LncRNA-RMRP Acts as an Oncogene in Lung Cancer

Qingjun Meng, Mingming Ren, Yanguang Li, Xiang Song*
Department of thoracic surgery, CangZhou central hospital, CangZhou, Hebei, China
* xiaoxi1 @163.com

Abstract

Accumulating studies have demonstrated that long noncoding RNAs (lncRNAs) act a crucial role in the development of tumors. However, the role of lncRNAs in lung cancer remains largely unknown. In this study, we demonstrated that the expression of RMRP was upregulated in lung adenocarcinoma tissues compared to the matched adjacent normal tissues. Moreover, of 35 lung adenocarcinoma samples, RMRP expression was upregulated in 25 cases (25/35; 71.4%) compared to the adjacent normal tissues. We also showed that RMRP expression was upregulated in lung adenocarcinoma cell lines (A549, SPC-A1, H1299 and H23) compared to the bronchial epithelial cell line (16HBE). Ectopic expression of RMRP promoted lung adenocarcinoma cell proliferation, colony formation and invasion. In addition, overexpression of RMRP inhibited the miR-206 expression in the H1299 cell and increased the KRAS, FMNL2 and SOX9 expression, which were the target genes of miR-206. Re-expression of miR-206 reversed the RMRP-induced the H1299 cell proliferation and migration. Our data proved that RMRP acted as an oncogene LncRNA to promote the expression of KRAS, FMNL2 and SOX9 by inhibiting miR-206 expression in lung cancer. These data suggested that RMRP might serve as a therapeutic target in lung adenocarcinoma.

Introduction

Lung cancer is the leading cause of tumor death worldwide, with about 1,400,000 deaths every year [1–4]. Non-small cell lung cancer (NSCLC), including squamous cell carcinoma and adenocarcinoma, accounts for about 85% of lung cancer [5–8]. The five-year overall survival rate of late-stage NSCLC patients is only 5–20% [9–13]. Despite the improvements in surgery, chemotherapy and radiotherapy, the overall survival of NSCLC is still not encouraging [14–16]. Therefore, it is important to identify noninvasive, new prognostic biomarkers for the NSCLC to develop new therapeutic target for NSCLC.

Long noncoding RNAs (lncRNAs) are noncoding RNAs longer than nucleotides. [17–20]. Increasing evidences show that lncRNAs play a critical role in the cell biology such as cell proliferation, metabolism, differentiation, development, invasion, migration and apoptosis [21–25]. Recently, many lncRNAs are proved to be deregulated in several tumors such as gastric cancer, ovarian carcinoma, colorectal tumor, hepatocellular carcinoma and also lung
cancer [19, 24, 26–28]. However, the expression, function and roles of lncRNAs are still not well studied.

RMRP is a long non-coding RNA that was expressed in various murine and human tissues [29]. Previous study showed that the expression of RMRP was deregulated in gastric cancer [30]. Moreover, Shao et al [31] showed that RMRP expression level was downregulated in gastric cancer tissues and gastric dysplasia. RMRP suppressed the expression of miR-206 and regulated the cell cycle by modulating Cyclin D2 expression in gastric cancer cell. However, the role of RMRP in lung cancer is still known. In this study, we demonstrated that RMRP expression was upregulated in lung adenocarcinoma tissues and cell lines. Ectopic expression of RMRP promoted lung adenocarcinoma cell proliferation, colony formation and invasion. Overexpression of RMRP inhibited the miR-206 expression in the H1299 cell and increased the expression of KRAS, FMNL2 and SOX9, which were the target genes of miR-206. Re-expression of miR-206 reversed the RMRP-induced H1299 cell proliferation and migration.

