

Original Article

MicroRNA-137 inhibits cell migration and invasion by targeting bone morphogenetic protein-7 (BMP7) in non-small cell lung cancer cells

Yan-Rong Yang, Yun-Xia Li, Xin-Yuan Gao, Sha-Sha Zhao, Shu-Zhi Zang, Zhi-Qiang Zhang

Department of Respiration, The First Affiliated Hospital of Xinxiang Medical University, Xinxiang Medical University, Weihui 453100, China

Received July 31, 2015; Accepted August 28, 2015; Epub September 1, 2015; Published September 15, 2015

Abstract: MicroRNA-137 (miR-137) was reported to be dysregulated in several human cancers. However, the function and mechanism of miR-137 in non-small cell lung cancer (NSCLC) is still unclear. In the current study, we explored the role of miR-137 in NSCLC progression. Using qRT-PCR, our data showed that miR-137 was significantly down-regulated in NSCLC tissues and cell lines. In vitro functional assay, we found that over-expression of miR-137 suppressed NSCLC cells proliferation, migration and invasion, indicating that miR-137 could act as a tumor suppressor in NSCLC progression. In addition, bone morphogenetic protein-7 (BMP7) was identified as a target of miR-137 in NSCLC cells, Luciferase reporter assay suggested that miR-137 directly targeted 3'-UTR of BMP7, and correlation analysis revealed that BMP7 inversely correlated with miR-137 in NSCLC tissues. Furthermore, Restoration of BMP7 remarkably reversed the tumor suppressive effects of miR-137 on NSCLC cell proliferation, migration, and invasion. Taken together, our findings suggested that miR-137/BMP7 axis could contribute to the progression of NSCLC, suggesting miR-137 as a potential therapeutic target for the treatment of NSCLC.

Keywords: Non-small cell lung cancer, miR-137, bone morphogenetic protein-7

Introduction

Lung cancer is one of the most common cancers and the leading cause of cancer-related deaths worldwide [1]. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for more than 85% of all lung cancer cases [2]. Despite recent advances in clinical and experimental oncology, the 5-year survival rate for NSCLC patients is still only 5-20% [3]. The distant metastases are responsible for the failure of lung cancer therapy and the poor prognosis of lung cancer [4]. Thus, a better understanding of the mechanisms of metastasis might be helpful to find new therapeutic targets for the treatment of NSCLC.

MicroRNAs (miRNAs) are small, non-coding RNAs that negatively regulate the expression of target genes via translational repression or mRNA degradation [5]. miRNAs play essential roles in a variety of biological processes, including cell proliferation, metastasis, differentiation, stress resistance, and fat metabolism

[6-8]. Recent studies revealed that dysregulation of miRNAs occur in a variety of cancers. For example, Li et al showed that down-regulated miR-646 in renal cell carcinoma was associated with tumor metastasis through MAPK pathway by targeting NOB1 [9]. Liu et al found that miR-506 suppressed proliferation and induces senescence by directly targeting the CDK4/6-FOXM1 axis in ovarian cancer [10]. Zhu et al reported that up-regulation of miR-106a in gastric cancer could exert oncogenic role with promoting effects on cell proliferation and metastasis by targeting TIMP2 [11]. Ma et al revealed that over-expression of miR-301a in breast cancer could promote tumor metastasis by targeting PTEN and activating Wnt/ β -catenin signaling [12]. These findings suggested that de-regulation of miRNA may be associated with NSCLC carcinogenesis.

In the current study, our results showed that miR-137 was significantly down-regulated in both NSCLC tissue samples and cell lines. Over-expression of miR-137 inhibited cell proliferation, migration and invasion of NSCLC cells.

