Int J Clin Exp Pathol 2020;13(9):2270-2279
www.ijcep.com /ISSN:1936-2625/IJCEP0113576

Original Article

microRNA-133b represses the progression of lung cancer through inhibiting SOX9/β-catenin signaling pathway

Shubin Liu[1*], Shasha Li[2*], Xiaoqiang Yu[4], Qiping Wang[5], Hui Sun[3]

Departments of [1]Oncology, [2]Respiratory Medicine, [3]Cardiothoracic Surgery, Ganzhou Peoples Hospital, Ganzhou 341000, Jiangxi, China; [4]Department of Thoracic Surgery, Ruijin Peoples Hospital, Ganzhou 342500, Jiangxi, China; [5]The Three Department of Medicine, Dayu County Peoples Hospital, Ganzhou 341500, Jiangxi, China. [*]Equal contributors.

Received April 30, 2020; Accepted June 11, 2020; Epub September 1, 2020; Published September 15, 2020

Abstract: MicroRNA-133b (miR-133b) has been shown to be down-regulated in lung cancer and functions as a tumor repressor. However, the underlying mechanisms of miR-133b in lung cancer are not clear. SOX9, a member of SOX family, serves as an oncogene in lung cancer by activating β-catenin signaling and was identified to be a direct target of miR-133b in breast cancer. Based on these data, the current study was performed to explore whether SOX9/β-catenin signaling is implicated in miR-133b-meditaed lung cancer repression. MiR-133b expression in lung cancer tissues and cells were detected by RT-PCR. CCK-8, colony formation, flow cytometry, transwell chamber and *in vivo* assays were carried out to determine cell proliferation, colony formation, apoptosis, cell cycle, invasion, and tumorigenesis. We found that miR-133b expression was decreased in lung cancer tissues and cells. Up-regulation of miR-133b reduced cell proliferation and colony formation, induced cell apoptosis and G0/G1 phase arrest, and decreased cell invasion. Besides, miR-133b up-regulation decreased the expression of β-catenin and SOX9. Cell viability inhibition and apoptosis promotion induced by miR-133b up-regulation were all impaired when SOX9 was up-regulated. Furthermore, miR-133b over-expression repressed the tumorigenesis of lung cancer cells with smaller tumor size and lower Ki-67 expression. Taken together, this study clarifies that miR-133b represses lung cancer progression by inhibiting SOX9/β-catenin signaling.

Keywords: MiR-133b, β-catenin, SOX9, lung cancer

Introduction

Lung cancer ranks 2th among the most frequent type of malignant tumors and is one of the leading causes of cancer-related deaths in the world [1]. Although advances have been made in lung cancer diagnose and treatment, the outcomes of patients remain poor, and only 15% of patients have more than 5 years' survival after diagnosis [2]. Therefore, it's essential to explore the mechanisms underlying lung cancer occurrence and development.

MicroRNAs (miRNAs) are small noncoding RNAs which modulate protein translation at the post-transcriptional level through inducing mRNA degradation or translation inhibition by binding to the 3' untranslated region (3'UTR) of their targeting mRNAs [3]. Alterations of miRNA expression have been identified to be strongly related to the tumorigenesis of majority of cancers including lung cancer [4], indicating the vital roles of miRNAs in cancer treatment. Among these miRNAs, miR-133b has been found to be lowly expressed in non-small cell lung cancer (NSCLC) tissues and cells, and miR-133b up-regulation inhibited lung cancer cell migration and invasion by targeting fascin1 [5], as well as repressing cell growth through interaction with the 3'UTR of epidermal growth factor receptor (EGFR) [6], suggesting that miR-133b functions as a tumor suppressive gene in lung cancer. However, the mechanisms underlying miR-133b in lung cancer remains largely unknown.

SOX9 is a member of SOX family and serves as a stem cell-related transcription factor in holding stem cell properties [7]. Zhou et al. [8] have demonstrated that SOX9 is over-expressed in lung cancer cell lines and tissues, which predicts poor prognosis and advanced clinical progression of patients with lung cancer. Wang et al. [9] have revealed that up-regulation of SOX9 significantly promotes lung cancer A549 cell growth, migration and invasion. Moreover, Guo et al. [10] have illustrated that inhibition of Wnt/β-catenin signaling abolishes the role of SOX9 up-regulation in lung cancer A549 cell proliferation enhancement and apoptosis repression, indicating that SOX9 serves as an oncogene in lung cancer through activating Wnt/β-catenin signaling, which is of importance in both normal embryonic development and tumorigenesis including lung cancer [11, 12]. Under normal conditions, β-catenin is wrapped in a destruction complex which is made up of adenomatous polyposis coli (APC), glycogen synthase kinase 3 (GSK3), axin 1, and casein kinase 1. This causes β-catenin to be phosphorylated and degraded through a ubiquitin-induced proteasomal pathway [13]. However, in human cancers, β-catenin separates from this complex and accumulates in the cytoplasm and nucleus, triggering the expression of its target genes such as cyclin D1 and c-Myc through activating TCF/LEF transcription factors [14]. Furthermore, miR-133b was reported to inhibit the tumorigenesis and metastasis of breast cancer through targeting SOX9 [15]. However, whether miR-133b represses the progression of lung cancer through the SOX9/β-catenin pathway remains unclear.

Overall, this study was performed to probe the effects of miR-133b/SOX9/β-catenin in lung cancer progression through both *in vitro* and *in vivo* assays.