Materials and Methods

Tissue samples and cell lines cultured and transfected

All lung adenocarcinoma tissues and matched adjacent normal tissues were handled at the Cangzhou Central Hospital. All lung adenocarcinoma patients did not receive radiotherapy or chemotherapy before surgery. All patients gave their written informed consent to participate in this study and the protocol of this study were approved by the Ethics Committee of Cangzhou central hospital. The lung adenocarcinoma cell lines (A549, SPC-A1, H1299 and H23) and 16HBE (bronchial epithelial cell line) were bought from the cell bank of the Chinese Academy of Sciences (Shanghai, China). These cell lines were kept in the RPMI 1640 medium. PDNA3.1-RMRP vector and control vector were synthesized by the GenePharma (Shanghai, China). Cell transfection was performed by the Lipofectamine 2000 reagent according to the manufacturer’s instructions.

Quantitative real-time PCR

Total RNAs were extracted from cells or tissues by the Trizol reagent (Invitrogen, USA) following to the manufacturer’s instruction. Real-time PCR was done to measure the lncRNA and mRNA expression using the SYBR Green PCR kit on the ABI 7300 system (Applied Biosystems, USA) following to the manufacturer’s information. GAPDH and 18S rRNA was used as the control for mRNA or lncRNA expression respectively. The primer sequences were used as following: LncRNA-RMRP, 5'–ACTCCAAAGTCCGCCAAGA–3' (forward) and 5'–TGGCTAACTAGGGAGCTGAC–3' (reverse); GAPDH, 5'–GGAGCCAAAAGGGT CAT3' (forward) and reverse primer: 5'–GAGTCCTTCCAGCATACCAA3' (reverse). 18S rRNA, 5'–ACACGGACGGATGACAGA–3' (forward) and 5'–GGACATCTAAGGCCATCACA–3' (reverse).

Cell proliferation, colony formation and invasion assays

The cells were cultured in the 96-well plate with 1x10^4 per well. MTT (5 mg/ml; Sigma, USA) were put into the each well for addition 4 hours. Optical density (OD) was measured by detecting the absorbance at the 490 nm using the microplate reader (Bio-Rad, USA). Cells were treated with PDNA3.1-RMRP or control vector for 24 hours and then cultured for colony formation in the 6-well plate for 14 days. The colonies were fixed with paraformaldehyde and then stained with crystal violet. Cells invasion were evaluated using Matrigel chambers (BD Biosciences, United Kingdom) following with the manufacturer’s information. Cell was
cultured in the upper chamber in DMEM medium without serum, and the lower chamber supplemented with FBS. After 24 hours, the noninvading cells were discarded using the cotton swab and the bottom cells were fixed with paraformaldehyde and stained with crystal violet.

**Western blot assay**

Protein was extracts from cell or tissue using the cell lysis reagent and measured by the BCA kit (Pierce, USA) according to the instruction’s information. Protein was separated from 12% SDS—PAGE and then transferred to the PVDF membranes. The blot was incubated with primary antibody (KRAS, FMNL2 and SOX9, Abcam) for 12 hours at 4˚C and then measured with secondary antibody with HRP-conjugated (1:10000). Signal was measured by using ECL (Millipore). GAPDH was used as the control for normalization.

**Statistical analysis**

Data was shown as the mean±SD and statistical analyse was performed using the SPSS 17.0 software (IBM SPSS, USA). The difference between two groups was assessed by the Student t-test and the difference between more than two groups was estimated by the one-way ANOVA. P<0.05 was considered statistically significant.

**Results**

The expression of RMRP was upregulated in the lung adenocarcinoma tissues

As shown in Fig 1A, RMRP expression was upregulated in the lung adenocarcinoma tissues compared to the matched adjacent normal tissues. Moreover, of 35 lung adenocarcinoma samples, RMRP was upregulated in 25 cases (25/35; 71.4%) compared to the adjacent normal tissues (Fig 1B). In addition, the RMRP expression was upregulated in lung adenocarcinoma cell lines (A549, SPC-A1, H1299 and H23) compared to the bronchial epithelial cell line (16HBE) (Fig 1C).