miR-137 targets BMP7 in NSCLC

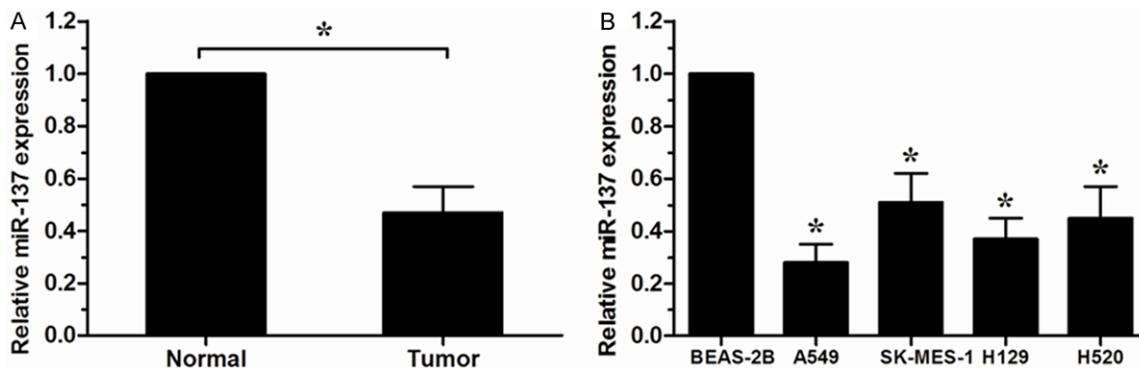


Figure 1. MiR-137 is down-regulated in NSCLC tissues and cell lines. A. The relative expression levels of miR-137 were significantly down-regulated in NSCLC tissues (Tumor) compared with the adjacent non-tumor tissues (Normal). B. The relative miR-137 expression levels were significantly decreased in four NSCLC cell lines (A549, SK-MES-1, H129, and H520) compared with the normal lung bronchus epithelial cell line BEAS-2B. * $P < 0.05$.

Moreover, we identified that bone morphogenetic protein-7 (BMP7) as direct target genes of miR-137 in NSCLC cells. Restoration of BMP7 remarkably reversed the tumor-suppressive effects of miR-137, indicating that miR-137 exerted its effect on the inhibition of cell proliferation, migration and invasion by down-regulating BMP7 in NSCLC cells.

Materials and methods

Patient samples and cell lines

A total of 32 pairs of human NSCLC tissues and adjacent non-tumor tissues were obtained from The First Affiliated Hospital of Xinxiang Medical University. None of the NSCLC patients had received radiation therapy or chemotherapy before the surgery, and the tissues were immediately snap-frozen in liquid nitrogen and stored at -80°C until used. 4 NSCLC cell lines (A549, SK-MES-1, H129, and H520) and a normal lung bronchus epithelial cell line BEAS-2B were obtained from American Type Culture Collection and cultured in DMEM supplemented with 10% fetal bovine serum (FBS, Invitrogen), and incubated in 5% CO_2 humid atmosphere at 37°C .

RNA extraction and quantitative real-time-PCR (qRT-PCR)

Total RNA was extracted by TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. SYBR® Premix Ex Taq (Takara) was used to detect gene expression on ABI Stepone plus (Applied Biosystems). miRNA was extracted using All-in-one microRNA extraction kit (GeneCopoeia). Primers for BMP7: sense, 5'-TCTCATTGATTGGAGCATT-3' and anti-sense, 5'-GGCAGTGGTAGGATAAAC-3'. Primers for GA-

PDH: sense, 5'-ACGGATTGGTCGATTG-3', and anti-sense, 5'-GGAAGATGGTGATGGGATT-3'. Primers for miR-137 and U6 were purchased from GeneCopoeia (Carlsbad). The expression of BMP7 was normalized with GAPDH, and the expression of miR-137 was normalized with U6.

Plasmids and luciferase activity assay

MiR-137, the scramble control mimics (miR-NC) and BMP7 overexpression plasmid, (pcDNA3-BMP7) were obtained from GenePharma. The BMP7 3'-UTR fragment containing putative miR-137 binding sites was amplified from complementary DNA (cDNA) of A549 cells. The 3'-UTR was inserted into pGL3 vector (Invitrogen), and named pGL3-BMP7 3'-UTR (Wt). Mutation of the miR-137 target sites in pGL3-BMP7 3'-UTR (Mut) was performed using a Stratagene mutation kit (Stratagene).

