 cells had significantly faster migration compared to their control cells (Figure 5A and 5B, upper).

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Figure 5. HOXA9 inhibits migratory and invasive capacities of HCC cells in vitro. HHL-5-shHOXA9 (A), HL7702-shHOXA9 (B), and their control vector cells were subjected to Transwellmigration (A and B, top), and Matrigel invasion assays (A and B, bottom), quantification of migrated cells through the membrane and invaded cells through Matrigel of each cell line are shown as proportions of their vectorcontrols. SMMC-7721-HOXA9 (C), SNU423-HOXA9 (D) and their control vector cells were subjected to Transwellmigration (C and D, top), and Matrigel invasion assays (C and D, bottom), quantification of migrated cells through the membrane and invaded cells through Matrigel of each cell line are shown as proportions of their vectorcontrols. **, $P < 0.01$ is based on the Student *t* test. All results are from three independent experiments. Error bars, SD.



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Figure 6. HOXA9 inhibits metastasis *in vivo*. (A) The total numbers of mice with distant metastasis at 60 days after injection of HHL-5-shHOXA9, SMMC-7721-HOXA9 or their control cells. (B) and (C) the numbers of metastatic foci per section in lung of individual mouse with injection of HHL-5-shHOXA9 (B), SMMC-7721-HOXA9 (C) or their control cells. $n=5$ for tail vein injection. **, $P<0.01$ is based on the Student *t* test. All results are from three independent experiments. Error bars, SD.