Ectopic expression of RMRP enhanced lung adenocarcinoma cell proliferation, colony formation and invasion

The expression of RMRP was significantly enhanced after treated with RMRP vector (Fig 2A). Ectopic expression of RMRP promoted lung adenocarcinoma cell line H1299 cell proliferation (Fig 2B). In line with this, overexpression of RMRP enhanced the expression of cyclin D1 and

![Fig 1. The expression of RMRP was upregulated in the lung adenocarcinoma tissues. (A) RMRP expression was measured in the lung adenocarcinoma tissues and the matched adjacent normal tissues using qRT-PCR. (B) The RMRP was upregulated in 25 cases (25/35; 71.4%) compared to the adjacent normal tissues. (C) The RMRP expression was upregulated in lung adenocarcinoma cell lines (A549, SPC-A1, H1299 and H23) compared to the bronchial epithelial cell line (16HBE).]("image")

doi:10.1371/journal.pone.0164845.g001
Fig 2. Ectopic expression of RMRP promoted lung adenocarcinoma cell proliferation, colony formation and invasion. (A) The expression of RMRP was measured in the H1299 cell after treated with RMRP vector. (B) Ectopic expression of RMRP promoted H1299 cell proliferation. (C) Overexpression of RMRP enhanced the cyclin D1 expression in the H1299 cell. (D) Ectopic expression of RMRP promoted ki-67 expression in the H1299 cell. (E) Overexpression of RMRP promoted the H1299 cell colony formation. (F) Overexpression of RMRP enhanced the H1299 cell invasion. *p<0.05, **p<0.01 and ***p<0.001.

doi:10.1371/journal.pone.0164845.g002
ki-67 (Fig 2C and 2D). Overexpression of RMRP promoted the H1299 cell colony formation (Fig 2E). Ectopic expression of RMRP increased the H1299 cell invasion (Fig 2F).

miR-206 expression was downregulated in the lung adenocarcinoma tissues

miR-206 expression was downregulated in lung adenocarcinoma cell lines (A549, SPC-A1, H1299 and H23) compared to the bronchial epithelial cell line (Fig 3A). As shown in Fig 3B, miR-206 expression was downregulated in lung adenocarcinoma tissues and the matched adjacent normal tissues by using qRT-PCR. (C) miR-206 expression was downregulated in 21 cases (21/35; 60%) compared to the adjacent normal tissues. (D) The expression of RMRP was negative correlated with the expression of miR-206 in lung adenocarcinoma tissues. doi:10.1371/journal.pone.0164845.g003

RMRP suppressed expression of miR-206 and increased the expression of KRAS, FMNL2 and SOX9

miR-206 was proved to act as a tumor suppressor miRNA in lung cancer through targeting the KRAS, FMNL2 and SOX9. Overexpression of RMRP inhibited the expression of miR-206 in
Moreover, ectopic expression of RMRP promoted the expression of KRAS, FMNL2 and SOX9 (Fig 4B–4G) in H1299 cell. RMRP exhibited an oncogenic activity through targeting miR-206. miR-206 expression was significantly upregulated in H1299 cell after treated with the miR-206 mimic (Fig 5A). miR-206 expression was decreased in H1299 cell after treated with RMRP vector (Fig 5B). CCK8 proliferation assay demonstrated that restoration of miR-206 suppressed cell proliferation in H1299 cell after treated with miR-206 mimic (Fig 5C). Moreover, invasion assay showed that restoration of miR-206 inhibited the cell invasion in H1299 cell after treated with miR-206 mimic (Fig 5D).