HEK293 cells were cultured in 24-well plates, and each well was co-transfected with 100-ng Wt or Mut, and 50-ng pGL3 (Promega) and 100-ng miR-137 mimics using Lipofectamine 2000. Renilla luciferase pGL3 was used as a control. Cells were collected 48 h after transfection and the dual-luciferase activity was examined with a Dual-Luciferase Reporter Assay (Promega) according to the manufacturer's instructions.

Cell proliferation assay

Transfected cells were seeded into 96-well plates with a density of 4000 cells/well, and cultured for different time. 10 μL of MTT was added into each well, and incubated for 4 h. Then the supernatant was discarded, and 200 μL of DMSO was added to each well. Optical

miR-137 targets BMP7 in NSCLC

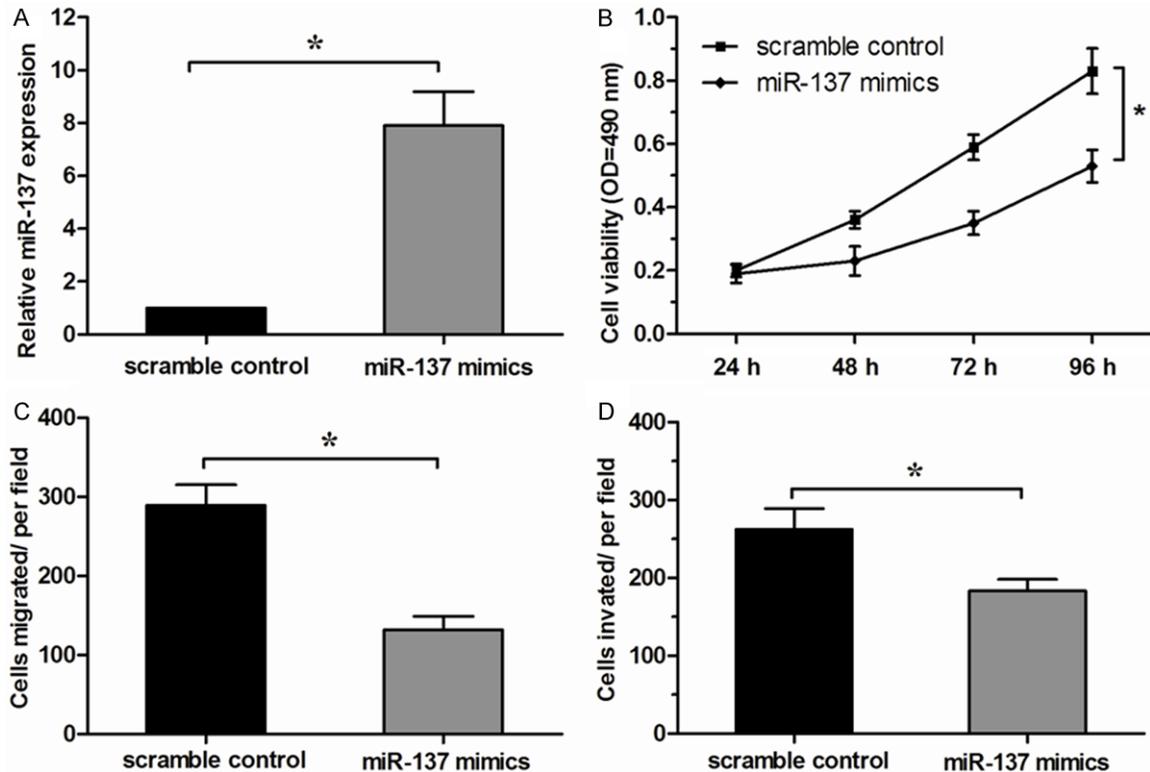


Figure 2. MiR-137 inhibits the proliferation, migration and invasion of NSCLC cells in vitro. A. A549 cells were transfected with miR-137 mimics or the scramble control mimics. The expression of miR-137 was analyzed by qRT-PCR. B. Cell proliferation of A549 cells were detected by MTT assay. C. Migration assay of A549 cells transfected with miR-137 mimics or the scramble control. D. Invasion assay of A549 cells transfected with miR-137 mimics or the scramble control. * $P < 0.05$.

density (OD) was detected at the wavelength of 490 nm.

