Two lung development-related microRNAs, miR-134 and miR-187, are differentially expressed in lung tumors

Article *in* Gene · November 2015

DOI: 10.1016/j.gene.2015.11.040

CITATIONS
13

READS
380

7 authors, including:

Fatemeh Mirzadeh
Università degli Studi di Torino
11 PUBLICATIONS **39** CITATIONS
SEE PROFILE

Parisa Naeli
Queen's University Belfast
19 PUBLICATIONS **80** CITATIONS
SEE PROFILE

Mahshid Malakootian
Rajaei Cardiovascular Medical and Research Center, Iran University of Medical Sci…
19 PUBLICATIONS **77** CITATIONS
SEE PROFILE

Azar Baradaran
Isfahan University of Medical Sciences
52 PUBLICATIONS **644** CITATIONS
SEE PROFILE

Gene xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Two lung development-related microRNAs, miR-134 and miR-187, are differentially expressed in lung tumors

F. Mirzadeh Azad [a], P. Naeli [a], M. Malakootian [b], A. Baradaran [c], M. Tavallaei [d], M. Ghanei [e], S.J. Mowla [a,*]

[a] Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
[b] Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran
[c] Department of Pathology, Alzahra Hospital, Isfahan University of Medical Sciences, Isfahan, Iran
[d] Genetic Research Center, Baqiatallah University of Medical Sciences, Tehran, Iran
[e] Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 8 June 2015
Received in revised form 18 November 2015
Accepted 26 November 2015
Available online xxxx

Keywords:
Lung cancer
Tumor marker
Development related miRNAs
miR-134
miR-187
Biomarkers

ABSTRACT

Introduction: MicroRNAs (miRNAs) are involved in various cellular events needed for embryonic development and tumorigenesis. As some of the development-specific gene expression patterns could be observed in cancers, we speculated that the expression pattern of lung development-specific miRNAs miR-134 and miR-187 might be altered in lung tumor samples. Lung cancer is the first cause of cancer related deaths worldwide, mostly due to its late diagnosis. Therefore, finding a reliable diagnostic tumor marker, based on molecular profile of tumorigenesis, would be critical in lowering lung cancer mortality.
Methods: We employed a real-time RT-PCR approach to evaluate the expression alteration of two lung development-related miRNAs in lung tumor tissues. The suitability of miRs expression alterations as lung tumor biomarkers was tested by receiver operating characteristic (ROC) curve analysis. The effect of miR-187 overexpression on a lung carcinoma cell cycle was assessed using flow cytometry analysis.
Results: Our data revealed a significant upregulation (7.8 times, $p < 0.02$) of miR-134 in lung tumors. However, its expression level failed to discriminate different tumor types and grades of malignancies from each other. Moreover, the ROC curves analysis did not give it a good score as a reliable biomarker (AUC = 0.522, P = 0.729). In contrast, miR-187 showed a significant down-regulation (P = 0.008) in lung tumors. Similarly, its expression level failed to differentiate different tumor types or grades of malignancies. Nevertheless, ROC curve analysis gave it an AUC score of 0.669 (P = 0.012), which suggests its suitability as a potential biomarker for lung cancer. Furthermore, ectopic expression of miR-187 in A549 cells caused a cell cycle arrest in G1 phase (P = 0.013).
Conclusion: Altogether, our data demonstrated an altered expression of two development-related miRNAs namely miR-134 and miR-187 in lung tumors for the first time. Moreover we have shown that miR-134 and miR-187 expression alternation were in accordance with their approved regulatory roles, therefore these miRNAs could serve as new biomarkers with potential usefulness in lung cancer diagnosis and treatments. In addition, miR-187 expression in tumor cells could perturb cell cycle which supported its possible role as tumor suppressor.

1. Introduction

Lung cancer is the leading cause of cancer-related deaths around the world (Herbst et al., 2008). Nearly 85% of all lung tumor cases are non-small cell carcinomas (NSCC). In Iran, it is among the five most frequent cancer types and its incidence is growing mostly due to the environmental pollutions and life style changes (Hosseini et al., 2009). The main reason for high mortality rate of lung cancer is its late diagnosis. In fact, 75% of all lung carcinomas are diagnosed at late stages, when tumors have already become invasive and refractor to treatments (Hoffman et al., 2000; Herbst et al., 2008; Ettinger et al., 2012; Zhang et al., 2013). Therefore, finding reliable lung cancer biomarkers with high sensitivity and specificity is of great importance (Brambilla et al., 2003).