Materials and methods

Tissue samples

Twenty paired lung cancer tissues and the adjacent normal lung tissues derived from lung cancer patients were used in this study. Each patient had undergone pneumonectomy without any form of chemoradiotherapy treatment and signed an informed consent.

Cell lines and culture

Normal lung epithelial cell line BEAS-2B and lung cancer cell lines A549, H1299, and SPC-A1 were all purchased from BeNa Culture Collection (Beijing, China). BEAS-2B cells were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM-H, Gibco, CA, USA); A549 cells were cultured in F-12K medium (Gibco, CA, USA); H1299 and SPC-A1 cells were cultured in RPMI-1640 medium (Gibco, CA, USA); supplemented with 10% fetal bovine serum (FBS, Gibco, USA). Cells were incubated in a humidified atmosphere at 37°C with 5% CO_2.

Western blot analysis

Protein was extracted from cells and tissue samples with lysis buffer (Roche, Shanghai, China) containing protease and phosphatase inhibitors (Solarbio, Beijing, China). After centrifugation at 4°C for 25 min, the protein samples were quantified by using a Bicinchoninic acid Protein Assay Reagent (Thermo Fisher Scientific, MA, USA). Then, protein samples were loaded into 10% SDS-polyacrylamide gel and separated by electrophoresis, followed by transformation to polyvinylidene difluoride membranes (PVDF, Millipore, MA, USA). After incubation with 5% non-fat milk for 1 h at room temperature, the membranes were probed with the indicated primary antibodies including β-catenin (No. MA1-2001, Invitrogen, MA, USA), SOX9 (No. ab26414, Abcam, MA, USA) at 4°C overnight and the secondary antibodies for 1 h at room temperature in succession. After washing three times with phosphate buffered saline (PBS), the bands were measured by ECL regent (Millipore, MA, USA) in a ProfiBlot-48 (Tecan, Switzerland), and gray-scale value analysis was carried out by ImageJ software.

Quantitative real time PCR (qPCR)

Total RNA was exacted from cells and cancer tissue samples with GenElute™ Total RNA Purification Kit (Sigma-Aldrich, MO, USA). After quantification, a total of 1 μg RNA was reverse transcribed (RT) into cDNA prior to qPCR analysis. All kits used in this experiment were bought from Takara (Dalian, China). U6 was used as an internal control for miR-133b, respectively. Relative gene levels were calculated by the $2^{-\Delta\Delta Ct}$ method. Primer sequences used in this

study were obtained from Invitrogen (CA, USA) and were as follows:

miR-133b: RT-5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTAGCTG-3', sense-5'-GATTTGGTCCCCTTCAAC-3', antisense-5'-GTGCAGGGTCCGAGGT-3'; U6: RT-5'-AACGCTTCACGAATTTGCGT-3', sense-5'-CTCGCTTCGGCAGCACA-3', anti-sense-5'-AACGCTTCACGAATTTGCGT-3'.

CCK-8 assay

H1299 cells (2×10^3/well) in logarithmic growth period was suspended in 100 μL culture medium and added into 96-well plates. Next day, the cells were given different treatments. After 1, 2, 3, 4, or 5 days of treatment, 10 μL of cell counting kit-8 (CCK-8) regent (Sangon Biotech, Shanghai, China) were added into each well and incubated for another 4 h at 37°C. Then, the optical density (OD) levels at 450 nm were examined using microplate reader.

Colony formation assay

Stable transfected H1299 cells were inoculated into a 5-cm dish at a density of 3×10^3/100 μL and were incubated at 37°C. The medium was replaced every 2 d until 14 d. Then, after being washed with PBS for three times, the cells were fixed with 5% paraformaldehyde for 15 min, and stained with 0.1% crystal violet solution for 20 min. The visible colonies were counted for cell colony formation ability evaluation.

Cell cycle and apoptosis detection

Flow cytometry was used to analyze cell cycle and apoptosis. After 48 h of different treatments, H1299 cells were harvested and washed with PBS for one time. Then, Annexin V-FITC and PI solution were added to each sample for 15 min in the dark. The fluorescent signals reflecting cell apoptosis were evaluated by flow cytometry within 1 h. Cells in $FITC^-/PI^-$ quadrant were identified as live cells, $FITC^+/PI^-$ were early apoptotic cells and $FITC^+/PI^+$ were late apoptotic cells.

For cell cycle detection, cells were first synchronized with serum-free medium for 12 h prior to different treatments. After 48 h of cell treatment, cells including floating and attached H1299 cells were collected and fixed in 70% ethanol at 4°C for 3 h. Then the cells were incubated with 20 μg/mL RNase for 30 min at 37°C and 5 μg/mL PI solution for 30 min in the dark at 4°C. Then, the cell cycle was evaluated by using flow cytometry.

Transwell assay

Cell invasion was analyzed on H1299 cells using the Transwell chamber pre-coated with material (8 μm pore size; Corning, NY, USA). 1×10^5 H1299 cells were re-suspended in 200 μL FBS-free RPMI-1640 medium and were seeded into the upper chamber. At the same time, 600 μL RPMI-1640 medium containing 10% FBS was added into the lower chamber. After incubation at 37°C for 48 h, cells attached in the lower chamber membrane were fixed in paraformaldehyde, and stained with 1% crystal violet for 10 min at room temperature. Cell numbers in six randomly selected fields were counted under an upright light microscope at ×200 magnification.

