Am J Cancer Res 2016;6(5):1099-1107
www.ajcr.us /ISSN:2156-6976/ajcr0024057

Original Article

LncRNA MALAT1 exerts oncogenic functions in lung adenocarcinoma by targeting miR-204

Jipeng Li[1], Jianhua Wang[1], Yin Chen[1], Shanfeng Li[1], Mingwei Jin[1], Huaying Wang[2], Zhe Chen[1], Wanjun Yu[2]

Departments of [1]Central Laboratory, [2]Respiratory and Critical Care Medicine, Yinzhou People's Hospital, Ningbo 315040, China

Received January 14, 2016; Accepted February 29, 2016; Epub May 1, 2016; Published May 15, 2016

Abstract: Accumulating evidence indicates that the lncRNAs play a critical role in cancer progression and metastasis. In this study, we found that MALAT1 upregulation was associated with larger tumor size and lymph-node metastasis, and also correlated with shorter overall survival of lung adenocarcinoma patients. Furthermore, MALAT1 promotes EMT and metastasis of lung adenocarcinoma cells in vitro and in vivo. In particular, MALAT1 upregulated the expression of miR-204 target gene SLUG through competitively 'spongeing' miR-204. In summary we unveil a branch of the MALAT1/miR-204/SLUG pathway that regulates the progression of lung adenocarcinoma.

Keywords: Lung adenocarcinoma, MALAT1, miR-204, SLUG

Introduction

Lung cancer is the most common cancer worldwide in terms of both incidence and mortality [1], with very poor prognosis and high possibilities of tumor invasion and migration. Although invasion and migration have been acknowledged as the most lethal attributes of solid tumors, the molecular mechanism underlying them is still limited. Recent researches have demonstrated that epithelial-to-mesenchymal transition (EMT) is a key process contributing to cancer metastasis, characterized by the loss of the epithelial marker E-cadherin, an increase in the mesenchymal markers vimentin and N-cadherin, and an increase in the migratory and invasive behavior [2, 3].

Long noncoding RNAs (lncRNAs) are a class of transcripts longer than 200 nucleotides with limited protein coding ability [4]. Recently, many studies have shown that lncRNAs are frequently dysregulation in various tumors and have multiple functions in a wide range of biological processes, such as the proliferation, apoptosis, cell cycle arrest or cell migration and invasion [5, 6]. LncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) located on chromosome 11q13 has been identified to be involved in tumorigenesis of several cancers such as lung cancer, pancreatic cancer, and cervical cancer [7-12]. Hiroshi Hirata et al also have found that long noncoding RNA MALAT1 promotes aggressive renal cell carcinoma through Ezh2 and interacts with miR-205 [8]. However, how MALAT1 function in lung adenocarcinoma pathogenesis remains largely unknown.

In this study, we found MALAT1 is upregulated in lung adenocarcinoma. Functional assays demonstrated that MALAT1 promotes invasion and EMT in lung adenocarcinoma cells. Particularly, mechanistic analysis reveals that MALAT1 may function as a competing endogenous RNA (ceRNA) to regulate the expression of SLUG through competition for miR-204, thus playing an oncogenic role in lung pathogenesis. Together, these data contribute to the characterization of the molecular mechanisms of lung adenocarcinoma progression.

Materials and methods

Tissue collection

Fresh-frozen and paraffin-embedded lung adenocarcinoma tissues and corresponding adja-

cent non-tumorous lung samples were obtained from Chinese patients at Yinzhou People's Hospital (Ningbo, China) between January 2009 and March 2010. Informed consent was obtained from all subjects and this study was approved by the Review Board of Hospital Ethics Committee. All cases were reviewed by pathologist and histologically confirmed as lung adenocarcinoma based on histopathological evaluation.

Cell culture and transfection

The lung adenocarcinoma cell lines, including A549, H1299, H460, and H446, and one cultured human lung epithelial cell (BEAS-2B), were grown in Dulbecco's modified Eagle medium (DMEM) (GIBCO BRL, Grand Island, NY) containing 10% fetal bovine serum (FBS) with 100 μg/ml penicillin/streptomycin at 37°C with 5% CO_2. miR-204 mimics, negative control mimics (NC), miR-204 inhibitors (anti-miR-204) and negative control inhibitors (anti-NC) were synthesized by GenePharma Company (Shanghai, China). Transfection was performed with Lipofectamine 2000 (Invitrogen, CA, USA) according to the manufacturer's protocol. Total RNA and protein were prepared 48 h after transfection and were used for qRT-PCR or western blot analysis.

RNA extraction and qRT-PCR

Total RNA was extracted from tissues or cultured cells using TRIZOL reagent (Invitrogen, Carlsbad, Calif). For qRT-PCR, RNA was reverse transcribed to cDNA by using a Reverse Transcription Kit (Takara, Dalian, China). Real-time PCR analyses were performed with Power SYBR Green (Takara, Dalian China). Results were normalized to the expression of GAPDH. For miR-204 expression detection, reverse transcription was performed following Applied Biosystems TaqMan MicroRNA Assay protocol, U6 snoRNA was validated as the normalizer. The primers were listed in Supplementary Table S1. qRT-PCR and data collection were performed on ABI 7500.