Discussion

In this study, we demonstrated that RMRP expression was upregulated in lung adenocarcinoma tissues compared to the matched adjacent normal tissues. Moreover, of 35 lung adenocarcinoma samples, RMRP expression was upregulated in 25 cases (25/35; 71.4%) compared to the adjacent normal tissues. We also showed that RMRP expression was upregulated in lung adenocarcinoma cell lines (A549, SPC-A1, H1299 and H23) compared to the bronchial epithelial cell line (16HBE). Ectopic expression of RMRP increased lung adenocarcinoma cell proliferation, colony formation and invasion. In addition, overexpression of RMRP inhibited miR-206 expression in the H1299 cell and increased the expression of KRAS, FMNL2 and SOX9, which were the target genes of miR-206. Re-expression of miR-206 reversed the RMRP-induced the H1299 cell proliferation and migration. These data suggested that RMRP acted as...
an oncogene LncRNA to promote the KRAS, FMNL2 and SOX9 by inhibiting miR-206 expression in the lung cancer.

LncRNAs play critical roles in the development of various cancers [32–34]. Previous study showed that RMRP was deregulated in gastric cancer [30]. Moreover, Shao et al [31]. demonstrated that RMRP expression was downregulated in gastric cancer tissues and gastric dysplasia. RMRP inhibited the expression of miR-206 and regulated the cell cycle through modulating Cyclin D2 expression in gastric cancer cell. However, the role of RMRP in lung cancer is still uncovered. In this study, we firstly measured RMRP expression in 35 pairs of lung adenocarcinoma tissues and matched adjacent normal tissues. Our results showed that RMRP expression was upregulated in lung adenocarcinoma tissues compared to the matched adjacent normal tissues. Of 35 lung adenocarcinoma samples, RMRP expression was upregulated in 25 cases (25/35; 71.4%) compared to the adjacent normal tissues. In line with this, RMRP expression was upregulated in lung adenocarcinoma cell lines compared to the bronchial epithelial cell line. Moreover, ectopic expression of RMRP promoted lung adenocarcinoma cell proliferation.
colony formation and invasion. These data suggested that RMRP acted as a oncogenic lncRNA in lung cancer.

LncRNAs regulate gene expression epigenetically through competing for shared the miRNA response elements, therefore decreasing the binding of miRNA to its target genes [35–38]. Previous study showed that RMRP increased aggressive gastric cancer by regulating Cyclin D2 as the ceRNA for miR-206 [31]. In line with this, we demonstrated that overexpression of RMRP suppressed miR-206 expression in lung adenocarcinoma cell. In addition, our data showed that ectopic expression of RMRP promoted the expression of KRAS, FMNL2 and SOX9 in lung adenocarcinoma cell. Previous study suggested that miR-206 expression was downregulated in gastric cancer tissues compared to the normal adjacent mucosa [39–42]. Zhang et al [43] demonstrated that miR-206 expression was downregulated in non small cell lung cancer tissues compared with adjacent normal tissues. Overexpression of miR-206 suppressed the cell proliferation, invasion and migration of NSCLC cells through targeting SOX9. In addition, Ren et al [44] showed that miR-206 expression was decreased in colorectal cancer (CRC) tissues and associated with lymphatic metastasis, differentiation and serosal invasion. Overexpression of miR-206 inhibited CRC cell proliferation and increased the cell apoptosis by inhibiting FMNL2 expression. Keklikoglou et al. demonstrated that miR-206 was abrogated in pancreatic ductal adenocarcinoma cell lines and tissues. They showed that miR-206 suppressed the pancreatic ductal adenocarcinoma cell cycle progression, migration, invasion and proliferation by targeting KRAS and annexin a2 (ANXA2) [45]. In our study, we also demonstrated that ectopic expression of RMRP promoted the expression of KRAS, FMNL2 and SOX9 in lung adenocarcinoma cell, which were the direct target gene of miR-206. Furthermore, RMRP exhibited an oncogenic activity through targeting miR-206 in lung adenocarcinoma cell.

In conclusion, we demonstrated that RMRP expression was upregulated in lung adenocarcinoma tissues and overexpression of RMRP promoted lung adenocarcinoma cell proliferation, colony formation and invasion. RMRP exhibited an oncogenic activity through targeting miR-206 in lung adenocarcinoma cell. Therefore, RMRP might serve as a therapeutic target in lung adenocarcinoma.