In vivo xenograft assay

Twenty male BALB/c nude mice with 4-week-old were purchased from the animal center of air force medical university (Shanghai, China) and were housed under specific pathogen free (SPF) conditions with free water and food available for 1 week. H1299 cells with miR-133b stable over-expression or not were established through selecting with 2 μg/ml puromycin (Sigma-Aldrich, MO, USA) for 7 days. After 1 weeks of breeding, mice were injected 1×10^6 H1299 stable transfected cells around the left side of the neck, with 10 mice in each group. Mice were sacrificed and tumors were removed 28 days post-injection.

Immunohistochemistry

For immunohistochemistry, tumor tissues cut into 4-μm slides were deparaffinized, rehydrated, and blocked with 5% goat serum. Then the slides were subjected to immunohistochemical staining with Ki-67 antibody (No. #12202, Cell Signaling Technology, CA, USA) and incubated for overnight at 4°C, followed with incubation with the secondary antibodies. Finally, the slides were visualized by staining with chromogen 3, 3'-diaminobenzidine tetrachloride. Protein expression was evaluated by grey val-

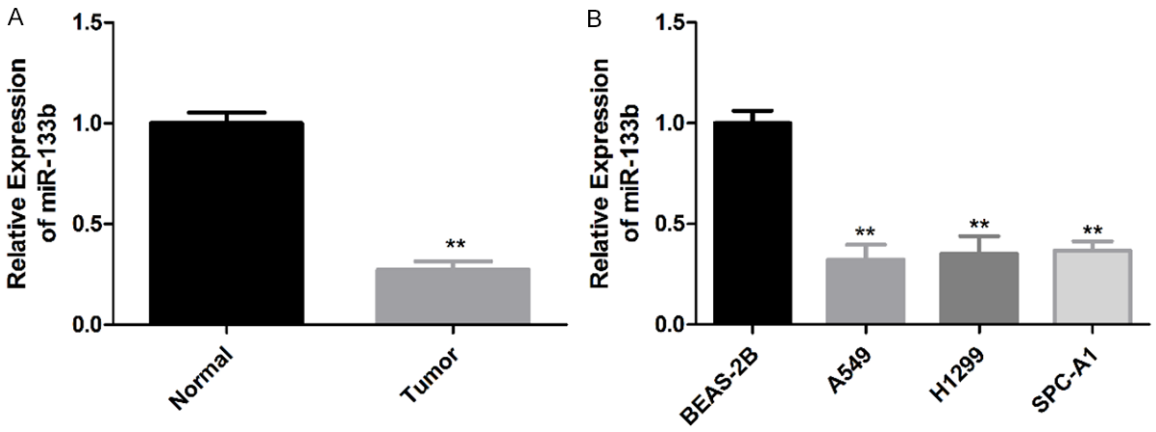

Figure 1. Expression of miR-133b was decreased in lung cancer tissues and cell lines. A. QPCR analysis of the levels of miR-133b in 20 paired lung cancer tissues and adjacent normal lung tissues. B. QPCR analysis of the levels of miR-133b in normal lung cell line BEAS-2B and lung cancer cell lines A549, H1299, and SPC-A1. (**P<0.01).

ues at 400× magnification under a microscope.

Statistical analysis

Data were analyzed by SPSS23.0 software and are presented as mean ± SD. Data comparison was performed by Student's t test for two groups and two-way ANOVA with Bonferroni post-tests for multiple groups. A *p* value less than 0.05 was considered significant.

Results

Down-regulation of miR-133b in lung cancer tissues and cell lines

To explore the function of miR-133b in the occurrence and development of lung cancer, we first detected the expression level of miR-133b in the lung cancer tissues and cell lines. Compared with the normal tissues, the expression of miR-133b was obviously decreased in the lung cancer tissues (**Figure 1A**). Similarly, miR-133b was lowly expressed in lung cancer cell lines A549, H1299, and SPC-A1 when compared to that of the normal lung cell line BEAS-2B (**Figure 1B**). These results illustrated that the down-regulation of miR-133b might play a crucial role in lung cancer progression.

Up-regulation of miR-133b inhibits the malignant phenotype transformation of lung cancer cells

Then, we explored the function of miR-133b in lung cancer progression through a gain-of-function assay in vitro. As the expression of miR-133b was decreased in A549, H1299, and SPC-A1 cells, we chose H1299 cells for further study. Results confirmed that transfection with the mimic of miR-133b significantly increased miR-133b expression in H1299 cells (**Figure 2A**). miR-133b up-regulation decreased cell colony formation (**Figure 2B**) and proliferation abilities (**Figure 2C**) and reduced cell apoptosis (**Figure 2D**) in H1299 cells. Moreover, miR-133b up-regulation induced G0/G1 phase increase and S phase reduction (**Figure 2E**), and impaired the invasion ability of H1299 cells (**Figure 2F**). Taken above, these findings illustrated that miR-133b functioned as a tumor suppressor in lung cancer progression.

miR-133b inhibits lung cancer progression through negative regulation of SOX9/β-catenin signaling

Next, we explored whether SOX9/β-catenin signaling was implicated in lung cancer progression in which miR-133b functioned as a tumor suppressor. Western blot results showed that up-regulation of miR-133b with mimic infection obviously decreased both β-catenin and SOX9 expression in H1299 cells (**Figure 3A**). Subsequently, we overexpressed SOX9 with OE-SOX9 plasmid transfection. **Figure 3B** showed its transfection efficiency. However, miR-133b-induced cell colony formation repression and cell apoptosis promotion were all abolished when SOX9 was up-regulated in H1299 cells (**Figure 3C**, **3D**), suggesting that miR-133b repressed lung cancer progression through