MicroRNAs (miRNAs) are a group of small (18–22 nucleotides) non-coding RNAs that post-transcriptionally regulate gene expression. By binding to their target mRNAs, they either repress translation or degrade their targets (Bartel, 2004; Ketting, 2011). Regulating more than 60% of human protein-coding genes, miRNAs profoundly control different cellular pathways and biological events (Friedman et al., 2009). Two important cellular events that are under tight control of these small regulators are embryonic development and oncogenesis (Lin et al., 2010).

Several previous investigations have emphasized on significant similarities between lung tumorigenesis and lung embryonic development.

Abbreviations: miRNA, microRNA; RT-PCR, reverse transcriptase polymerase chain reaction; ROC curve, receiver operating characteristic curve; AUC, area under curve; NSCC, non-small cell carcinomas; rRNA, ribosomal RNA.

* Corresponding author.
E-mail address: sjmowla@modares.ac.ir (S.J. Mowla).

http://dx.doi.org/10.1016/j.gene.2015.11.040
0378-1119/

In fact, some processes such as cell growth, division, and differentiation recruit the same genetic machinery in both states. In other words, genes which have strategic roles in developmental events, most likely have a part in tumorigenesis as well (Bonner et al., 2004; Liu and Kohane, 2009; Whitsett et al., 2011). MiRNAs that are down-regulated during embryonic lung development are proved to function as tumor suppressor, while those with elevated level in developing lung are mostly considered as oncomiRs (Mendell, 2008; Liu and Kohane, 2009).

First indications of miRNAs orchestrating lung development came from studies on conditional knockout mice for Dicer in which affected epithelial cells were unable to succeed epithelial branching (Harris et al., 2006). Furthermore, analyzing miRNAs expression pattern in different stages of lung development exhibited a unique profile for each stage (Williams et al., 2007; Bhaskaran et al., 2009). These miRNAs are responsible for fine-tuning expression of growth and transcription factors or act as molecular switches for cellular events (Brennecke et al., 2003; Tay et al., 2008; Croce, 2009; Shenouda and Alahari, 2009; Liu et al., 2010).

MiR-134 and miR-187 were previously reported as lung development-related miRNAs, with different expression status in mature and developing lung in human and mice (Williams et al., 2007). MiR-187 is reported to be down-regulated in developing lung. Moreover, its ectopic expression in renal cell lines affects cellular proliferation rate (Zhao et al., 2013). MiR-134 is known as a modulator of cell growth, apoptosis, and migration and is upregulated in developing lung (Williams et al., 2007; Zhang et al., 2012). Considering the important role of miRNAs in cancers, several studies have investigated miR-134 and miR-187 expressions and functions in different tumors; however, their expression alterations in lung cancer are still unclear.

2. Materials and methods

2.1. Tissues samples

36 FFPE samples of lung tumor along with their adjacent apparently normal tissues were obtained from Al-zahra hospitals pathology archive (Isfahan, Iran). Clinical and pathological characteristics of obtained tissues were as shown in Table 1. Tissue blocks were examined by an expert pathologist and the representative tumor and non-tumor areas were punched off and sectioned into 10 μm sections by a microtome

2.2. RNA extraction

FFPE tissues were deparaffinized with xylene, followed by absolute ethanol wash. Then tissues were digested by proteinase K (Fermentas, UK) for 3 h at 56 °C. Total RNA was isolated using RiboEx reagent (GeneAll, South Korea), according to the manufacturer's instructions, and stored at −80 °C until further investigations.

Table 1
Clinico-pathological characteristics of the patients with lung cancer.

Variables		No. of individuals
All samples		36
Gender	Male	25
	Female	11
Tumor types	Adenocarcinoma	15
	Large cell carcinoma	9
	Squamous cell carcinoma	12
Tumors differentiation grade	G 1	7
	G 2	10
	G 3	11
	G 4	8

2.3. MicroRNA quantification

MiR-134, miR-187 and 5S rRNA, as an internal control, were reverse transcribed using PARSGENOME MiR-Amp kit (Parsgenome, Iran) following manufacturer's instruction. Briefly, 2 μg of total RNA sample was polyadenylated by poly (A) polymerase and reversely transcribed into cDNA using reverse transcriptase enzyme and adaptor primers. Real-time qPCR was done in 20-μl PCR reaction using HOT FIREPol Eva Green qPCR Mix (Solis BioDyne, Estonia) and specific primer mix (Parsgenome, Iran) via an ABI 7500 Instrument (Applied Biosystems, USA). After analyzing melt curves, PCR products were sequenced to validate the accuracy of amplification. Relative expressions of miRs to 5S rRNA were calculated using the $2^{-\Delta\Delta Ct}$ method. All reactions were performed in duplicate.