Vector construction

To construct luciferase reporter vectors, MALAT1 cDNA fragment containing the predicted potential miR-204 binding site was amplified by PCR, and then subcloned downstream of the luciferase gene in the pGL3-control vector (Promega, Madison, WI). The primers were listed in Supplementary Table S1. MALAT1 knockdown was performed using lentivirus-encoded short hairpin RNA (shRNA), the shMALAT1 and shCtrl targeting sequences were GGGCTTCTCTTAACATTTA and TTCTCCGAACGTGTCACGT, respectively.

Western blot and immunohistochemistry analysis

Protein extracts were prepared by a modified RIPA buffer with 0.5% sodium dodecyl sulfate (SDS) in the presence of proteinase inhibitor cocktail (Complete mini, Roche, Indianapolis, IN, USA). Polyacrylamide gel electrophoresis, tank-based transfer to Immobilon Hybond-C membranes (Amersham Biosciences) and immunodetection were performed with standard techniques. For immunohistochemistry analysis, Tumors from mice were immunostained, the signal was amplified and visualized with 3'-diaminobenzidine chromogen, followed by counterstaining with hematoxylin. Antibodies against SLUG (Novus Biologicals, CO, USA), β-actin (Beijing Zhongshan Biotechnology, Beijing, China), E-cadherin, N-cadherin and vimentin (Santa Cruz, CA) were used in western and immunohistochemistry analysis in accordance with the manufacturer's instruction.

Luciferase assay

H1299 cells were transfected in 24-well plates using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). The transfection mixtures contained 100 ng of firefly luciferase reporter plasmid and 5 pmol of miRNAs. pRL-TK (Promega, Madison, WI) was also transfected as a normalisation control. Cells were collected 48 h after transfection, and luciferase activity was measured using a dual-luciferase reporter assay system (Promega, Madison, WI).

RNA-binding protein immunoprecipitation (RIP) assay

RNA-binding protein immunoprecipitation (RIP) assay was performed using the EZ-Magna RIP Kit (Millipore, Billerica, MA, USA) according to the manufacturer's instruction. Briefly, cells were lysed with the use of RIP lysis buffer, followed by incubated with RIP buffer containing magnetic beads conjugated with human anti-Ago2 antibody (Millipore) or negative control Normal Mouse IgG (Millipore). Proteinase K was

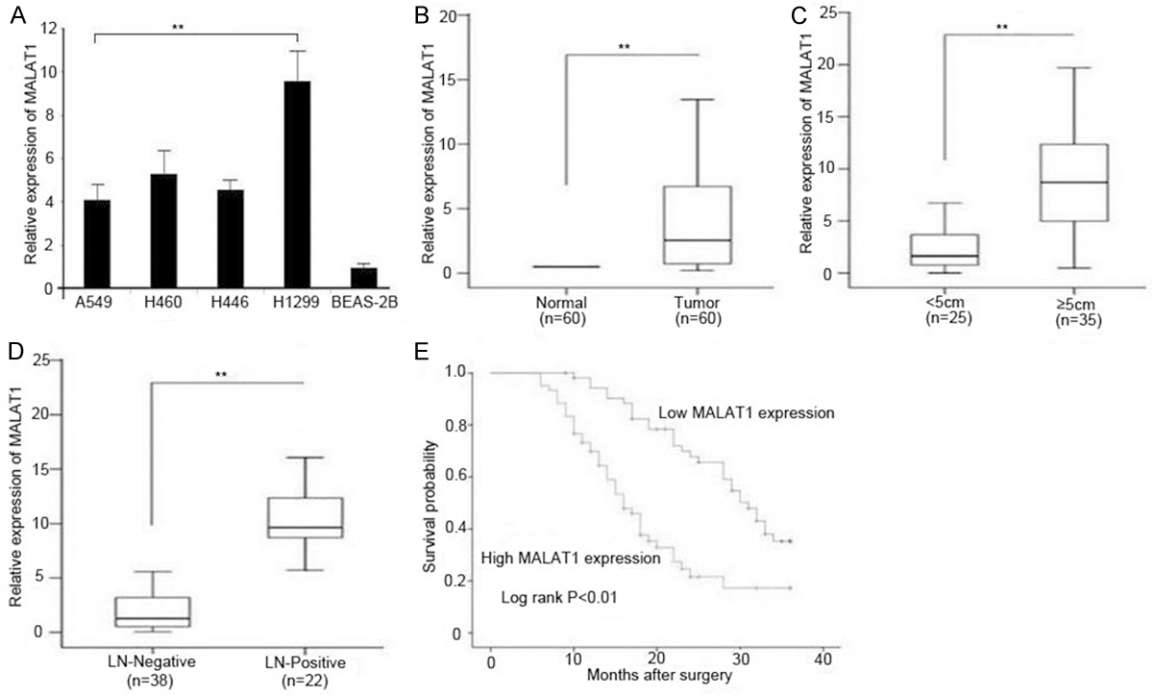

Figure 1. MALAT1 expression was up-regulated in lung adenocarcinoma cells and tissues. (A) Expression of MALAT1 in four lung adenocarcinoma cells and one cultured human lung epithelial cells. (B) MALAT1 relative expression levels were determined in 60 paired lung adenocarcinoma and their corresponding normal samples. Upregulation of MALAT1 in lung adenocarcinoma was associated with tumor size (C) and lymph-node metastasis (D). Lung adenocarcinoma patients with higher expression of MALAT1 had shorter overall survival time than those with lower expression of MALAT1 (E). $**P < 0.01$.