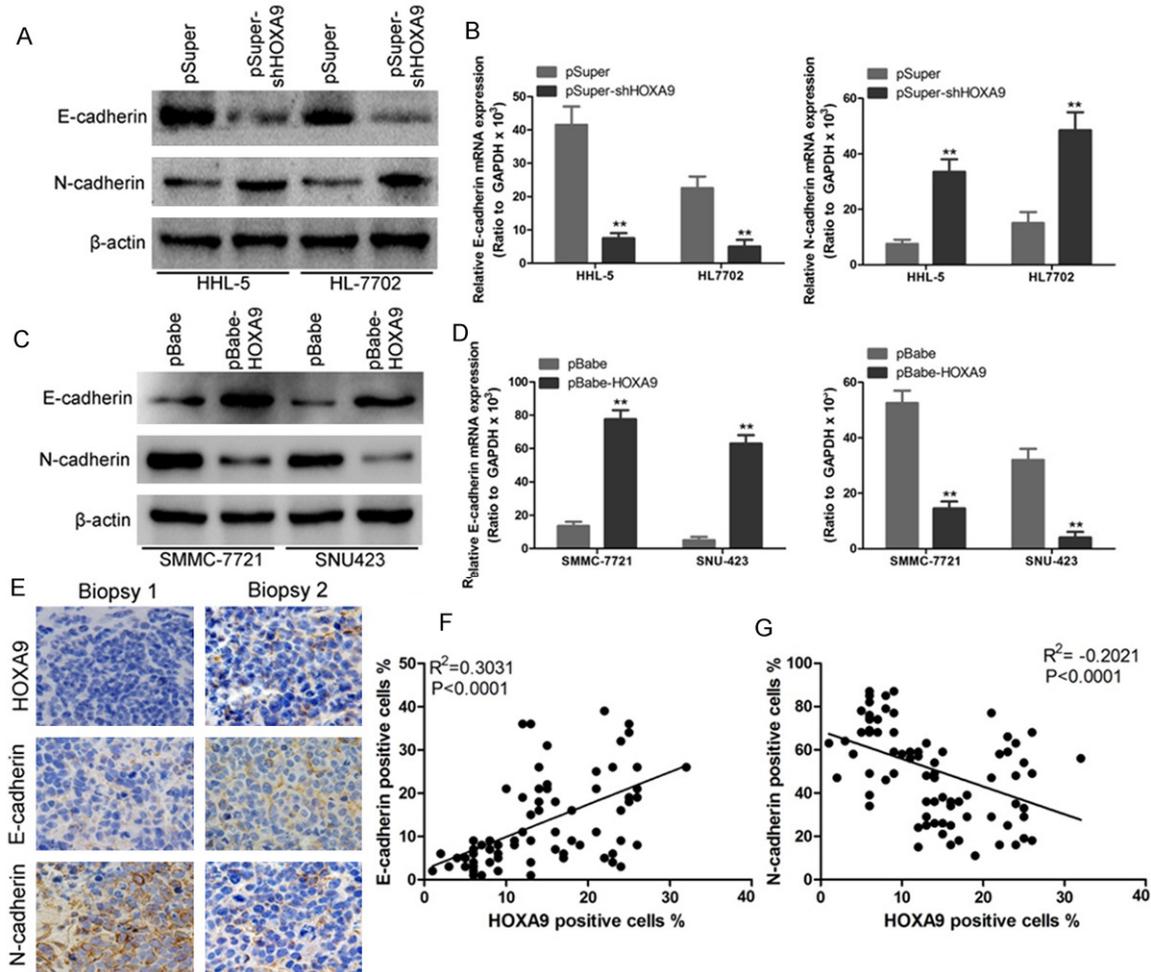


Figure 7. HOXA9 regulates the expression of E-cadherin and N-cadherin in HCC cells. A. Expression of E-cadherin and N-cadherin protein were analyzed by Western blot in HHL-5-shHOXA9, HL7702-shHOXA9, and their control vector cells. B. Expression of E-cadherin and N-cadherin mRNA were analyzed by qRT-PCR in HHL-5-shHOXA9, HL7702-shHOXA9, and their control vector cells. C. Expression of E-cadherin and N-cadherin protein were analyzed by Western blot in SMMC-7721-HOXA9, SNU423-HOXA9, and their control vector cells. D. Expression of E-cadherin and N-cadherin mRNA were analyzed by qRT-PCR in SMMC-7721-HOXA9, SNU423-HOXA9, and their control vector cells. E. HOXA9, E-cadherin, and N-cadherin protein expression was analyzed by immunohistochemical analysis in 82 cases HCC tissues, and the representative results were shown. F. E-cadherin expression was positively correlated with HOXA9 expression in HCC tissues. G. N-cadherin expression was negatively correlated with HOXA9 expression in HCC tissues. **, $P < 0.01$ is based on the Student *t* test. All results are from three independent experiments. Error bars, SD.

Moreover, HHL-5-shHOXA9 and HL7702-shHOXA9 cells showed a greater degree of invasion through Matrigel (Figure 5A and 5B, lower). In contrast, ectopic expression of HOXA9 dramatically reduced the migratory and invasive capacity of SMMC-7721 and SNU423 cells (Figure 5C and 5D). These results indicate that

down-expression of HOXA9 promotes migratory and invasive behaviors in HCC cells.

HOXA9 inhibits metastasis *in vivo*

We then investigated the functional relevance of HOXA9 for metastasis *in vivo*. HHL-5-shHOXA9, SMMC-7721-HOXA9 and their corre-

sponding control cells were injected into nude mice through the tail vein. Silencing HOXA9 not only significantly increased the number of mice with distant metastasis (**Figure 6A**), but also dramatically increased the number of metastatic tumors in lung of each mouse (**Figure 6B**). Ectopic expression of HOXA9 in SMMC-7721 cells inhibited metastatic behavior, both in terms of the number of mice with distant metastasis (**Figure 6A**) and the number of metastatic tumors in the lung of each mouse (**Figure 6C**). Therefore, the *in vivo* results further demonstrate the critical role of HOXA9 in HCC metastasis.

HOXA9 regulates the expression of E-cadherin and N-cadherin in HCC cells

Previous studies had shown that down-regulation of epithelial cell marker and up-regulation of mesenchymal cell marker had an essential role in HCC motility and invasion [20]. E-cadherin is the epithelial cells marker, and N-cadherin is the mesenchymal cells marker [20]. To investigate whether HOXA9 regulates E-cadherin and N-cadherin expression in HCC cell lines, we observed the protein expression changes and found that silencing HOXA9 decreased the levels of E-cadherin and increased the levels of N-cadherin (**Figure 7A**). At the same silencing HOXA9 also decreased the mRNA levels of E-cadherin and increased the mRNA levels of N-cadherin (**Figure 7B**). Conversely, HOXA9 overexpression increased expression of E-cadherin, and decreased expression of N-cadherin both in protein (**Figure 7C**) and mRNA (**Figure 7D**) levels. To recognize any clinical correlation of HOXA9 and EMT markers, we analyzed E-cadherin and N-cadherin expression in the same human HCC tissues (**Figure 7E**). Highly positive correlation between HOXA9 and E-cadherin expression was drawn (**Figure 7F**). Conversely, highly negative correlation between HOXA9 and N-cadherin expression was drawn (**Figure 7G**). Taken together, these findings suggest that HOXA9 plays an important role in regulating migratory and invasive behaviors in HCC cells may via controlling the expression of E-cadherin and N-cadherin expression.

Discussion

Hepatocellular carcinoma (HCC) is the sixth most common cancer and ranks as high as

third for cancer-related deaths worldwide [21]. Although tumor resection and liver transplantation are effective treatments for selected HCC patients, tumor recurrence remains a main concern [22]. Furthermore, surgical treatment is not applicable for patients with advanced tumor stages. Development of a new approach to prevent tumor recurrence and improve prognosis is an urgent need for HCC patients [22]. Metastasis continues to be the main obstacle to the effective treatment of HCC [23]. Therefore, there is an urgent need to identify novel molecular factors that lead to the invasiveness and metastasis of HCC. In the present manuscript, we identified HOXA9 as a candidate target gene for the inhibition of HCC metastasis.