2.4. Construction of miR-187 overexpressing vector

Genomic sequence of miR-187 precursor was amplified by Pfu polymerase (GeneAll, South Korea), using GGGTACCCATGCACAGCAAGTC GGATT as forward primer and GGGCCCTGTGTCGAGTCCCTC as reverse primer. The amplified sequence was directly cloned into pTracer™-SV40 vector (Invitrogen, USA) and transformed to DH5α competent cells (TaKaRa, Japan). Positive selection against zeocin™ (Life Technologies, USA) was used to identify recombinant colonies. The accuracy of the recombinant vectors was confirmed by direct DNA sequencing (Macrogen, South Korea).

2.5. Cell cycle analysis

The human lung adenocarcinoma (A549) cell line was obtained from Stem Cell Technology Company (Tehran, Iran). Cells were cultured at 37 °C with 5% humidified CO_2 in high glucose Dulbecco's Modified Eagle Medium (DMEM; Invitrogen, USA) supplemented with 10% FBS (Invitrogen, USA) and 1% Penicillin/Streptomycin solution (Biowest, Canada). For transfection, A549 cells were plated overnight in 24-well plates (7×10^4 cells per well). After complete adhesion, cells were transfected with 1 μg of miR-187 overexpressing vector using Lipofectamin 2000 (Invitrogen, USA), according to the manufacturer instruction. Transfection rate was checked by visualizing GFP signal and by performing real-time PCR against miR-187. To determine cell cycle distribution, cells were harvested 24 h post transfection using trypsin (Gibco, USA) treatment. After ethanol fixation, cells were stained by 60 mg/mL propidium iodide solution containing RNaseA (20 mg/mL) and analyzed by FACScan cell sorter (Partec Flow Cytometry, Deutschland). Flowing software (version 2.5) was used in order to determine cell cycle profile.

2.6. Statistical analysis

Statistical analyses were performed using GraphPadPrism 6 software. Student t-test, one way ANOVA and receiver operating characteristic (ROC) curve analysis were employed for examining the statistical significance of observed miRNAs expression differences, and also to test whether they have enough sensitivity and specificity to distinguish tumor from non-tumor states. All values were expressed as mean ± standard error (SE), and P values less than 0.05 were considered as statistically significant.

3. Results

3.1. MiR-134 is significantly upregulated in lung tumor tissues

A real-time quantitative RT-PCR approach was applied to investigate the expression pattern of miR-134 in different types of lung tumors (adenocarcinoma, large cell carcinoma, and squamous cell carcinoma). Using a poly-A tailing method, we amplified miR-134 mature form in

Please cite this article as: Mirzadeh Azad, F., et al., Two lung development-related microRNAs, miR-134 and miR-187, are differentially expressed in lung tumors, Gene (2015), http://dx.doi.org/10.1016/j.gene.2015.11.040

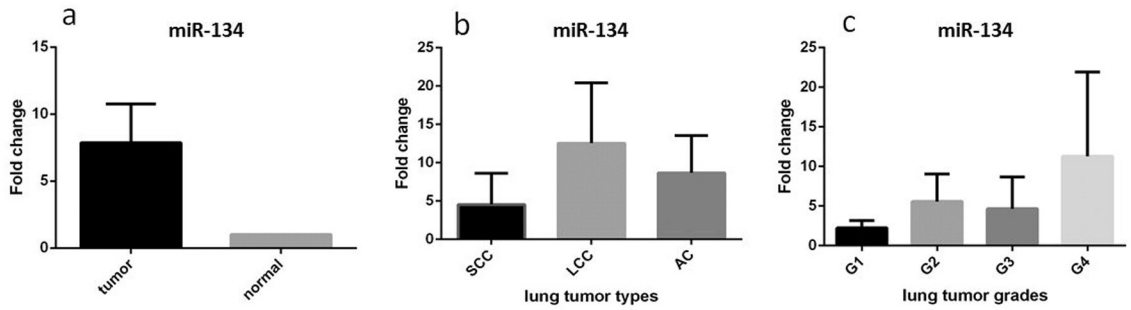

Fig. 1. Relative expression of miR-134 in tumor and non-tumor samples of lung, normalized to that of 5S rRNA. a) Real-time PCR revealed a significant up-regulation of miR-134 in lung tumor tissues (P value = 0.02). b,c) miR-134 expression alterations were not statistically significant among different types of lung cancer (squamous cell carcinoma (SCC), large cell carcinoma (LCC), adenocarcinoma (AC)) and different grades of malignancies.