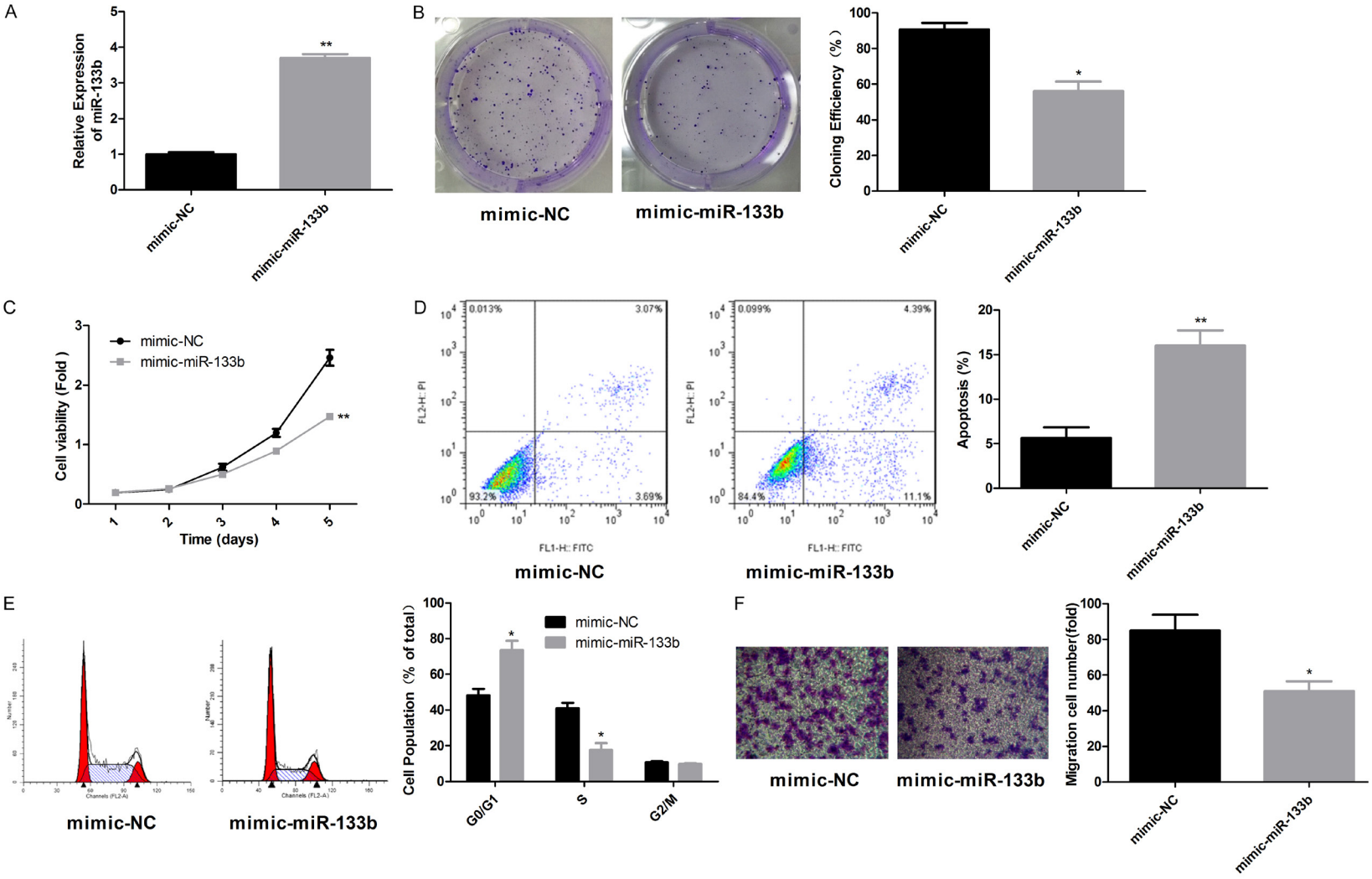

Figure 2. miR-133b inhibited the malignant phenotypic switching of lung cancer cells. H1299 cells were infected with mimic-miR-133b or mimic-NC, then: A. The expression of miR-133b was detected by QPCR. B. Cell colony formation ability was determined by colony formation assay. C. Cell viability was determined by CCK-8 assay. D, E. Cell cycle and apoptosis were evaluated by flow cytometry with PI staining and Annexin V/PI double staining, respectively. F. Cell invasion was assessed by transwell chamber with Matrigel coated. (n=3, $^{*}P<0.05$, $^{**}P<0.01$).