Cell migration and invasion assay

For migration assay, 5×10^4 transfected cells in serum-free medium were added into the upper chamber of an insert (8- μm pore size, Millipore). For invasion assays, 1×10^5 cells in serum-free medium were placed into the upper chamber of an insert coated with Matrigel (Sigma). Medium containing 10% FBS were added to the lower chamber as a chemoattractant. After 24 h of incubation, cells remaining on the upper membrane were removed, whereas cells which had migrated or invaded to the lower membrane were stained with 0.1% crystal violet, imaged, and counted using a microscope (Olympus).

Western blot

Treated cells were washed twice with PBS and proteins were extracted with SDS lysis buffer

(Beyotime), and separated by 10% SDS-PAGE gel. Protein samples were transferred to PVDF membrane (Millipore), and were probed with primary antibodies against BMP7 or GAPDH (Abcam). Membranes were incubated at 4°C overnight, followed by incubation with AP-conjugated secondary antibodies and detected by ECL (Amersham).

Statistical analysis

All statistical analyses were performed using SPSS version 18.0 software (IBM). Data are expressed as the mean \pm SD from at least three separate experiments. ANOVA or Student's t test was used to examine the difference. $P < 0.05$ was considered statistically significant.

Results

miR-137 is significantly down-regulated in NSCLC tissues and cell lines

Expression of miR-137 in 32 paired NSCLC patient tissues and the adjacent non-tumor tis-