used to digest the protein and then immunoprecipitated RNA was isolated. NanoDrop spectrophotometer was used to measure the RNA concentration. Purified RNA was subjected to qRT-PCR analysis.

Cell invasion assay

For the invasion assays, 2×10^5 cells were added into the upper chamber of the insert precoated with Matrigel (ECM gel, Sigma-Aldrich, St. Louis, MO). Cells were plated in medium without serum, and medium containing 10% fetal bovine serum in the lower chamber served as chemoattractant. After several hours of incubation, the cells that did not invade through the pores were carefully wiped out with cotton wool. Then the inserts were fixed with 95% ethanol, stained with 0.2% crystal violet, and counted (five high-power fields/chamber) using an inverted microscope.

In vivo metastasis assay

For in vivo metastasis assays, H1299 cells infected with either the shMALAT1 lentivirus or the mock lentivirus (shCtrl) were transplanted into nude mice (five-week-old BALB/c-nu/nu, six per group, 1×10^6 cells for each mouse) through the lateral tail vein. After 7 weeks, mice were sacrificed. Their lungs were removed and subjected to hematoxylin & eosin (H&E) staining. All research involving animal complied with protocols approved by the Zhejiang Medical Experimental Animal Care Commission.

Statistical analysis

Statistical analyses were performed using SPSS 16.0 software (SPSS Inc.). Data were presented as mean ± SD from at least three separate experiments. Overall survival rates were calculated actuarially according to the Kaplan-Meier method and survival curves were plotted. The relationship between MALAT1 and miR-204 or SLUG expressions was tested with two-tailed Pearson's correlation. $P < 0.05$ was considered statistically significant.

Results

MALAT1 expression is upregulated in human lung adenocarcinoma cells and tissues

To explore whether MALAT1 is detectable and altered in lung adenocarcinoma, qRT-PCR was

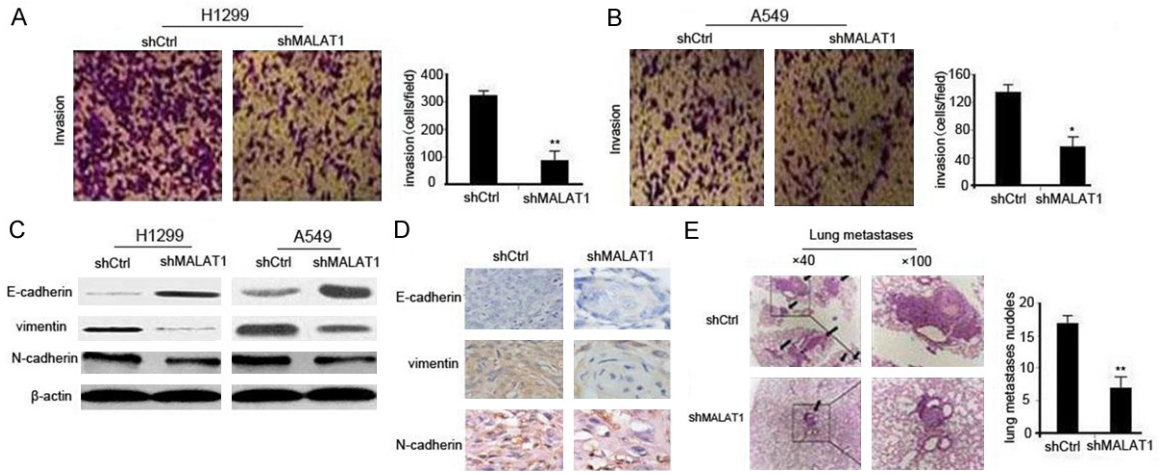

Figure 2. Knockdown of MALAT1 inhibited cell EMT and metastasis in vitro and vivo. Knockdown of MALAT1 significantly impeded abilities of cell invasion in H1299 (A) and A549 (B) cells after infection with shMALAT1 or shCtrl lentivirus. (C) Western blotting was used to detect E-cadherin, vimentin and N-cadherin expression, β-actin was used as an internal control. Representative immunohistochemistry (D) and H&E (E) stained sections of the lung tissues isolated from mice that injected with H1299-shCtrl or H1299-shMALAT1 cells through the lateral tail vein. Arrow head points to the tumor focus formed in the lung. The numbers of metastases in the lungs were counted. $*P < 0.05$, $**P < 0.01$.