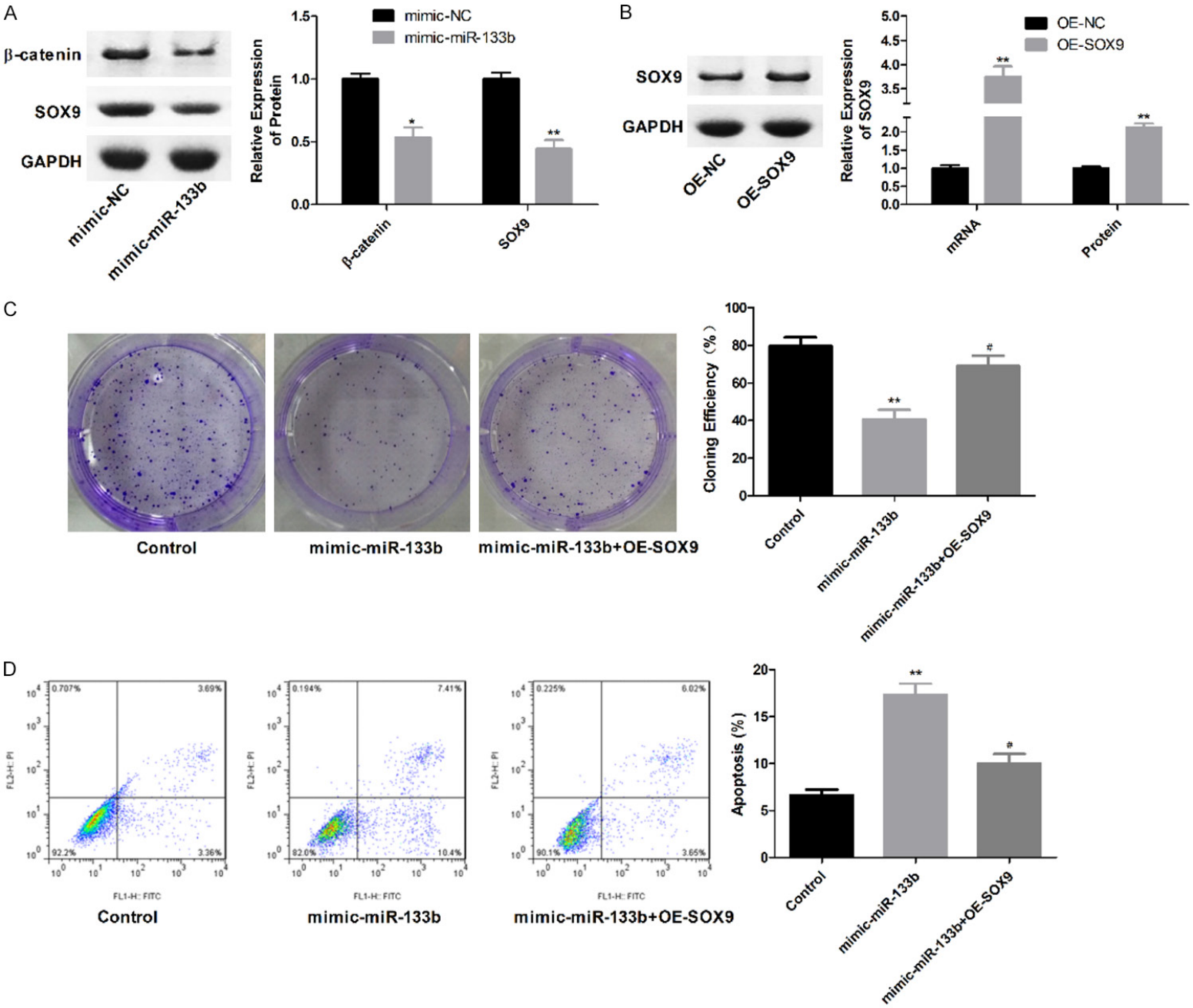
A
β-catenin
SOX9
GAPDH
mimic-NC
mimic-miR-133b
Relative Expression of Protein
mimic-NC
mimic-miR-133b
β-catenin
SOX9
B
SOX9
GAPDH
OE-NC
OE-SOX9
Relative Expression of SOX9
OE-NC
OE-SOX9
mRNA
Protein
C
Control
mimic-miR-133b
mimic-miR-133b+OE-SOX9
Cloning Efficiency (%)
Control
mimic-miR-133b
mimic-miR-133b+OE-SOX9
D
Control
mimic-miR-133b
mimic-miR-133b+OE-SOX9
Apoptosis (%)
Control
mimic-miR-133b
mimic-miR-133b+OE-SOX9

Figure 3. miR-133b repressed the progression of lung cancer through down-regulating β-catenin and SOX9 expression in H1299 cells. A. Western blot analysis of the protein expression of β-catenin and SOX9 after 48 h of H1299 cells infection with mimic-NC or mimic-miR-133b (n=3, $^{*}P<0.05$, $^{**}P<0.01$). B. Western blot analysis of the transfection efficiency of OE-SOX9 (n=3, $^{**}P<0.01$). C. Cell colony formation ability was determined by colony formation assay after H1299 cells were treated with mimic-miR-133b or mimic-miR-133b + OE-SOX9. D. Cell apoptosis was evaluated by flow cytometry after H1299 cells were treated with mimic-miR-133b or mimic-miR-133b + OE-SOX9. (n=3, $^{**}P<0.01$, compared with control group; $^{\#}P<0.05$, compared with mimic-miR-133b group).