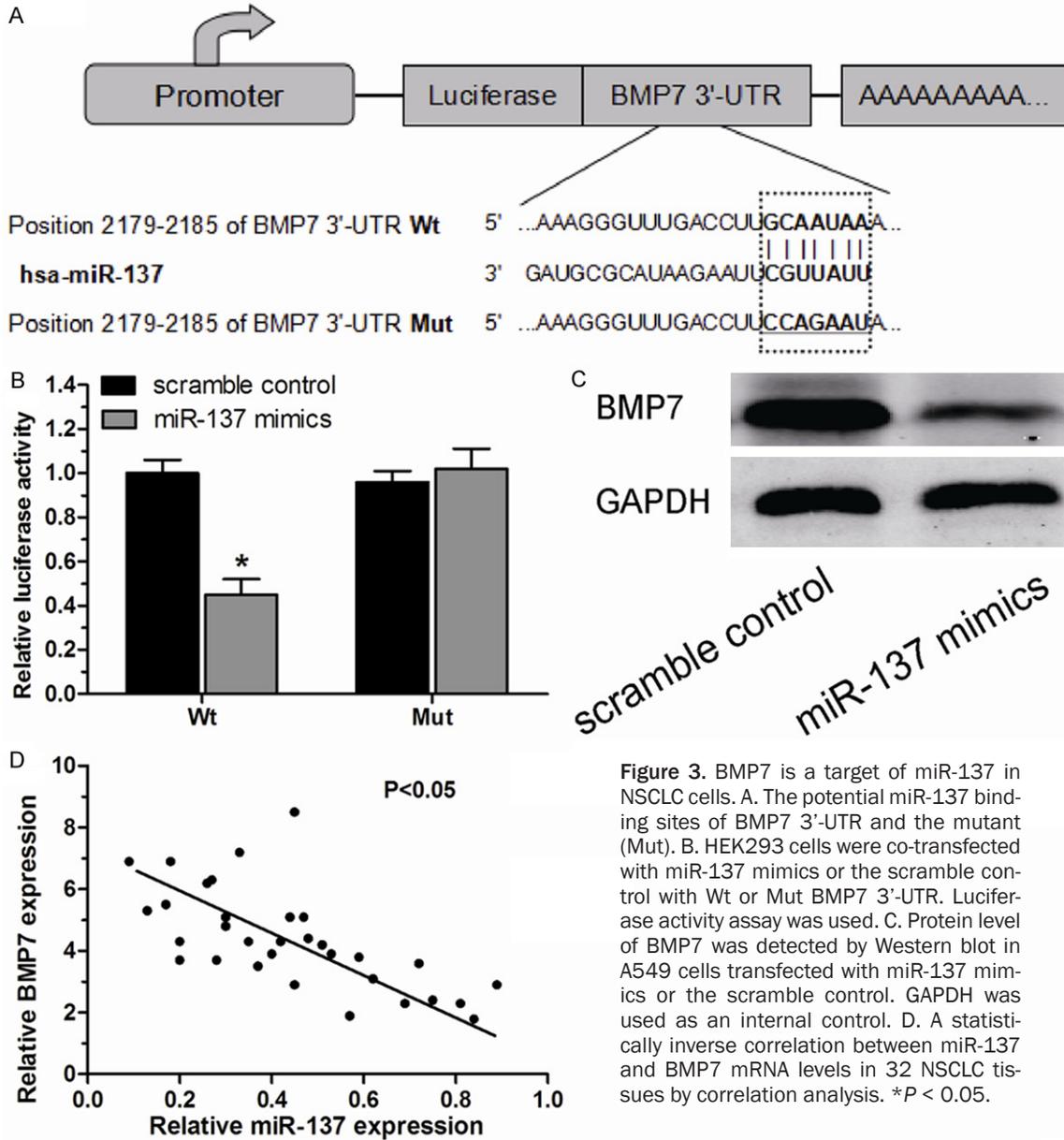


Figure 3. BMP7 is a target of miR-137 in NSCLC cells. **A.** The potential miR-137 binding sites of BMP7 3'-UTR and the mutant (Mut). **B.** HEK293 cells were co-transfected with miR-137 mimics or the scramble control with Wt or Mut BMP7 3'-UTR. Luciferase activity assay was used. **C.** Protein level of BMP7 was detected by Western blot in A549 cells transfected with miR-137 mimics or the scramble control. GAPDH was used as an internal control. **D.** A statistically inverse correlation between miR-137 and BMP7 mRNA levels in 32 NSCLC tissues by correlation analysis. * $P < 0.05$.

sues was examined by qRT-PCR. Our data showed that the expression level of miR-137 was significantly decreased in NSCLC tissues, as compared with that in adjacent non-tumor tissues (**Figure 1A**). In addition, we investigated miR-137 expression in four NSCLC cell lines. As shown in **Figure 1B**, miR-137 were significantly down-regulated in NSCLC cell lines (A549, SK-MES-1, H129, and H520) compared with normal lung bronchus epithelial cell line BEAS-2B. Taken together, these data indicated that miR-137 may be associated with NSCLC carcinogenesis.

miR-137 inhibits NSCLC cell proliferation, migration and invasion

We further studied the effects of miR-137 on cell proliferation, migration and invasion of NSCLC cells. A549 cells were transfected with miR-137 mimics or scramble control mimics (miR-NC), and the expression of miR-137 was determined by qRT-PCR (**Figure 2A**). MTT assay revealed that miR-137 over-expression significantly decreased the proliferation ability of A549 cells (**Figure 2B**). In addition, in vitro migration and invasion assays found that miR-