36 lung tumor samples along with the apparently normal tissues obtained from the margin of same tumors. Our data revealed a significant upregulation of miR-134 in tumor samples, with a mean fold increase of 7.8 times and a P value less than 0.02 (Fig. 1a). However, the expression alteration of miR-134 was not statistically significant among different tumor types (Fig. 1b) and different grades of malignancies (Fig. 1c).

3.2. MiR-187 is down-regulated in lung tumor samples

The aforementioned real-time quantitative RT-PCR, using specific primers for miR-187, was carried out to determine miR-187 expression in lung tumor samples. The normalized expression data for miR-187 in 36 NSCLC samples revealed its significant down-regulation in tumor tissues (P value = 0.008; Fig. 2a). However, the obtained data failed to show a significant difference among different tumor types or different grades of malignancy (Fig. 2b and c).

3.3. The specificity and sensitivity of miR-134 and miR-187 expression levels in discriminating tumors from non-tumor samples

To investigate a potential suitability of miR-134 and miR-187 in discriminating tumor vs. non-tumor states of lung samples, we calculated their sensitivities and specificities by means of ROC curve analysis. Despite a significant difference in gene expression alteration of miR-134 in lung tumor tissues, its expression level had a low AUC (area under curve) of 0.522 (P value = 0.729, 95% confidence interval: 0.394 to 0.650) for differentiating tumor from non-tumor samples (Fig. 3a). In contrast, ROC analysis measured an AUC = 0.669 for miR-187 (P value = 0.012, 95% confidence interval: 0.546 to 0.791, Fig. 3b), which is a score very close to the cutoff (0.7) needed for a considerable biomarker.

3.4. Ectopic expression of miR-187 induces G1 cell cycle arrest

A significant decline in the expression of miR-187 in tumor tissues suggested a probable role for it as a tumor suppressor in lung cancer. In order to examine whether miR-187 expression could affect cell cycle progression in lung cancer cells, A549 cells were transfected with a recombinant vector containing the sequence of mir-187 precursor. Using flow cytometry, we then determined cell cycle progression in cells transfected with either miR-187-expressing or empty vectors (Fig. 4). As early as 24 h after transfecting, a significant accumulation of cells in G1 phase (62.07% compared to 47.2% for mock and 45.12% for untreated cells, P value: 0.013) was observed in the cells overexpressing miR-187.

4. Discussion

MicroRNAs are generally known as transcription regulators, primarily act as fine-tuners of many signaling pathways and cellular events (Bartel, 2004). MicroRNAs expression pattern is connected to various biological and physiological conditions, and hence obvious changes in their expression are being used as valuable biomarkers for diagnosis, prognosis, and also treatment of cancers (Croce, 2009; Shenouda and Alahari, 2009). Herein, we have investigated a potential expression alteration of two lung development-related microRNAs, miR-134 and miR-187, in lung tumor samples.

Down-regulation of miR-187 is already reported in developing lung as well as in several solid tumors including renal cell carcinoma and ovarian tumors (Williams et al., 2007; Chao et al., 2012; Zhao et al., 2013). Our data also revealed a down-regulation of miR-187 in lung tumor tissues, regardless of tumors type and grades of malignancy. We also determined that ectopic expression of miR-187 could induce a G1 cell cycle arrest in human lung carcinoma cells. This observation suggests that miR-187 could perturb cell cycle in tumor cells and inhibit

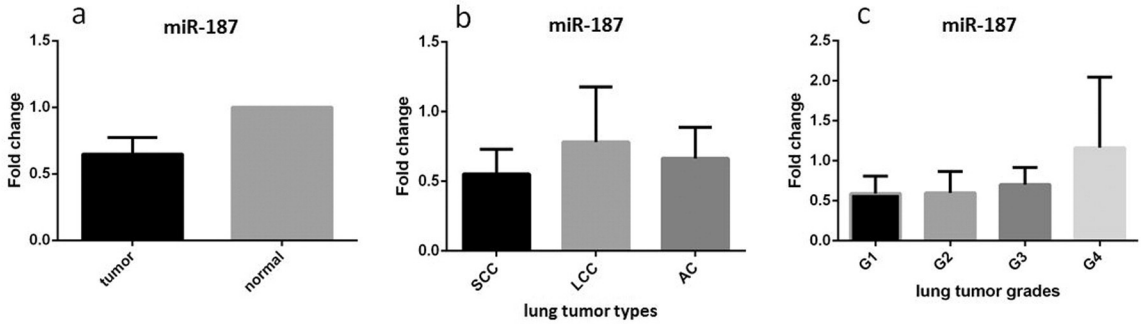

Fig. 2. miR-187 expression level in tumor and non-tumor tissue samples of lung, normalized to that of 5S rRNA used as an internal control. a) Note that miR-187 is significantly down-regulated in lung tumor tissues (P value = 0.008). b,c) miR-187 relative expression was not significantly different between various tumor types and grades of malignancy.