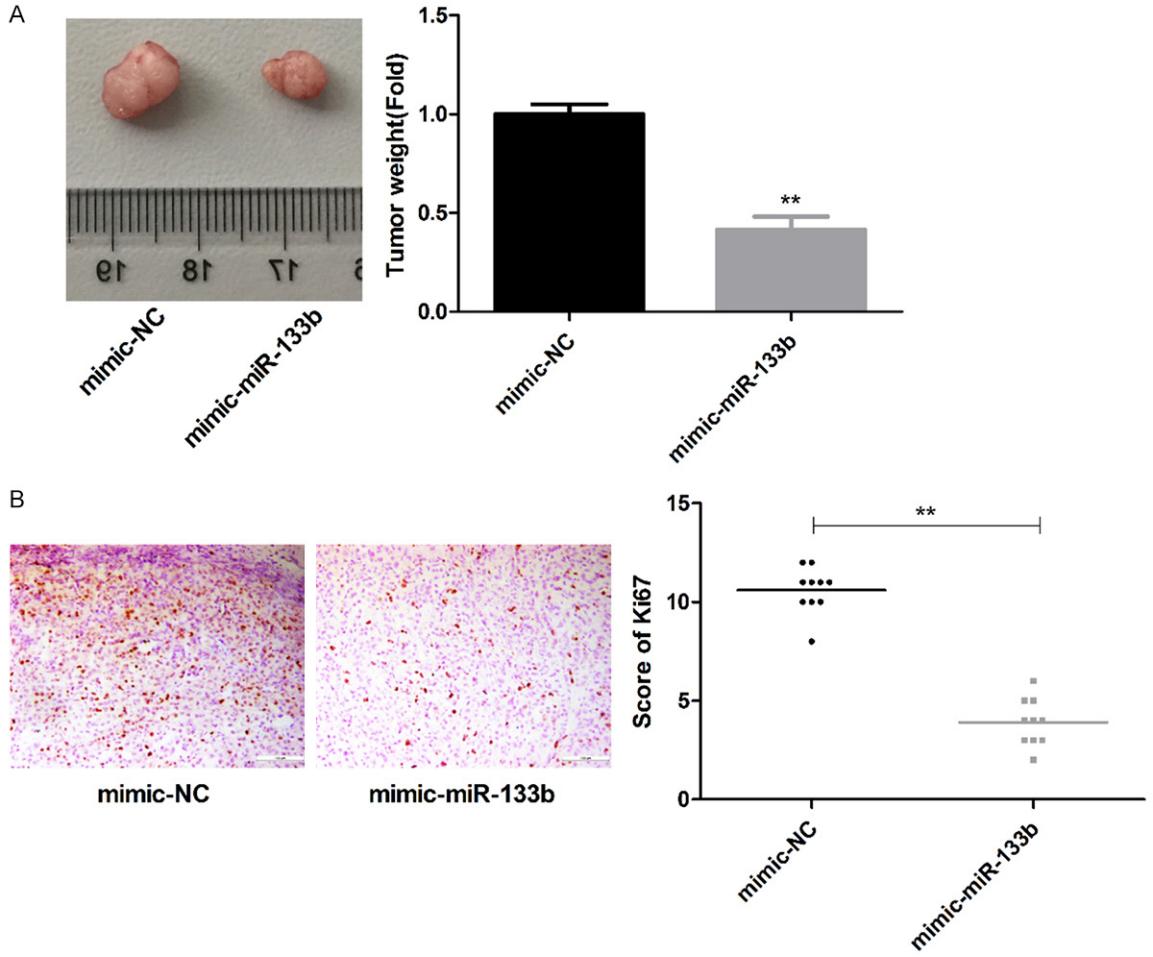

Figure 4. miR-133b reduced the tumor formation ability of lung cancer cells *in vivo*. H1299 cells were stably transfected with mimic-miR-133b or mimic-NC, then these stably transfected cell lines were injected into nude mice to test their tumorigenesis. A. Tumor weights were measured. B. The expression of Ki-67 in tumor tissues was evaluated by immunohistochemistry (n=10 $^{**}P<0.01$).

negative regulation of SOX9/β-catenin signaling.

miR-133b up-regulation represses the tumorigenesis of lung cells in vivo

Subsequently, we used a mice tumor-bearing assay to investigate the role of miR-133b in lung cancer. Compared with the control group, up-regulation of miR-133b decreased the tumorigenesis of H1299 cells with smaller tumor size and weight (**Figure 4A**) and decreased expression of Ki-67 in tumor tissues (**Figure 4B**).

Discussion

miR-133b, a muscle-specific microRNA, appears to exert different roles in different kinds of cancer cells. On one hand, low expression of miR-133b is associated with shorter survival and metastasis rate and functions as a tumor

repressor in colorectal cancer [16-18], gastric cancer [19, 20], bladder cancer [21], ovarian cancer [22], breast cancer [15], as well as lung cancer [23, 24]. However, Li et al. [25] revealed that miR-133b improved tumor-promoting properties in less aggressive prostate cancer LNCaP cells, whereas it acted as a tumor suppressor in more aggressive prostate cancer PC-3 cells. In the present study, we showed that miR-133b was down-regulated in lung cancer cell lines and tissues as compared with the normal lung epithelial cell line BEAS-2B and the para-carcinoma normal lung tissues. Up-regulation of miR-133b to lung cancer H1299 cells significantly reduced cell colony formation, growth, invasion, and tumorigenesis capacities while increasing the cell apoptosis rate and induced G0/G1 phase increase and S phase reduction, suggesting that miR-133b served as a tumor suppressor in lung cancer progression, which was consistent with previous studies [23, 24]. Moreover, a study also indicated that high expression of miR-133b was also associated with high chemosensitivity of NSCLC patients to erlotinib in the second or third line [26].