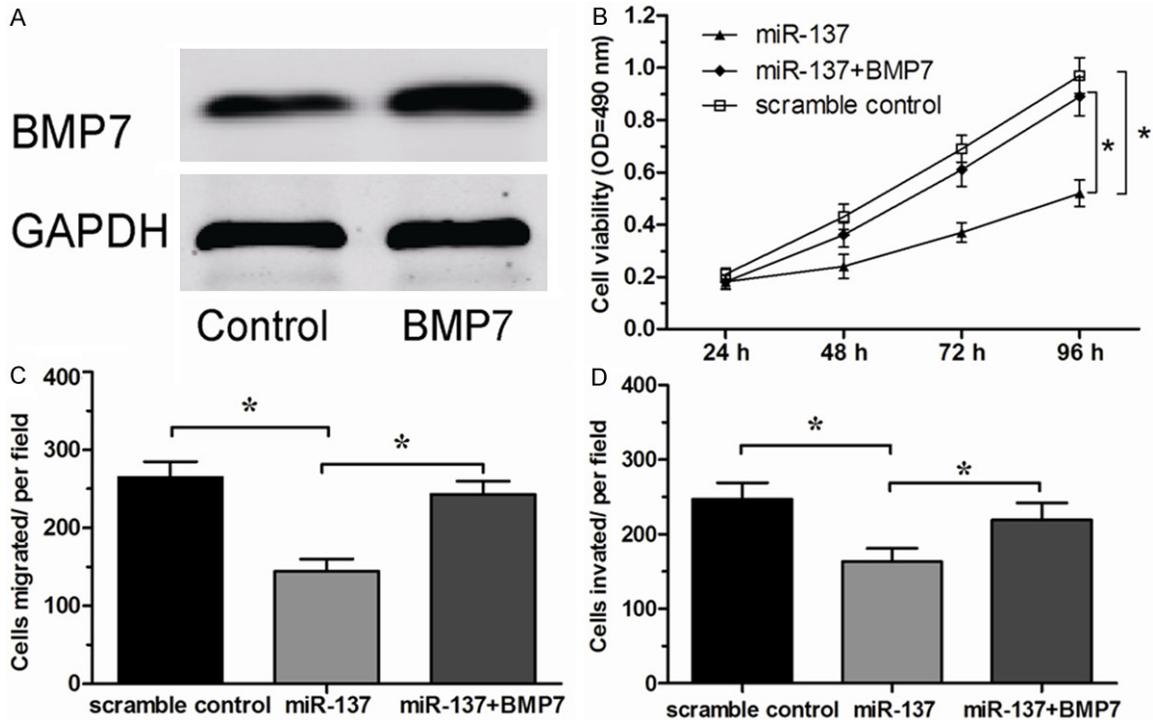


Figure 4. Overexpression of BMP7 abolishes the effects of miR-137. (A) A549 cells were transfected with pcDNA3-BMP7 or the vector, and the expression of BMP7 was examined by western blot. (B-D) A549 cells were co-transfected with miR-137 mimics or scramble control with pcDNA3-BMP7, MTT assay (B), migration assay (C), and invasion assay were performed. * $P < 0.05$.

miR-137 overexpression markedly inhibited the migration and invasion ability of A549 cells (Figure 2C, 2D). These data demonstrated that miR-137 acted as a tumor suppressor in NSCLC progression and development.

miR-137 targets BMP7 in NSCLC cells

To elucidate the molecular mechanism by which miR-137 exerted its inhibitory effect on NSCLC cells, we used TargetScan 6.2 to screen the target gene of miR-137. Bone morphogenetic protein-7 (BMP7) was predicted to be a target of miR-137 (Figure 3A). Luciferase activity assay revealed that overexpression of miR-137 significantly decreased the relative luciferase activity of wild type (Wt) 3'-UTR of BMP7 in A549 cells, but had no effect on the mutant (Mut) 3'-UTR of BMP7 (Figure 3B). Western blot analysis revealed that overexpression of miR-137 significantly reduced the expression of BMP7 in A549 cells (Figure 3C). In addition, there was a significant inverse correlation between expression levels of miR-137 and BMP7 mRNAs in NSCLC tissues (Figure 3D). Taken together, these data suggested that BMP7 was a target of miR-137 in NSCLC cells.

Restoration of BMP7 abolished the effects of miR-137

We further explored whether restoration of BMP7 could reverse the tumor suppressive effects of miR-137. The effect of pcDNA3-BMP7 was confirmed by western blot (Figure 4A). MTT, migration, and invasion assays revealed that restoration of BMP7 significantly abolished the tumor suppressive effects of miR-137 in NSCLC cells (Figure 4B-D).

Discussion

In this study, we characterized the function of miR-137 in NSCLC progression. We found that the expression levels of miR-137 were significantly decreased in NSCLC tissues and cell lines. In addition, we found that ectopic expression of miR-137 significantly inhibited NSCLC cell proliferation, migration, and invasion. We further identified BMP7 as a direct target of miR-137 in NSCLC cells. Moreover, over-expression of BMP7 abolished the tumor suppressive effects of miR-137. Our data illustrated the possible role of miR-137 and BMP7 in the progression of NSCLC.