Please cite this article as: Mirzadeh Azad, F., et al., Two lung development-related microRNAs, miR-134 and miR-187, are differentially expressed in lung tumors, Gene (2015), http://dx.doi.org/10.1016/j.gene.2015.11.040

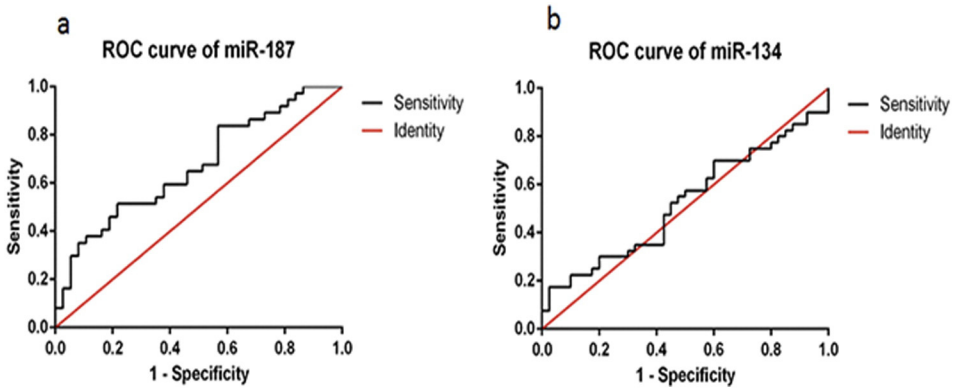

Fig. 3. The receiver-operator characteristic (ROC) curves for miR-187 and miR-134 expression in tumor and non-tumor lung samples. a) miR-187 could significantly discriminates tumor from non-tumor samples by an AUC of 0.669 (P value = 0.012). b) ROC curve analysis calculated a low and insignificant capability for miR-134 expression level to distinguish tumor vs. non-tumor states of the samples.

cancer progression by blocking G1 to S transition. Our finding on a tumor suppressor role of miR-187 is in agreement with its developmental activity status and also the previous reports of its expression in other cancers.

Two validated targets of miR-187 are B7-H3 and Dab2 transcripts. B7-H3 is an oncogene that activates jak2/STAT3 pathway and facilitates cancer progression and metastasis (Hofmeyer et al., 2008; Chao et al., 2012; Zhao et al., 2013; Nygren et al., 2014). B7-H3 silencing led to cell cycle arrest at the G0/G1 phase and reduces cancer cell growth (Zhang et al., 2015). Lung tumors show a high level of B7-H3 expression (Chen et al., 2013), which might be caused by a down-regulation of miR-187, as a negative regulator. Our results indicated that overexpression of miR-187 (a negative regulator of B7-H3) in lung carcinoma cells caused cell cycle arrest at sub-G1/G1 phase. This finding was in accordance with B7-H3 knock down result. Thus it is possible that miR-187 performs its tumor suppressor functions by controlling G1 to S progression through B7-H3 post transcriptional regulation.

Dab2 is generally considered as a tumor suppressor, however, it promotes tumor progression through TGF-β induced epidermal mesenchymal transition (EMT) (Prunier and Howe, 2005; Thiery and Sleeman, 2006). Ectopic expression of miR-187 in cancer cells suppressed Dab2 and inhibited proliferation and migration of the cells (Chao et al., 2012).Therefore, the observed down-regulation of miR-187 could be a strategy used by lung tumor cells to recover Dab2 expression level in favor of tumor progression.

Based on our data, down-regulated miR-187 could be considered as a reliable biomarker for diagnosis of lung tumors. Moreover, considering its impact on cell cycle and validated targets, miR-187 could have critical impact on lung tumorigenesis. Thus, a significant alteration in its expression level could potentially reflect changes in molecular signaling pathways that control cellular transformation in lung.

MiR-134 is reported to be involved in cortical neural development and memory formation (Gao et al., 2010; Gaughwin et al., 2011). Considered as a powerful inducer of embryonic stem cells differentiation, miR-134 could promote cellular proliferation and counteract apoptosis in neural progenitor cells (Tay et al., 2008; Gaughwin et al., 2011; Poitz et al., 2013). According to the previous reports, miR-134 is upregulated in developing lung and modulates cell proliferation, apoptosis, and migration during lung septation (Williams et al., 2007; Zhang et al., 2012).