To further explore the molecular mechanisms underlying miR-133b in lung cancer, we asked whether SOX9/β-catenin signaling was involved in miR-133b-mediated the inhibition of lung cancer progression. Results showed that the expression of β-catenin and SOX9 were all decreased when miR-133b was up-regulated in H1299 cells. Additionally, we found that SOX9 up-regulation abolished the roles of miR-133b up-regulation in the repression of cell colony formation and the enhancement of cell apoptosis, demonstrating that miR-133b repressed the progression of lung cancer in a SOX9 down-regulation-dependent manner. SOX9 has been identified to play an important role in lung development, as Schafer et al. [27] demonstrated that infants with mutant SOX9 usually die from respiratory distress shortly after birth and Li et al. [28] revealed that SOX9 was required for lung function recovery after acute lung injury. Until now, SOX9 has been shown to be deregulated and involved in the carcinogenesis of many kinds of cancers. In most cancers such as prostate [29], esophageal [30], colorectal [31], gastric [32] and lung cancers [9, 33], SOX9 is characterized to be an oncogene. The high expression of SOX9 predicts a shorter overall survival of lung cancer patients [8]. However, SOX9 functions as a tumor suppressor in melanoma and endometrial carcinoma [34, 35], suggesting that SOX9 roles in tumorigenesis are dependent on cell contents. Moreover, SOX9 positively regulates the expression of low density lipoprotein receptor-related protein 6 (LRP6) and T-cell factor 4 (TCF4), two major components of the Wnt/β-catenin pathway, then supports Wnt/β-catenin activity [36]. In lung cancer, SOX9 significantly enhanced A549 cell proliferation and reduced cell apoptosis through activating Wnt/β-catenin signaling [10], suggesting that SOX9/β-catenin signaling plays a key role in lung cancer progression.

Collectively, this study clarifies that miR-133b exerts a tumor-inhibiting role in lung cancer through inactivating SOX9/β-catenin signaling.

Acknowledgements

This study was funded by the Key Project of Ganzhou Science and Technology Bureau (No. GZ2015ZSF205). The experiment protocols involving human or animal samples were approved by the Animal Experimentation Ethics Committee of Ganzhou Peoples Hospital.

Disclosure of conflict of interest

None.

Address correspondence to: Hui Sun, Department of Cardiothoracic Surgery, Ganzhou Peoples Hospital, Ganzhou 341000, Jiangxi, China. Tel: +86-15297772820; E-mail: Liaoyongbin711@163.com

References

[1] McGuire S. World Cancer Report 2014. Geneva, Switzerland: World Health Organization, international agency for research on cancer, WHO Press, 2015. Adv Nutr 2016; 7: 418-419.

[2] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[3] Krol J, Loedige I and Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010; 11: 597-610.

[4] Del Vescovo V and Denti MA. microRNA and lung cancer. Adv Exp Med Biol 2015; 889: 153-177.

[5] Yang X, Lei P, Huang Y, Zhang Z and Zhang Y. MicroRNA-133b inhibits the migration and invasion of non small cell lung cancer cells by

targeting FSCN1. Oncol Lett 2016; 12: 3619-3625.
[6] Liu L, Shao X, Gao W, Zhang Z, Liu P, Wang R, Huang P, Yin Y and Shu Y. MicroRNA-133b inhibits the growth of non-small-cell lung cancer by targeting the epidermal growth factor receptor. FEBS J 2012; 279: 3800-3812.
[7] Marshall OJ and Harley VR. Molecular mechanisms of SOX9 action. Mol Genet Metab 2000; 71: 455-462.
[8] Zhou CH, Ye LP, Ye SX, Li Y, Zhang XY, Xu XY and Gong LY. Clinical significance of SOX9 in human non-small cell lung cancer progression and overall patient survival. J Exp Clin Cancer Res 2012; 31: 18.
[9] Wang X, Ju Y, Zhou MI, Liu X and Zhou C. Upregulation of SOX9 promotes cell proliferation, migration and invasion in lung adenocarcinoma. Oncol Lett 2015; 10: 990-994.
[10] Guo YZ, Xie XL, Fu J and Xing GL. SOX9 regulated proliferation and apoptosis of human lung carcinoma cells by the Wnt/beta-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2018; 22: 4898-4907.
[11] Stewart DJ. Wnt signaling pathway in non-small cell lung cancer. J Natl Cancer Inst 2014; 106: djt356.
[12] Jamieson C, Sharma M and Henderson BR. Targeting the beta-catenin nuclear transport pathway in cancer. Semin Cancer Biol 2014; 27: 20-29.
[13] Kimelman D and Xu W. beta-catenin destruction complex: insights and questions from a structural perspective. Oncogene 2006; 25: 7482-7491.
[14] Xie XM, Zhang ZY, Yang LH, Yang DL, Tang N, Zhao HY, Xu HT, Li QC and Wang EH. Aberrant hypermethylation and reduced expression of disabled-2 promote the development of lung cancers. Int J Oncol 2013; 43: 1636-1642.
[15] Wang QY, Zhou CX, Zhan MN, Tang J, Wang CL, Ma CN, He M, Chen GQ, He JR and Zhao Q. MiR-133b targets Sox9 to control pathogenesis and metastasis of breast cancer. Cell Death Dis 2018; 9: 752.
[16] Akcakaya P, Ekelund S, Kolosenko I, Caramuta S, Ozata DM, Xie H, Lindforss U, Olivecrona H and Lui WO. miR-185 and miR-133b deregulation is associated with overall survival and metastasis in colorectal cancer. Int J Oncol 2011; 39: 311-318.
[17] Hu G, Chen D, Li X, Yang K, Wang H and Wu W. miR-133b regulates the MET proto-oncogene and inhibits the growth of colorectal cancer cells in vitro and in vivo. Cancer Biol Ther 2010; 10: 190-197.
[18] Duan FT, Qian F, Fang K, Lin KY, Wang WT and Chen YQ. miR-133b, a muscle-specific microRNA, is a novel prognostic marker that participates in the progression of human colorectal cancer by regulation of CXCR4 expression. Mol Cancer 2013; 12: 164.
[19] Wen D, Li S, Ji F, Cao H, Jiang W, Zhu J and Fang X. miR-133b acts as a tumor suppressor and negatively regulates FGFR1 in gastric cancer. Tumour Biol 2013; 34: 793-803.
[20] Zhao Y, Huang J, Zhang L, Qu Y, Li J, Yu B, Yan M, Yu Y, Liu B and Zhu Z. MiR-133b is frequently decreased in gastric cancer and its overexpression reduces the metastatic potential of gastric cancer cells. BMC Cancer 2014; 14: 34.
[21] Chen XN, Wang KF, Xu ZQ, Li SJ, Liu Q, Fu DH, Wang X and Wu B. MiR-133b regulates bladder cancer cell proliferation and apoptosis by targeting Bcl-w and Akt1. Cancer Cell Int 2014; 14: 70.
[22] Yang L, Hou J, Cui XH, Suo LN and Lv YW. MiR-133b regulates the expression of CTGF in epithelial-mesenchymal transition of ovarian cancer. Eur Rev Med Pharmacol Sci 2017; 21: 5602-5609.
[23] Zhen Y, Liu J, Huang Y, Wang Y, Li W and Wu J. miR-133b inhibits cell growth, migration, and invasion by targeting MMP9 in non-small cell lung cancer. Oncol Res 2017; 25: 1109-1116.
[24] Xia H, Jing H, Li Y and Lv X. Long noncoding RNA HOXD-AS1 promotes non-small cell lung cancer migration and invasion through regulating miR-133b/MMP9 axis. Biomed Pharmacother 2018; 106: 156-162.
[25] Li X, Wan X, Chen H, Yang S, Liu Y, Mo W, Meng D, Du W, Huang Y, Wu H, Wang J, Li T and Li Y. Identification of miR-133b and RB1CC1 as independent predictors for biochemical recurrence and potential therapeutic targets for prostate cancer. Clin Cancer Res 2014; 20: 2312-2325.
[26] Bisagni A, Pagano M, Maramotti S, Zanelli F, Bonacini M, Tagliavini E, Braglia L, Paci M, Mozzarelli A and Croci S. Higher expression of miR-133b is associated with better efficacy of erlotinib as the second or third line in non-small cell lung cancer patients. PLoS One 2018; 13: e0196350.
[27] Schafer AJ, Dominguez-Steglich MA, Guioli S, Kwok C, Weller PA, Stevanovic M, Weissenbach J, Mansour S, Young ID, Goodfellow PN, et al. The role of SOX9 in autosomal sex reversal and campomelic dysplasia. Philos Trans R Soc Lond B Biol Sci 1995; 350: 271-277; discussion 277-278.
[28] Li L, Zhang H, Min D, Zhang R, Wu J, Qu H and Tang Y. Sox9 activation is essential for the recovery of lung function after acute lung injury. Cell Physiol Biochem 2015; 37: 1113-1122.
[29] Huang Z, Hurley PJ, Simons BW, Marchionni L, Berman DM, Ross AE and Schaeffer EM. Sox9