Author Contributions

Conceptualization: QM MR YL XS.
Data curation: QM MR YL XS.
Formal analysis: QM MR YL XS.
Funding acquisition: QM MR YL XS.
Investigation: QM MR YL XS.
Methodology: QM MR YL XS.
Project administration: QM MR YL XS.
Resources: QM MR YL XS.
Software: QM MR YL XS.
Supervision: QM MR YL XS.
Validation: QM MR YL XS.
Visualization: QM MR YL XS.
References

1. Plaimee P, Weerapreeyakul N, Thumanu K, Tanthanu W, Barusrux S. Melatonin induces apoptosis through biomolecular changes, in SK-LU-1 human lung adenocarcinoma cells. Cell proliferation. 2014; 47(6):564–77. Epub 2014/10/28. doi: 10.1111/cpr.12140 PMID: 25345555

2. Postiglione I, Chiaviello A, Aloj SM, Palumbo G. 5-aminolaevulinic acid/photo-dynamic therapy and gefitinib in non-small cell lung cancer cell lines: a potential strategy to improve gefitinib therapeutic efficacy. Cell proliferation. 2013; 46(4):382–95. Epub 2013/07/23. doi: 10.1111/cpr.12040 PMID: 23522940

3. Li CY, Wang Y, Wang HL, Shi Z, An N, Liu YX, et al. Molecular mechanisms of Lycoris aurea agglutinin-induced apoptosis and G2/M cell cycle arrest in human lung adenocarcinoma A549 cells, both in vitro and in vivo. Cell proliferation. 2013; 46(3):272–82. Epub 2013/05/23. doi: 10.1111/cpr.12034 PMID: 23692086

4. Falcone D, Gallelli L, Di Virgilio A, Tucci L, Scaramuzzino M, Terracciano R, et al. Effects of simvastatin and rosuvastatin on RAS protein, matrix metalloproteinases and NF-kappaB in lung cancer and in normal pulmonary tissues. Cell proliferation. 2013; 46(2):172–82. Epub 2013/03/21. doi: 10.1111/cpr.12018 PMID: 23510472

5. Xie X, Liu H, Wang M, Ding F, Xiao H, Hu F, et al. miR-342-3p targets RAP2B to suppress proliferation and invasion of non-small cell lung cancer cells. Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine. 2015. Epub 2015/02/11.

6. Wei J, Ma Z, Li Y, Zhao B, Wang D, Jin Y. miR-143 inhibits cell proliferation by targeting autophagy-related 2B in non-small cell lung cancer H1299 cells. Molecular medicine reports. 2015; 11(1):571–6. Epub 2014/10/18. doi: 10.3892/mmr.2014.2675 PMID: 25322940

7. Zhang ZY, Fu SL, Xu SQ, Zhou X, Liu XS, Xu YJ, et al. By downregulating Ku80, hsa-miR-526b suppresses non-small cell lung cancer. Oncotarget. 2015; 6(3):1462–77. Epub 2015/01/19. doi: 10.18632/oncotarget.2808 PMID: 2556743

8. Zhang J, Xu L, Yang Z, Lu H, Hu D, Li W, et al. MicroRNA-10b indicates a poor prognosis of non-small cell lung cancer and targets E-cadherin. Clinical & translational oncology: official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico. 2015; 17(3):209–14. Epub 2014/09/13.