used to detect the expression of MALAT1 in four lung cancer cell lines. As shown in **Figure 1A**, MALAT1 expression was significantly higher in all four lung cancer cell lines compared with that in human lung epithelial cells. We then examined the expression of MALAT1 in 60 paired lung adenocarcinoma samples and adjacent normal lung tissues. As presented in **Figure 1B**, MALAT1 expression was significantly upregulated in cancerous tissues compared with normal counterparts. Examination of the correlation between MALAT1 expression and clinical pathological features showed that MALAT1 upregulation was correlated with larger tumor size and lymph-node metastasis (**Figure 1C** and **1D**), With regard to overall survival, patients with high MALAT1 expression had a significantly poorer prognosis than those with low MALAT1 expression (**Figure 1E**). These results imply that MALAT1 overexpression may be useful in the development of novel prognostic or progression markers for lung adenocarcinoma.

Knockdown of MALAT1 inhibited cell EMT and metastasis in vitro and vivo

To investigate the potential role of MALAT1 on lung adenocarcinoma cells, A549 and H1299 cells were selected for next study. We stably inhibited MALAT1 in two lung adenocarcinoma cell lines H1299 and A549 with lentiviruses carrying shRNA for MALAT1 and a control non-specific shRNA (shCtrl). The invasion assay showed that knockdown of MALAT1 significantly inhibited the invasion of lung adenocarcinoma cells compared with the control (**Figure 2A** and **2B**). Since EMT is well known to be involved in invasion of cancer cells, we assessed the EMT markers by western blot. Interestingly, we found that the expression level of E-cadherin increased, while the levels of N-cadherin and vimentin decreased, when MALAT1 was inhibited in lung adenocarcinoma cells (**Figure 2C**). Next, H1299 stably expressing shMALAT1 were delivered into nude mice by tail vein injection. Histological analysis confirmed that E-cadherin expression increased, while N-cadherin and vimentin decreased (**Figure 2D**). Moreover, the total signal of metastatic nodules in the lung was dramatically decreased in shMALAT1 groups when compared with shCtrl group (**Figure 2E**). Taken together, these results suggest that the level of MALAT1 expression is significantly associated with metastasis capacity of lung adenocarcinoma.

MALAT1 is a target of miR-204

Bioinformatics analysis of miRNA recognition sequences on MALAT1 revealed the presence of miR-204 binding site (**Figure 3A**). To further

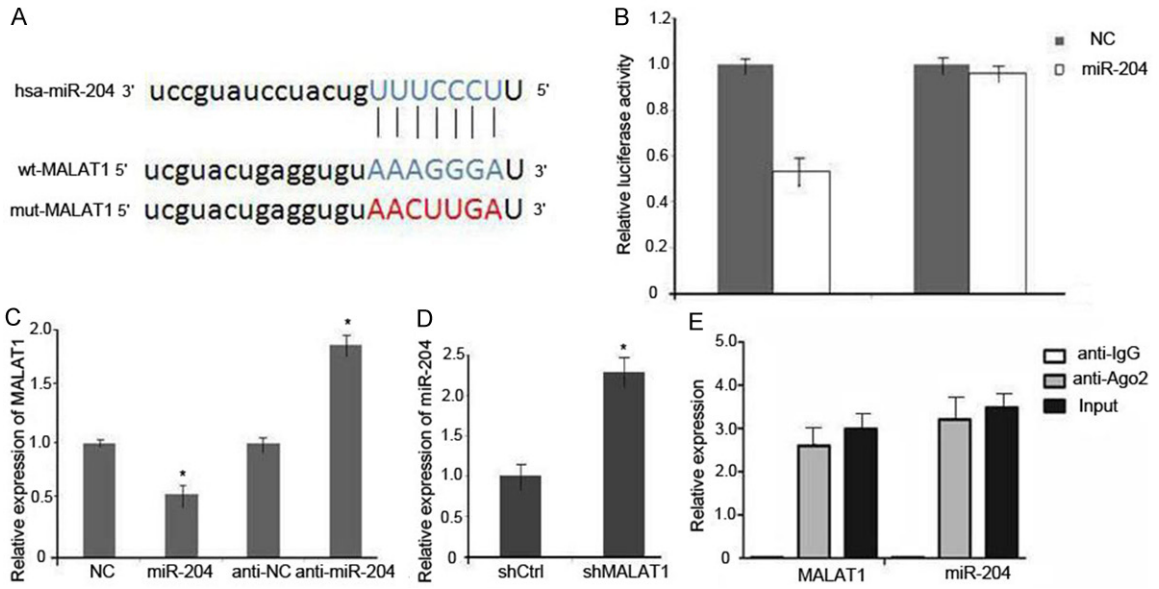

Figure 3. MALAT1 is a target of miR-204. A. The binding site of miR-204 on MALAT1. B. Co-transfection of miR-204 and wt-MALAT1 strongly decreased the luciferase activity, while co-transfection of miR-204 and mut-MALAT1 did not change the luciferase activity. C. MiR-204 negatively regulated MALAT1 expression. D. Knockdown of MALAT1 increased miR-204 expression. E. MALAT1 and miR-204 were enriched in Ago2 immunoprecipitates relative to control IgG immunoprecipitates. *$P < 0.05$.