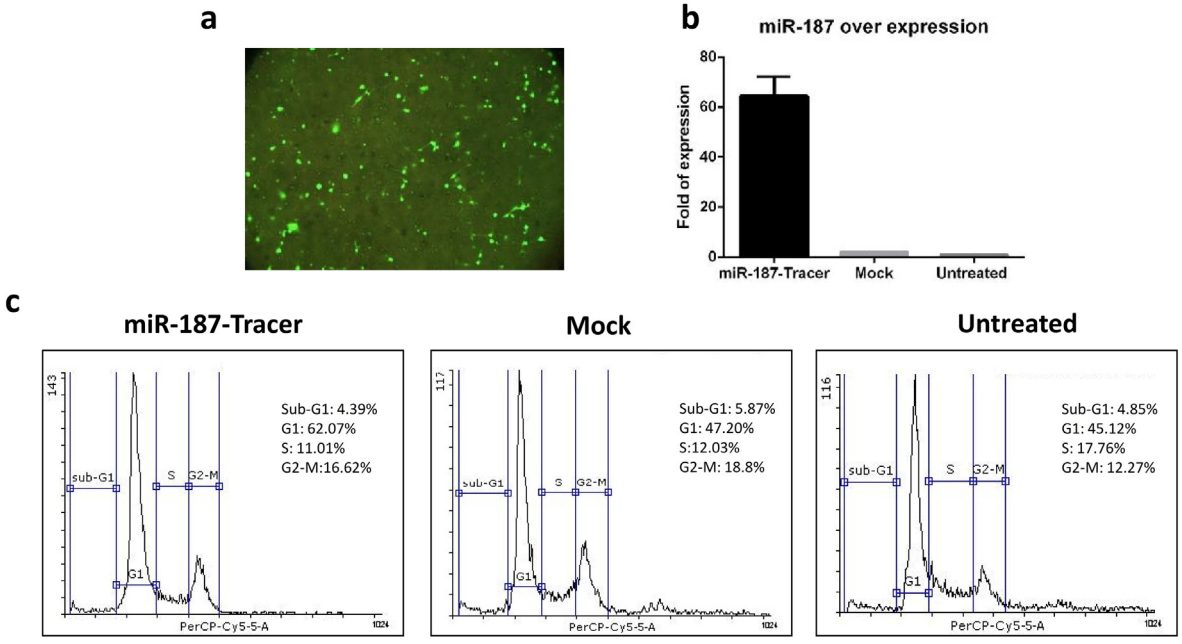

Fig. 4. Effect of miR-187 ectopic expression on lung carcinoma cell cycle. a) 24 h after transfecting A549 cells by miR-187-Tracer vector, cells were probed for positive GFP signal. b) The increase in miR-187 expression level in transfected cells was assessed by real time-PCR. C) Note that overexpression of miR-187 arrested the A549 cells at G1 phase.

Please cite this article as: Mirzadeh Azad, F., et al., Two lung development-related microRNAs, miR-134 and miR-187, are differentially expressed in lung tumors, Gene (2015), http://dx.doi.org/10.1016/j.gene.2015.11.040

Our finding on upregulation of miR-134 is in accordance with its developmental expression pattern, and highlights its probable oncogenic role in lung tissue. Recent studies have determined that miR-134 critically contributes to TGFβ-induced EMT in lung cancer cells. Its expression level increases after TGFβ treatment, and negatively regulates a tumor suppressor gene known as MAGI2 (Kitamura et al., 2014). MAGI2 act as a PTEN stabilizer and its expression leads to PI3K/Akt pathway inactivation (Tolkacheva et al., 2001; Valiente et al., 2005). Therefore, the observed upregulation of miR-134 could be considered as an initiator recruited for PI3K/Akt activation in lung cancerous cells.

Another validated target for miR-134 is WWOX, which codes for a tumor suppressor protein (Liu et al., 2014). WWOX has a decreased expression level in various solid tumors including lung carcinomas (Yendamuri et al., 2003; Fabbri et al., 2005; Iliopoulos et al., 2005). It functions as a pro-apoptotic factor which contributes to cellular proliferation regulatory pathways through interaction with ErBb2 and P73 (Aqeilan et al., 2004; Fabbri et al., 2005). Based on this functional analysis and our qPCR data, it could be hypothesized that transforming lung cells overexpress miR-134 in order to demolish WWOX effects.