Recently studies revealed that miR-137 play essential roles in tumorigenesis. For example, Xiao et al revealed that miR-137 modulates pancreatic cancer cell growth, invasion and sensitivity to chemotherapy [13]. Hao et al showed that miR-137 was significantly down-regulated in melanoma and inhibited proliferation of melanoma cells by targeting PAK2 [14]. Smith et al showed that miR-137 was decreased in colorectal cancer tissues and miR-137 inhibited cell growth, colony formation, and tumor-sphere growth of colon cancer cell by targeting Musashi-1 [15]. Zhang et al found that miR-137 was down-regulated in NSCLC tissues and inhibited NSCLC cell proliferation and motility by directly targeting SLC22A18 [16]. Herein, we found that over-expression of miR-137 could inhibit NSCLC cell proliferation, migration, and invasion by targeting BMP7. Our study expanded the tumor suppressive role of miR-137 in NSCLC progression.

Bone morphogenetic proteins (BMPs) are important signaling molecules that were first identified by their ability to induce bone and cartilage [17]. BMP is a member of the TGF- β superfamily known to regulate cell proliferation, apoptosis, and differentiation during human development [18]. Recent studies showed that BMP7 play important roles in tumor progression and development. For example, Li et al showed that high-expression level of BMP7 in hepatocellular carcinoma (HCC) was important in tumor progression and served as an independent biomarker for poor survival of HCC patients [19]. Xu et al found that BMP7 modulated the expression of E-cadherin and MMP-9, and by which mechanism it might regulate cell migration and metastasis of esophageal squamous cell carcinoma [20]. Naber et al found that BMP-7 inhibited TGF- β -induced invasion of breast cancer cells through inhibition of integrin β (3) expression [21]. Here, our results showed that BMP7 was a target of miR-137 in NSCLC cells, and BMP7 was inversely correlated with miR-137 in NSCLC tissues. Furthermore, we found that supplement of BMP7 dramatically abolished the tumor suppressive effects of miR-137 in NSCLC cells. Taken together, those results indicated that BMP7 could be involved in NSCLC progression.

In conclusion, our results revealed that miR-137 was down-regulated in NSCLC and enforced expression of miR-137 inhibited NSCLC

cell proliferation, migration and invasion through directly targeting BMP7. This novel miR-137/BMP7 axis may provide new insights into the mechanisms underlying tumor progression, and restoration of miR-137 may be a potential therapeutic strategy for the treatment of NSCLC in the future.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yan-Rong Yang, Department of Respiration, The First Affiliated Hospital of Xinxiang Medical University, Xinxiang Medical University, Weihui 453100, China. E-mail: yanrongyang@163.com

References

- [1] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; 61: 69-90.
- [2] Ettinger DS, Akerley W, Borghaei H, Chang AC, Cheney RT, Chirieac LR, D'Amico TA, Demmy TL, Ganti AK, Govindan R, Grannis FW Jr, Horn L, Jahan TM, Jahanzeb M, Kessinger A, Komaki R, Kong FM, Kris MG, Krug LM, Lennes IT, Loo BW Jr, Martins R, O'Malley J, Osarogigabon RU, Otterson GA, Patel JD, Pinder-Schenck MC, Pisters KM, Reckamp K, Riely GJ, Rohren E, Swanson SJ, Wood DE, Yang SC, Hughes M and Gregory KM. Non-small cell lung cancer. *J Natl Compr Canc Netw* 2012; 10: 1236-1271.
- [3] Goldstraw P, Ball D, Jett JR, Le Chevalier T, Lim E, Nicholson AG and Shepherd FA. Non-small-cell lung cancer. *Lancet* 2011; 378: 1727-1740.
- [4] Molina JR, Yang P, Cassivi SD, Schild SE and Adjei AA. Non-small cell lung cancer: Epidemiology, risk factors, treatment, and survivorship. *Mayo Clin Proc* 2008; 83: 584-594.
- [5] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; 116: 281-297.
- [6] Hwang HW and Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer* 2007; 96 Suppl: R40-44.
- [7] Chen CZ, Li L, Lodish HF and Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. *Science* 2004; 303: 83-86.
- [8] Ambros V. MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. *Cell* 2003; 113: 673-676.
- [9] Li W, Liu M, Feng Y, Xu YF, Huang YF, Che JP, Wang GC, Yao XD and Zheng JH. Downregulated miR-646 in clear cell renal carcinoma correlated with tumor metastasis by targeting the