investigate whether MALAT1 was a functional target of miR-204, we cloned reporter plasmid containing the predicted miR-204 binding site (wt-MALAT1) and plasmid that the miR-204 seed region binding site was mutated (mut-MALAT1). The results showed that co-transfection of miR-204 and wt-MALAT1 strongly decreased the luciferase activity, while co-transfection of miR-204 and mut-MALAT1 did not change the luciferase activity (**Figure 3B**). We next assessed whether miR-204 was able to negatively regulate MALAT1 expression. As shown in **Figure 3C**, the MALAT1 expression was decreased in miR-204 mimics group, whereas that in anti-miR-204 group was increased. To determine whether miR-204 is regulated by MALAT1, we detected the expression of miR-204 after knockdown of MALAT1. The results showed that the miR-204 expression was increased in shMALAT1 group compared with shctrl groups (**Figure 3D**). It is documented that miRNAs exert their gene silencing functions through a ribonucleoprotein complex called the RNA induced silencing complex (RISC). The core component of the RISC was Ago2 [13]. RIP experiments were performed to determine whether MALAT1 and miR-204 are in the same RISC complex. qRT-PCR was used to determine RNA levels in immunoprecipitates. We found that MALAT1 and miR-204 were enriched in Ago2 immunoprecipitates relative to control IgG immunoprecipitates (**Figure 3E**). These data indicated that both MALAT1 and miR-204 are probably in the same RISC complex, consistent with our bioinformatic analysis and luciferase assays.

miR-204 mediated the effect of MALAT1 on EMT and invasion

To dissect the importance of miR-204 binding in MALAT1-mediating lung adenocarcinoma progression, we treated A549 and H1299 cells stably expressing shMALAT1with anti-miR-204. As illustrated in **Figure 4A**, ectopic transfection of anti-miR-204 led to a dramatic decrease in E-cadherin expression and a significant increase in vimentin expression. Consistent with this, the effect of shMALAT1 was partially attenuated by miR-204 inhibitor on cell invasion (**Figure 4B**). These results showed that MALAT1 promotes tumor cell EMT and invasion in part via competitively binding miR-204.

MALAT1 modulated expression of endogenous miR-204 targets SLUG

Studies have reported that SLUG is a direct target of miR-204 in human intrahepatic cholan-

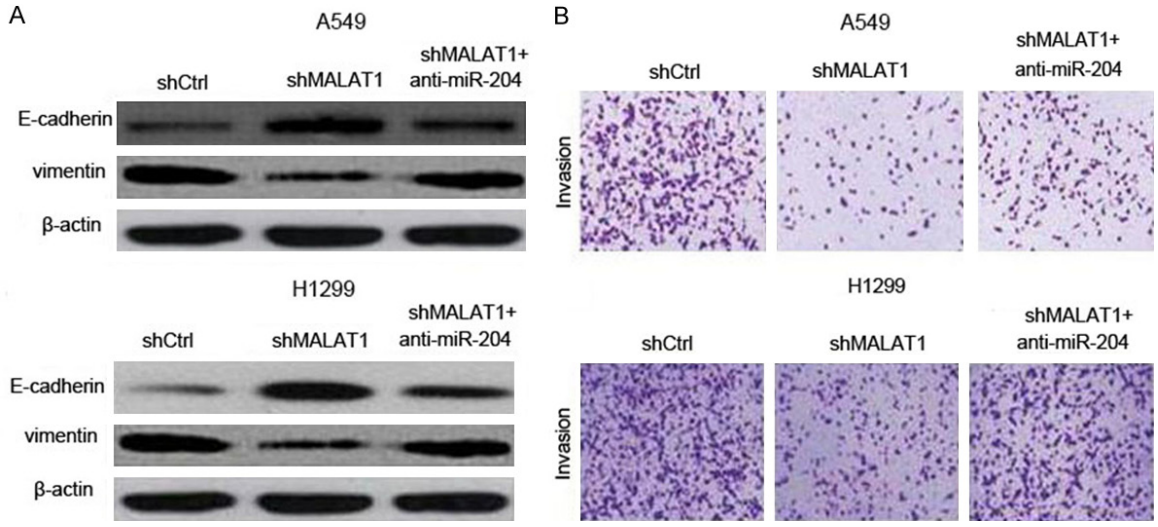

Figure 4. miR-204 mediated the effect of MALAT1 on EMT and invasion. The alter of EMT related markers (A) and invasion ability (B), caused by MALAT1 down-regulation, were rescued by anti-miR-204 treatment.