9. Sabarinathan R, Wenzel A, Novotny P, Tang X, Kalari KR, Gorodkin J. Transcriptome-wide analysis of UTRs in non-small lung cancer reveals cancer-related genes with SNV-induced changes on RNA secondary structure and miRNA target sites. PloS one. 2014; 9(1):e82699. Epub 2014/01/15. doi: 10.1371/journal.pone.0082699 PMID: 24416147

10. Tejero R, Navarro A, Campayo M, Vinolas N, Marrades RM, Cordeiro A, et al. miR-141 and miR-200c as markers of overall survival in early stage non-small cell lung cancer adenocarcinoma. PloS one. 2014; 9(7):e101899. Epub 2014/07/09. doi: 10.1371/journal.pone.0101899 PMID: 25003366

11. Tsay JC, Li Z, Yie TA, Wu F, Segal L, Greenberg AK, et al. Molecular characterization of the peripheral airway field of cancerization in lung adenocarcinoma. PloS one. 2015; 10(2):e0118132. Epub 2015/02/24. doi: 10.1371/journal.pone.0118132 PMID: 25705890

12. Zhang C, Chi YL, Wang PY, Wang YQ, Zhang YX, Deng J, et al. miR-511 and miR-1297 inhibit human lung adenocarcinoma cell proliferation by targeting oncogene TRIB2. PloS one. 2012; 7(10):e46090. Epub 2012/10/17. doi: 10.1371/journal.pone.0046090 PMID: 23071539

13. Zhang H, Su Y, Xu F, Kong J, Yu H, Qian B. Circulating microRNAs in relation to EGFR status and survival of lung adenocarcinoma in female non-smokers. PloS one. 2013; 8(11):e81408. Epub 2013/11/28. doi: 10.1371/journal.pone.0081408 PMID: 24282590

14. Zhang HH, Pang M, Dong W, Xin JX, Li YJ, Zhang ZC, et al. miR-511 induces the apoptosis of radioreistant lung adenocarcinoma cells by triggering BAX. Oncology reports. 2014; 31(3):1473–9. Epub 2014/01/10. doi: 10.3892/or.2014.2973 PMID: 24402374

15. Ma Y, Li X, Cheng S, Wei W, Li Y. MicroRNA-106a confers cisplatin resistance in non-small cell lung cancer A549 cells by targeting adenosine triphosphatase-binding cassette A1. Molecular medicine reports. 2015; 11(1):625–32. Epub 2015/10/24. doi: 10.3892/mmr.2015.2888 PMID: 25339370

16. Saito M, Shiraiishi K, Matsumoto K, Schetter AJ, Ogata-Kawata H, Tsuchiya N, et al. A three-microRNA signature predicts responses to platinum-based doublet chemotherapy in patients with lung adenocarcinoma. Clinical cancer research: an official journal of the American Association for Cancer Research. 2014; 20(18):4784–93. Epub 2014/08/22.
17. Zhang J, Zhang P, Wang L, Piao HL, Ma L. Long non-coding RNA HOTAIR in carcinogenesis and metastasis. Acta biochimica et biophysica Sinica. 2014; 46(1):1–5. Epub 2013/10/30. doi: 10.1093/abbs/gmt117 PMID: 24165275

18. Nakagawa T, Endo H, Yokoyama M, Abe J, Tamai K, Tanaka N, et al. Large noncoding RNA HOTAIR enhances aggressive biological behavior and is associated with short disease-free survival in human non-small cell lung cancer. Biochemical and biophysical research communications. 2013; 436(2):319–24. Epub 2013/06/08. doi: 10.1016/j.bbrc.2013.05.101 PMID: 23743197

19. Endo H, Shiroki T, Nakagawa T, Yokoyama M, Tamai K, Yamanami H, et al. Enhanced expression of long non-coding RNA HOTAIR is associated with the development of gastric cancer. PloS one. 2013; 8(10):e77070. Epub 2013/10/17. doi: 10.1371/journal.pone.0077070 PMID: 24130837

20. Yu X, Li Z. Long non-coding RNA growth arrest-specific transcript 5 in tumor biology. Oncology letters. 2015; 10(4):1953–8. Epub 2015/12/02. doi: 10.3892/ol.2015.3553 PMID: 26622780

21. Wang D, Ding L, Wang L, Zhao Y, Sun Z, Karnes RJ, et al. LncRNA MALAT1 enhances oncogenic activities of EZH2 in castration-resistant prostate cancer. Oncotarget. 2015. Epub 2015/10/31.