Homeobox genes regulate embryonic development and tissue patterning, and their expression is frequently perturbed and often aberrantly increased in tumors [24]. Until recently, the prevailing dogma has been that inappropriate expression of homeobox genes promotes tumor progression [24]. Consistently, the homeobox genes that are highly expressed during early embryogenesis, that promote cell proliferation and survival, and that induce migration are those that are most often over expressed in transformed cells and tissues [25]. These are the homeobox genes that have been implicated in altered growth receptor signaling, deregulated cell cycle control, and the elevated growth and apoptosis resistance of cancer cells and that appear to regulate tumor invasion and metastasis and promote angiogenesis [25].

However, recently studies showing that some homeobox genes could function as a tumor suppression gene. PITX1 [26], which is frequently downregulated in prostate, bladder, and colon cancers, could function as a tumor suppression gene by inhibiting oncogenic Ras signaling [26, 27]. HOXA5, which is lost in greater than 60% of mammary tumors and breast cancer cell lines, may restrict breast cancer by regulating levels of the p53 [28]. HOXA9 inhibits cell growth and survival and promotes morphogenesis in normal and transformed breast cancer cells by directly regulating expression of the BRCA1 [11]. Previous studies also demonstrated that HOXA9 gene was also frequently methylated in HCC [12]. Consistent with these researches, we showed that reducing levels of HOXA9 in non-

malignant liver cells enhanced motility and invasion property. Although investigators have previously reported that HOXA9 is methylated and that its expression is reduced in breast, lung, and ovarian cancers [29], this is the first study to our knowledge to assess the clinical relationship of HOXA9 loss to HCCs, to analyze the functional relevance of HOXA9 to the malignant behavior of HCC cells in culture, in vivo and in clinical specimens. In this respect, we found that HOXA9 restricts the aggressive and malignant behavior of HCC cells may by modulating E-cadherin and N-cadherin expression. These findings are consistent with the notion that developmental regulators, such as the HOX family of transcription factors, influence adult tissue homeostasis by regulating the expression and/or activity of key tumor suppression genes that regulate cell growth and survival and morphogenesis.

Tumor suppressor genes can inhibit tumor growth and invasive and metastatic potential [25]. Loss of tumor suppressor genes may lead to a malignant cancer phenotype. Previous studies have reported that the expression levels of tumor suppressor genes were decreased in tumors compared with normal tissues [21]. To confirm the tumor suppressor function of HOXA9 in HCC, we first examined the levels of HOXA9 in HCC samples and normal liver tissue samples using IHC. We found that HOXA9 was significantly reduced in cancers, which suggested that HOXA9 was a candidate tumor suppressor gene in HCC. To further explore the role of HOXA9 in HCC, we transfected HCC cells either to ectopically express HOXA9 or to inhibit its expression using RNA interference. Knockdown of HOXA9 in vitro significantly enhanced the migration and invasion of HCC cells, while overexpression of HOXA9 inhibited cell mobility. Our in vivo experiment also demonstrated that HOXA9 markedly inhibited metastasis to the lungs. These data further supported the tumor suppressor role of HOXA9 in HCC.

It has been demonstrated that down-regulation of epithelial cell marker and up-regulation of mesenchymal cell marker had an essential role in HCC motility and invasion [20]. The E-cadherin is the important epithelial cell marker and the N-cadherin is the important mesenchymal cell marker [20]. Therefore, we investi-

gated whether HOXA9 suppressed metastasis in HCC is relevant to E-cadherin and N-cadherin. Our results indicated that the level of E-cadherin was significantly decreased and N-cadherin was significantly increased in HOXA9 knock-down cells. Conversely, HOXA9 over expression increased levels of E-cadherin, and decreased levels of N-cadherin. All of these data revealed that HOXA9 suppresses the migration and invasion activities of HCC cells may via controlling the expression of E-cadherin and N-cadherin expression. However, how HOXA9 might regulate the E-cadherin and N-cadherin expression is still unknown. So in our following studies, we will reveal the detailed mechanism about how HOXA9 might regulate E-cadherin and N-cadherin expression in HCC by using the global expression profile microarray.

In conclusion, we found that HOXA9 expression was generally lower in HCC lesions compared with non-tumor tissues. Our in vitro and in vivo data demonstrate that HOXA9 has a vital function in inhibiting cell mobility, which is partially by the regulation of E-cadherin and N-cadherin expression. Thus, we propose that the candidate tumor suppressor gene HOXA9 may be an effective novel therapeutic target in the management of HCC.

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Disclosure of conflict of interest

None.

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