Considering the fact that an ideal biomarker should have a high sensitivity and specificity(Hartwell et al., 2006), our data demonstrated that miR-134 is not a suitable biomarker for lung cancer. This could be due to its expression pattern which reached to high level in some samples (including late grade samples). This scattered high values demands for higher threshold in ROC analysis that leads to lower sensitivity. Nevertheless, the observed upregulation of miR-134 in lung tumor samples is in accordance with its validated oncogenic functions.

In conclusion, we have indicated the re-activation of two lung-development related microRNAs expression patterns, miR-187 and miR-134, in lung tumor tissues. Our data revealed a significant down-regulation of miR-187 in lung tumors, its contribution to cell cycle regulation and that it could be applied as a potential biomarker for lung cancer detection. We also demonstrated a significant upregulation of miR-134 in lung tumor tissues. While its differential expression pattern did not reach to the score needed for a reliable biomarker, the important role of its validated targets in tumorigenesis suggest its potential role in lung cancer initiation and progression.

Conflict of interest

Authors declare no conflict of interests.

Acknowledgments

We would like to thank Ms. Mirzaei for her technical assistance. This work was funded by a research grant from Research Deputy of Ministry of Health and Medical Education of Iran (grant number 931208).

References

Aqeilan, R.I., Pekarsky, Y., Herrero, J.J., Palamarchuk, A., Letofsky, J., Druck, T., Trapasso, F., Han, S.Y., Melino, G., Huebner, K., et al., 2004. Functional association between Wwox tumor suppressor protein and p73, a p53 homolog. Proc. Natl. Acad. Sci. U. S. A. 101 (13), 4401–4406.

Bartel, D.P., 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116 (2), 281–297.

Bhaskaran, M., Wang, Y., Zhang, H., Weng, T., Baviskar, P., Guo, Y., Gou, D., Liu, L., 2009. MicroRNA-127 modulates fetal lung development. Physiol. Genomics 37 (3), 268–278.

Bonner, A.E., Lemon, W.J., Devereux, T.R., Lubet, R.A., You, M., 2004. Molecular profiling of mouse lung tumors: association with tumor progression, lung development, and human lung adenocarcinomas. Oncogene 23 (5), 1166–1176.

Brambilla, C., Fievet, F., Jeanmart, M., de Fraipont, F., Lantuejoul, S., Frappat, V., Ferretti, G., Brichon, P.Y., Moro-Sibilot, D., 2003. Early detection of lung cancer: role of biomarkers. Eur. Respir. J. Suppl. 39, 36 s–44 s.

Brennecke, J., Hipfner, D.R., Stark, A., Russell, R.B., Cohen, S.M., 2003. bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. Cell 113 (1), 25–36.

Chao, A., Lin, C.Y., Lee, Y.S., Tsai, C.L., Wei, P.C., Hsueh, S., Wu, T.I., Tsai, C.N., Wang, C.J., Chao, A.S., et al., 2012. Regulation of ovarian cancer progression by microRNA-187 through targeting Disabled homolog-2. Oncogene 31 (6), 764–775.

Chen, C., Shen, Y., Qu, Q.X., Chen, X.Q., Zhang, X.G., Huang, J.A., 2013. Induced expression of B7-H3 on the lung cancer cells and macrophages suppresses T-cell mediating anti-tumor immune response. Exp. Cell Res. 319 (1), 96–102.

Croce, C.M., 2009. Causes and consequences of microRNA dysregulation in cancer. Nat. Rev. Genet. 10 (10), 704–714.

Ettinger, D.S., Akerley, W., Borghaei, H., Chang, A.C., Cheney, R.T., Chirieac, L.R., D'Amico, T.A., Demmy, T.L., Ganti, A.K.P., Govindan, R., et al., 2012. Non-small cell lung cancer. J. Natl. Compr. Cancer Netw. 10 (10), 1236–1271.

Fabbri, M., Iliopoulos, D., Trapasso, F., Aqeilan, R.I., Cimmino, A., Zanesi, N., Yendamuri, S., Han, S.Y., Amadori, D., Huebner, K., et al., 2005. WWOX gene restoration prevents lung cancer growth in vitro and in vivo. Proc. Natl. Acad. Sci. U. S. A. 102 (43), 15611–15616.

Friedman, R.C., Farh, K.K., Burge, C.B., Bartel, D.P., 2009. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 19 (1), 92–105.

Gao, J., Wang, W.Y., Mao, Y.W., Graff, J., Guan, J.S., Pan, L., Mak, G., Kim, D., Su, S.C., Tsai, L.H., 2010. A novel pathway regulates memory and plasticity via SIRT1 and miR-134. Nature 466 (7310), 1105–1109.