is required for prostate development and prostate cancer initiation. Oncotarget 2012; 3: 651-663.
[30] Song S, Ajani JA, Honjo S, Maru DM, Chen Q, Scott AW, Heallen TR, Xiao L, Hofstetter WL, Weston B, Lee JH, Wadhwa R, Sudo K, Stroehlein JR, Martin JF, Hung MC and Johnson RL. Hippo coactivator YAP1 upregulates SOX9 and endows esophageal cancer cells with stem-like properties. Cancer Res 2014; 74: 4170-4182.
[31] Qian Y, Xia S and Feng Z. Sox9 mediated transcriptional activation of FOXK2 is critical for colorectal cancer cells proliferation. Biochem Biophys Res Commun 2017; 483: 475-481.
[32] Sun M, Uozaki H, Hino R, Kunita A, Shinozaki A, Ushiku T, Hibiya T, Takeshita K, Isogai M, Takada K and Fukayama M. SOX9 expression and its methylation status in gastric cancer. Virchows Arch 2012; 460: 271-279.
[33] Jiang SS, Fang WT, Hou YH, Huang SF, Yen BL, Chang JL, Li SM, Liu HP, Liu YL, Huang CT, Li YW, Jang TH, Chan SH, Yang SJ, Hsiung CA, Wu CW, Wang LH and Chang IS. Upregulation of SOX9 in lung adenocarcinoma and its involvement in the regulation of cell growth and tumorigenicity. Clin Cancer Res 2010; 16: 4363-4373.
[34] Passeron T, Valencia JC, Namiki T, Vieira WD, Passeron H, Miyamura Y and Hearing VJ. Upregulation of SOX9 inhibits the growth of human and mouse melanomas and restores their sensitivity to retinoic acid. J Clin Invest 2009; 119: 954-963.
[35] Saegusa M, Hashimura M, Suzuki E, Yoshida T and Kuwata T. Transcriptional up-regulation of Sox9 by NF-kappaB in endometrial carcinoma cells, modulating cell proliferation through alteration in the p14(ARF)/p53/p21(WAF1) pathway. Am J Pathol 2012; 181: 684-692.
[36] Wang H, He L, Ma F, Regan MM, Balk SP, Richardson AL and Yuan X. SOX9 regulates low density lipoprotein receptor-related protein 6 (LRP6) and T-cell factor 4 (TCF4) expression and Wnt/beta-catenin activation in breast cancer. J Biol Chem 2013; 288: 6478-6487.