22. Cai X, Liu Y, Yang W, Xia Y, Yang C, Yang S, et al. Long noncoding RNA MALAT1 as a potential therapeutic target in osteosarcoma. Journal of orthopaedic research: official publication of the Orthopaedic Research Society. 2015. Epub 2015/11/18.

23. Zhou X, Liu S, Cai G, Kong L, Zhang T, Ren Y, et al. Long Non Coding RNA MALAT1 Promotes Tumor Growth and Metastasis by inducing Epithelial-Mesenchymal Transition in Oral Squamous Cell Carcinoma. Scientific reports. 2015; 5:15972. Epub 2015/11/03. doi: 10.1038/srep15972 PMID: 26522444

24. Qi P, Xu MD, Ni SJ, Huang D, Wei P, Tan C, et al. Low expression of LOC285194 is associated with poor prognosis in colorectal cancer. Journal of translational medicine. 2013; 11:122. Epub 2013/05/18. doi: 10.1186/1479-5876-11-122 PMID: 23680400

25. Liu Q, Huang J, Zhou N, Zhang Z, Zhang A, Lu Z, et al. LncRNA loc285194 is a p53-regulated tumor suppressor. Nucleic acids research. 2013; 41(9):4976–87. Epub 2013/04/06. doi: 10.1093/nar/gkt182 PMID: 23587494

26. Hua L, Wang CY, Yao KH, Chen JT, Zhang JJ, Ma WL. High expression of long non-coding RNA ANRIL is associated with poor prognosis in hepatocellular carcinoma. International journal of clinical and experimental pathology. 2015; 8(3):3076–82. Epub 2015/06/06. PMID: 26045820

27. Naemura M, Murasaki C, Inoue Y, Okamoto H, Kotake Y. Long Noncoding RNA ANRIL Regulates Proliferation of Non-sm all Cell Lung Cancer and Cervical Cancer Cells. Anticancer research. 2015; 35(10):5377–82. Epub 2015/09/27. PMID: 26408699

28. Rosenbluh J, Nijhawan D, Chen Z, Wong KK, Masutomi K, Hahn WC. RMRP is a non-coding RNA essential for early murine development. PloS one. 2011; 6(10):e26270. Epub 2011/11/01. doi: 10.1371/journal.pone.0026270 PMID: 22039455

29. Song H, Sun W, Ye G, Ding X, Liu Z, Zhang S, et al. Long non-coding RNA expression profile in human gastric cancer and its clinical significances. Journal of translational medicine. 2013; 11:225. Epub 2013/09/26. doi: 10.1186/1479-5876-11-225 PMID: 24063685

30. Shao Y, Ye M, Li Q, Sun W, Ye G, Zhang X, et al. LncRNA-RMRP promotes carcinogenesis by acting as a miR-206 sponge and is used as a novel biomarker for gastric cancer. Oncotarget. 2016. Epub 2016/05/19.

31. Nie FQ, Sun M, Yang JS, Xie M, Xu TP, Xia R, et al. Long noncoding RNA ANRIL promotes non-small cell lung cancer cell proliferation and inhibits apoptosis by silencing KLF2 and P21 expression. Molecular cancer therapeutics. 2015; 14(1):268–77. Epub 2014/12/17. doi: 10.1158/1535-7163.MCT-14-0492 PMID: 25504755

32. Hu X, Bao J, Wang Z, Zhang Z, Gu P, Tao F, et al. The plasma lncRNA acting as fingerprint in non-small-cell lung cancer. Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine. 2015. Epub 2015/10/11.