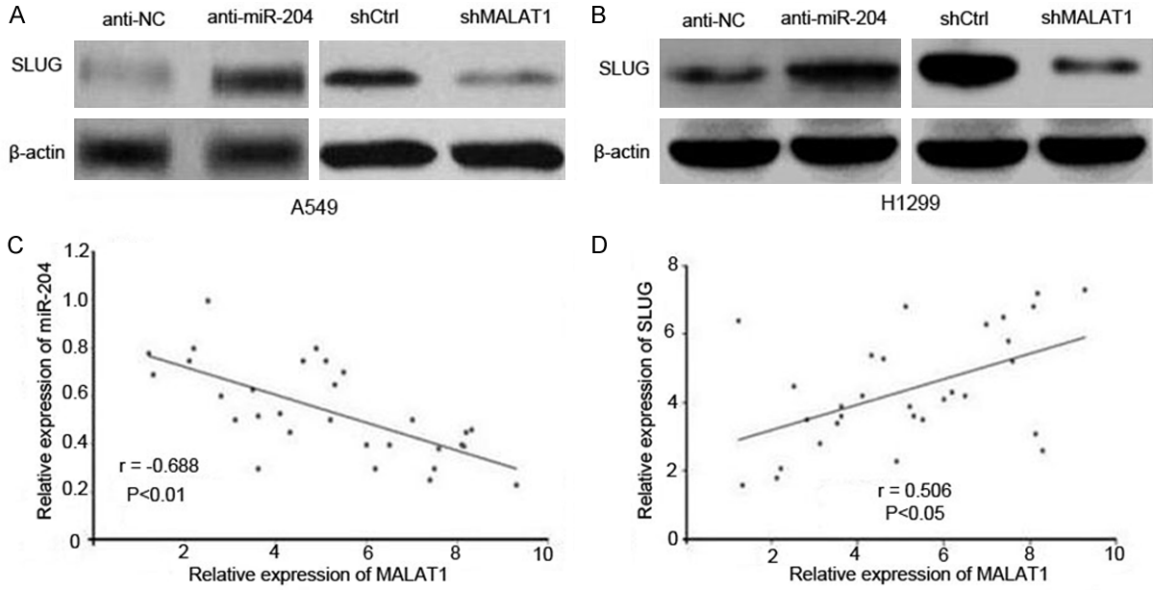

Figure 5. MALAT1 modulated expression of endogenous miR-204 targets SLUG. After transfected with anti-miR-204 or shMALAT1, the protein level of SLUG in A549 (A) and H1299 (B) cells was examined by western blot. Expression levels of MALAT1 were negative correlation with miR-204 (C) and positive correlation with SLUG mRNA (D) among lung adenocarcinoma samples (n = 30) as indicated by two-tailed Pearson's correlation analysis.

giocarcinoma [14]. To validate whether miR-204 targets SLUG in lung adenocarcinoma, western blot was carried out and showed that SLUG was increased in A549 and H1299 cells transfected with miR-204 inhibitor compared with the control. Moreover, the expression of SLUG protein were inhibited after transfected with shMALAT1 compared with the control (**Figure 5A** and **5B**). Furthermore, the expression of miR-204, MALAT1 and SLUG were examined in 30 tumor specimens that were used above. As shown in **Figure 5C**, MALAT1 was negative correlation with miR-204. On the contrary, SLUG mRNA expression was positive correlation with MALAT1 (**Figure 5D**).

Taken together, these results showed that MALAT1 eliminates the repression on SLUG

induced by miR-204 and exerts oncogenic functions by modulating miR-204/SLUG.

Discussion

Long non-coding RNA MALAT1, also known as nuclear-enriched abundant transcript 2 (NEAT2), is a highly expressed lncRNA in mammalian species [15]. Aberrant expression of MALAT1 was discovered in a group of human tumor tissues and many vital biological processes including tumor cell proliferation, apoptosis, invasion and metastasis are closely associated with MALAT1 [7-12]. Studies have shown that MALAT1 is upregulated in lung cancer and contributes to metastasis [10, 16, 17]. Nevertheless, tumorigenic properties and mechanistic heterogeneity of MALAT1 are far from being fully elucidated in lung cancer.

In this study, we tested the expression of MALAT1 in lung adenocarcinoma samples and their surrounding normal lung tissues. We also identified the function of MALAT1 in lung adenocarcinoma cells by applying loss-of-function approach. The results demonstrated that MALAT1 was upregulated in lung adenocarcinoma tissues, and that MALAT1 upregulation correlated with larger tumor size and lymph-node metastasis. Moreover, the overall survival time of patients with lower MALAT1 expression levels was significantly longer than that of patients with moderate or strong MALAT1 expression levels. Furthermore, knockdown of MALAT1 inhibited cell EMT and metastasis in vitro and in vivo. These findings suggest that MALAT1 plays a direct role in the modulation of oncogenic properties and lung adenocarcinoma progression.