miR-137 targets BMP7 in NSCLC

- nine one binding protein (NOB1). *Br J Cancer* 2014; 111: 1188-1200.
- [10] Liu G, Sun Y, Ji P, Li X, Cogdell D, Yang D, Parker Kerrigan BC, Shmulevich I, Chen K, Sood AK, Xue F and Zhang W. MiR-506 suppresses proliferation and induces senescence by directly targeting the CDK4/6-FOXM1 axis in ovarian cancer. *J Pathol* 2014; 233: 308-318.
- [11] Zhu M, Zhang N, He S, Lui Y, Lu G and Zhao L. MicroRNA-106a targets TIMP2 to regulate invasion and metastasis of gastric cancer. *FEBS Lett* 2014; 588: 600-607.
- [12] Ma F, Zhang J, Zhong L, Wang L, Liu Y, Wang Y, Peng L and Guo B. Upregulated microRNA-301a in breast cancer promotes tumor metastasis by targeting PTEN and activating Wnt/beta-catenin signaling. *Gene* 2014; 535: 191-197.
- [13] Xiao J, Peng F, Yu C, Wang M, Li X, Li Z, Jiang J and Sun C. microRNA-137 modulates pancreatic cancer cells tumor growth, invasion and sensitivity to chemotherapy. *Int J Clin Exp Pathol* 2014; 7: 7442-7450.
- [14] Hao S, Luo C, Abukiwan A, Wang G, He J, Huang L, Weber CE, Lv N, Xiao X, Eichmuller SB and He D. miR-137 inhibits proliferation of melanoma cells by targeting PAK2. *Exp Dermatol* 2015; [Epub ahead of print].
- [15] Smith AR, Marquez RT, Tsao WC, Pathak S, Roy A, Ping J, Wilkerson B, Lan L, Meng W, Neufeld KL, Sun XF and Xu L. Tumor suppressive microRNA-137 negatively regulates Musashi-1 and colorectal cancer progression. *Oncotarget* 2015; 6: 12558-12573.
- [16] Zhang B, Liu T, Wu T, Wang Z, Rao Z and Gao J. microRNA-137 functions as a tumor suppressor in human non-small cell lung cancer by targeting SLC22A18. *Int J Biol Macromol* 2015; 74: 111-118.
- [17] Cheng H, Jiang W, Phillips FM, Haydon RC, Peng Y, Zhou L, Luu HH, An N, Breyer B, Vanichakarn P, Szatkowski JP, Park JY and He TC. Osteogenic activity of the fourteen types of human bone morphogenetic proteins (BMPs). *J Bone Joint Surg Am* 2003; 85-A: 1544-52.
- [18] Xiao YT, Xiang LX and Shao JZ. Bone morphogenetic protein. *Biochem Biophys Res Commun* 2007; 362: 550-553.
- [19] Li W, Cai HX, Ge XM, Li K, Xu WD and Shi WH. Prognostic significance of BMP7 as an oncogene in hepatocellular carcinoma. *Tumor Biol* 2013; 34: 669-674.
- [20] Xu G, Tang S, Yang J, Chen K, Kang J, Zhao G, Feng F, Yang X, Zhao L, Lu Q, Sun L, Hong L, Gong T and Zhang H. BMP7 expression in esophageal squamous cell carcinoma and its potential role in modulating metastasis. *Dig Dis Sci* 2013; 58: 1871-1879.
- [21] Naber HP, Wiercinska E, Pardali E, van Laar T, Nirmala E, Sundqvist A, van Dam H, van der Horst G, van der Pluijm G and Heckmann B. BMP-7 inhibits TGF- β -induced invasion of breast cancer cells through inhibition of integrin β 3 expression. *Cell Oncol (Dordr)* 2012; 35: 19-28.