33. Lin L, Gu ZT, Chen WH, Cao KJ. Increased expression of the long non-coding RNA ANRIL promotes lung cancer cell metastasis and correlates with poor prognosis. Diagnostic pathology. 2015; 10:14. Epub 2015/04/19. doi: 10.1186/s13000-015-0247-7 PMID: 25989788

34. Xiao H, Tang K, Liu P, Chen K, Hu J, Zeng J, et al. LncRNA MALAT1 functions as a competing endogenous RNA to regulate ZEB2 expression by sponging miR-200s in clear cell kidney carcinoma. Oncotarget. 2015; 6(35):38005–15. Epub 2015/10/16. doi: 10.18632/oncotarget.6397 PMID: 26461224
36. Tang Y, Jin X, Xiang Y, Chen Y, Shen CX, Zhang YC, et al. The lncRNA MALAT1 protects the endothelium against ox-LDL-induced dysfunction via upregulating the expression of the miR-22-3p target genes CXCR2 and AKT. FEBS letters. 2015; 589(20 Pt B):3189–96. Epub 2015/09/15. doi: 10.1016/j.febslet.2015.08.046 PMID: 26364720

37. Lu L, Luo F, Liu Y, Liu X, Shi L, Lu X, et al. Posttranscriptional silencing of the lncRNA MALAT1 by miR-217 inhibits the epithelial-mesenchymal transition via enhancer of zeste homolog 2 in the malignant transformation of HBE cells induced by cigarette smoke extract. Toxicology and applied pharmacology. 2015; 289(2):276–85. Epub 2015/09/30. doi: 10.1016/j.taap.2015.09.016 PMID: 26415832

38. Ma J, Wang P, Yao Y, Liu Y, Li Z, Liu X, et al. Knockdown of long non-coding RNA MALAT1 increases the blood-tumor barrier permeability by up-regulating miR-140. Biochimica et biophysica acta. 2015. Epub 2015/12/02.

39. Chen QY, Jiao DM, Wu YQ, Chen J, Wang J, Tang XL, et al. MiR-206 inhibits HGF-induced epithelial-mesenchymal transition and angiogenesis in non-small cell lung cancer via c-MET/PI3k/ Akt/mTOR pathway. Oncotarget. 2016. Epub 2016/02/27.

40. Chen QY, Jiao DM, Wang J, Hu H, Tang X, Chen J, et al. miR-206 regulates cisplatin resistance and EMT in human lung adenocarcinoma cells partly by targeting MET. Oncotarget. 2016. Epub 2016/03/26.

41. Chen QY, Jiao DM, Yan L, Wu YQ, Hu HZ, Song J, et al. Comprehensive gene and microRNA expression profiling reveals miR-206 inhibits MET in lung cancer metastasis. Molecular bioSystems. 2015; 11 (8):2290–302. Epub 2015/06/16. doi: 10.1039/c4mb00734d PMID: 26075299

42. Cui Y, Xie S, Luan J, Zhou X, Han J. Quantitative proteomics and protein network analysis of A549 lung cancer cells affected by miR-206. Bioscience trends. 2013; 7(6):59–63. Epub 2014/01/07. PMID: 24390363

43. Zhang YJ, Xu F, Li HB, Han JC, Li L. miR-206 inhibits non small cell lung cancer cell proliferation and invasion by targeting SOX9. International journal of clinical and experimental medicine. 2015; 8 (6):9107–13. Epub 2015/08/27. PMID: 26309565

44. Ren XL, He GY, Li XM, Men H, Yi LZ, Lu GF, et al. MicroRNA-206 functions as a tumor suppressor in colorectal cancer by targeting FMNL2. Journal of cancer research and clinical oncology. 2016; 142 (3):581–92. Epub 2015/10/31. doi: 10.1007/s00432-015-2053-8 PMID: 26515696

45. Keklikoglou I, Hosaka K, Bender C, Bott A, Koerner C, Mitra D, et al. MicroRNA-206 functions as a pleiotropic modulator of cell proliferation, invasion and lymphangiogenesis in pancreatic adenocarcinoma by targeting ANXA2 and KRAS genes. Oncogene. 2015; 34(37):4867–78. Epub 2014/12/17. doi: 10.1038/onc.2014.408 PMID: 25500842