Emerging evidence suggests that lncRNAs act as endogenous miRNA sponges to bind to miRNAs and regulate their function [18-21]. To find out whether MALAT1 serves as a miRNA sponge, we performed the bioinformatics analysis and found that MALAT1 contained binding site for miR-204. miR-204 is often down-regulated in cancer tissues and restoration of miR-204 suppressed cancer cell invasion by reversing the EMT phenotype [14, 22-24]. As expected, we discovered miR-204 could induce translational repression of a wt-MALAT1 reporter gene. Moreover, qRT-PCR analysis showed that miR-204 expression was inversely correlated with MALAT1 expression in lung adenocarcinoma. Furthermore, the effect of shMALAT1 was partially attenuated by miR-204 inhibitor on cell EMT and invasion. Taken together, these data are consistent with our hypothesis and indicate that MALAT1 may interact with miRNAs to link miRNAs and the post-transcriptional network in lung pathogenesis.

SLUG, a direct target of miR-204 [13], is known to play a diverse number of roles in the cell, and its deregulation has been observed in a variety of cancers including lung [25-27]. Recent reports highlighted that SLUG was closed related to EMT [28-30], suggesting SLUG is a key factor in promoting the initiation and development of cancer. Our data revealed that MALAT1 elevated SLUG expression by negatively regulating miR-204 expression. It is possible that the miR-204/SLUG axis mediated MALAT1 function on EMT and metastasis.

In summary, our findings confirmed that MALAT1 promoted lung adenocarcinoma cell EMT and metastasis. In addition, we demonstrated that MALAT1 negatively regulated miR-204 expression by acting as a miRNA sponge. Our findings might facilitate the development of lncRNA-directed diagnostics and therapeutics against lung adenocarcinoma.

Acknowledgements

This work was supported by Zhejiang Provincial Natural Science Foundation of China (Grant No. LY14H160002), Zhejiang Provincial Medicine and Health Science Research Foundation of China (Grant No. 2014KYB248) and Yinzhou Science and Technology bureau (2013-107).

Disclosure of conflict of interest

None.

Address correspondence to: Wanjun Yu, Department of Respiratory and Critical Care Medicine, Yinzhou People's Hospital, 251 East Baizhang Road, Ningbo 315040, China. Fax: + 86 574 87016948; E-mail: nbyuwanjun@163.com

References

[1] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62: 10-29.

[2] Kraljevic Pavelic S, Sedic M, Bosnjak H, Spaventi S, Pavelic K. Metastasis: new per-

spectives on an old problem. Mol Cancer 2011; 10: 22.
[3] Yap AS. The morphogenetic role of cadherin cell adhesion molecules in human cancer: a thematic review. Cancer Invest 1998; 16: 252-261.
[4] Yuan JH, Yang F, Wang F, Ma JZ, Guo YJ, Tao QF, Liu F, Pan W, Wang TT, Zhou CC, Wang SB, Wang YZ, Yang Y, Yang N, Zhou WP, Yang GS, Sun SH. A long noncoding RNA activated by TGF-beta promotes the invasion-metastasis cascade in hepatocellular carcinoma. Cancer Cell 2014; 25: 666-681.
[5] Mercer TR, Dinger ME, Mattick JS. Long noncoding RNAs: insights into functions. Nat Rev Genet 2009; 10: 155-159.
[6] Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes Dev 2009; 23: 1494-1504.
[7] Zhang HM, Yang FQ, Chen SJ, Che J, Zheng JH. Upregulation of long non-coding RNA MALAT1 correlates with tumor progression and poor prognosis in clear cell renal cell carcinoma. Tumour Biol 2015; 36: 2947-2955.
[8] Hirata H, Hinoda Y, Shahryari V, Deng G, Nakajima K, Tabatabai ZL, Ishii N, Dahiya R. Long noncoding RNA MALAT1 promotes aggressive renal cell carcinoma through Ezh2 and interacts with miR-205. Cancer Res 2015; 75: 1322-1331.
[9] Han Y, Liu Y, Zhang H, Wang T, Diao R, Jiang Z, Gui Y, Cai Z. Hsa-miR-125b suppresses bladder cancer development by down-regulating oncogene SIRT7 and oncogenic long noncoding RNA MALAT1. FEBS Lett 2013; 587: 3875-3882.
[10] Ji P, Diederichs S, Wang W, Böing S, Metzger R, Schneider PM, Tidow N, Brandt B, Buerger H, Bulk E, Thomas M, Berdel WE, Serve H, Müller-Tidow C. MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene 2003; 22: 8031-8041.
[11] Leucci E, Patella F, Waage J, Holmstrøm K, Lindow M, Porse B, Kauppinen S, Lund AH. microRNA-9 targets the long non-coding RNA MALAT1 for degradation in the nucleus. Sci Rep 2013; 3: 2535.
[12] Tripathi V, Shen Z, Chakraborty A, Giri S, Freier SM, Wu X, Zhang Y, Gorospe M, Prasanth SG, Lal A, Prasanth KV. Long noncoding RNA MALAT1 controls cell cycle progression by regulating the expression of oncogenic transcription factor B-MYB. PLoS Genet 2013; 9: e1003368.
[13] Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell 2005; 123: 631-640.
[14] Qiu YH, Wei YP, Shen NJ, Wang ZC, Kan T, Yu WL, Yi B, Zhang YJ. miR-204 inhibits epithelial to mesenchymal transition by targeting slug in intrahepatic cholangiocarcinoma cells. Cell Physiol Biochem 2013; 32: 1331-1341.
[15] Hutchinson JN, Ensminger AW, Clemson CM, Lynch CR, Lawrence JB, Chess A. A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains. BMC Genomics 2007; 8: 39.
[16] Gutschner T, Hämmerle M, Eissmann M, Hsu J, Kim Y, Hung G, Revenko A, Arun G, Stentrup M, Gross M, Zörnig M, MacLeod AR, Spector DL, Diederichs S. The non-coding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. Cancer Res 2013; 73: 1180-1189.
[17] Tano K, Mizuno R, Okada T, Rakwal R, Shibato J, Masuo Y, Ijiri K, Akimitsu N. MALAT-1 enhances cell motility of lung adenocarcinoma cells by influencing the expression of motility-related genes. FEBS Lett 2010; 584: 4575-4580.
[18] Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 2011; 146: 353-358.
[19] Wang J, Liu X, Wu H, Ni P, Gu Z, Qiao Y, Chen N, Sun F, Fan Q. CREB up-regulates long non-coding RNA, HULC expression through interaction with microRNA-372 in liver cancer. Nucleic Acids Res 2010; 38: 5366-5383.
[20] Wang Y, Xu Z, Jiang J, Xu C, Kang J, Xiao L, Wu M, Xiong J, Guo X, Liu H. Endogenous miRNA sponge lincRNA-RoR regulates Oct4, nanog, and Sox2 in human embryonic stem cell self-renewal. Dev Cell 2013; 25: 69-80.
[21] Liang WC, Fu WM, Wong CW, Wang Y, Wang WM, Hu GX, Zhang L, Xiao LJ, Wan DC, Zhang JF, Waye MM. The lncRNA H19 promotes epithelial to mesenchymal transition by functioning as miRNA sponges in colorectal cancer. Oncotarget 2015; 6: 22513-22525.
[22] Wu X, Zeng Y, Wu S, Zhong J, Wang Y, Xu J. MiR-204, down-regulated in retinoblastoma, regulates proliferation and invasion of human retinoblastoma cells by targeting CyclinD2 and MMP-9. FEBS Lett 2015; 589: 645-650.
[23] Shi L, Zhang B, Sun X, Lu S, Liu Z, Liu Y, Li H, Wang L, Wang X, Zhao C. MiR-204 inhibits human NSCLC metastasis through suppression of NUAK1. Br J Cancer 2014; 111: 2316-2327.
[24] Zhang L, Wang X, Chen P. MiR-204 down regulates SIRT1 and reverts SIRT1-induced epithelial-mesenchymal transition, anoikis resistance and invasion in gastric cancer cells. BMC Cancer 2013; 13: 290.
[25] Barrallo-Gimeno A, Nieto MA. The snail genes as inducers of cell movement and survival:

Implications in development and cancer. Development 2005; 132: 3151-3161.
[26] Uygur B, Wu WS. SLUG promotes prostate cancer cell migration and invasion via CXCR4/CXCL12 axis. Mol Cancer 2011; 10: 139.
[27] Jiang W, Pang XG, Wang Q, Shen YX, Chen XK, Xi JJ. Prognostic role of Twist, Slug, and Foxc2 expression in stage I non-small-cell lung cancer after curative resection. Clin Lung Cancer 2012; 13: 280-287.
[28] Shih JY, Yang PC. The EMT regulator slug and lung carcinogenesis. Carcinogenesis 2011; 32: 1299-1304.
[29] Guo Q, Ning F, Fang R, Wang HS, Zhang G, Quan MY, Cai SH, Du J. Endogenous Nodal promotes melanoma undergoing epithelial-mesenchymal transition via Snail and Slug in vitro and in vivo. Am J Cancer Res 2015; 5: 2098-2112
[30] Kumar B, Uppuladinne MV, Jani V, Sonavane U, Joshi RR, Bapat SA. Auto-regulation of Slug mediates its activity during epithelial to mesenchymal transition. Biochim Biophys Acta 2015; 1849: 1209-1218.

Table S1. Primers for qRT-PCR and plasmid construction

Primers for qRT-PCR analyses	
MALAT1 forward	5'-GGTAACGATGGTGTCGAGGTC-3'
MALAT1 reverse	5'-CCAGCATTACAGTTCTTGAACTG-3'
SLUG forward	5'-CCTGGTTGCTTCAAGGACAC-3'
SLUG reverse	5'-AGCAGCCAGATTCCTCATGT-3'
GAPDH forward	5'-GGGAGCCAAAAGGGTCAT-3'
GAPDH reverse	5'-GAGTCCTTCCACG ATACCAA-3'
U6 forward	5'-CTCGCTTCGGCAGCACA-3'
U6 reverse	5'-AACGCTTCACGAATTTGCGT-3'
Primers for plasmid construction	
pGL-MALAT1 forward	5'-GATCTGCAGAGGATCCTAGACCAGCATGC-3'
pGL-MALAT1 reverse	5'-GATCATATGCCGCCTCAGTTACACATCCA-3'