Gaughwin, P., Ciesla, M., Yang, H., Lim, B., Brundin, P., 2011. Stage-specific modulation of cortical neuronal development by Mmu-miR-134. Cereb. Cortex 21 (8), 1857–1869.

Harris, K.S., Zhang, Z., McManus, M.T., Harfe, B.D., Sun, X., 2006. Dicer function is essential for lung epithelium morphogenesis. Proc. Natl. Acad. Sci. U. S. A. 103 (7), 2208–2213.

Hartwell, L., Mankoff, D., Paulovich, A., Ramsey, S., Swisher, E., 2006. Cancer biomarkers: a systems approach. Nat. Biotechnol. 24 (8), 905–908.

Herbst, R.S., Heymach, J.V., Lippman, S.M., 2008. Lung cancer. N. Engl. J. Med. 359 (13), 1367–1380.

Hoffman, P.C., Mauer, A.M., Vokes, E.E., 2000. Lung cancer. Lancet 355 (9202), 479–485.

Hofmeyer, K.A., Ray, A., Zang, X., 2008. The contrasting role of B7-H3. Proc. Natl. Acad. Sci. 105 (30), 10277–10278.

Hosseini, M., Naghan, P.A., Karimi, S., SeyedAlinaghi, S., Bahadori, M., Khodadad, K., Mohammadi, F., Kaynama, K., Masjedi, M.R., 2009. Environmental risk factors for lung cancer in Iran: a case–control study. Int. J. Epidemiol. 38 (4), 989–996.

Iliopoulos, D., Guler, G., Han, S.Y., Johnston, D., Druck, T., McCorkell, K.A., Palazzo, J., McCue, P.A., Baffa, R., Huebner, K., 2005. Fragile genes as biomarkers: epigenetic control of WWOX and FHIT in lung, breast and bladder cancer. Oncogene 24 (9), 1625–1633.

Ketting, R.F., 2011. microRNA biogenesis and function: an overview. Adv. Exp. Med. Biol. 700, 1–14.

Kitamura, K., Seike, M., Okano, T., Matsuda, K., Miyanaga, A., Mizutani, H., Noro, R., Minegishi, Y., Kubota, K., Gemma, A., 2014. MiR-134/487b/655 cluster regulates TGF-beta-induced epithelial-mesenchymal transition and drug resistance to gefitinib by targeting MAGI2 in lung adenocarcinoma cells. Mol. Cancer Ther. 13 (2), 444–453.

Lin, P.Y., Yu, S.L., Yang, P.C., 2010. MicroRNA in lung cancer. Br. J. Cancer 103 (8), 1144–1148.

Liu, H., Kohane, I., 2009. Tissue and process specific microrna — mrna co-expression in mammalian development and malignancy. PLoS ONE 4 (5).

Liu, X., Sempere, L.F., Ouyang, H., Memoli, V.A., Andrew, A.S., Luo, Y., Demidenko, E., Korc, M., Shi, W., Preis, M., et al., 2010. MicroRNA-31 functions as an oncogenic microRNA in mouse and human lung cancer cells by repressing specific tumor suppressors. J. Clin. Invest. 120 (4), 1298–1309.

Liu, C.J., Shen, W.G., Peng, S.Y., Cheng, H.W., Kao, S.Y., Lin, S.C., Chang, K.W., 2014. miR-134 induces oncogenicity and metastasis in head and neck carcinoma through targeting WWOX gene. Int. J. Cancer (*Journal international du cancer*) 134 (4), 811–821.

Mendell, J., 2008. miRiad roles for the miR-17-92 cluster in development and disease. *Cell* 133 (2), 217–222.

Nygren, M.K., Tekle, C., Ingebrigtsen, V.A., Makela, R., Krohn, M., Aure, M.R., Nunes-Xavier, C.E., Perala, M., Tramm, T., Alsner, J., et al., 2014. Identifying microRNAs regulating B7-H3 in breast cancer: the clinical impact of microRNA-29c. Br. J. Cancer 110 (8), 2072–2080.

Poitz, D.M., Stolzel, F., Arabanian, L., Friedrichs, J., Docheva, D., Schieker, M., Fierro, F.A., Platzbecker, U., Ordemann, R., Werner, C., et al., 2013. MiR-134-mediated beta1 integrin expression and function in mesenchymal stem cells. Biochim. Biophys. Acta 1833 (12), 3396–3404.

Prunier, C., Howe, P.H., 2005. Disabled-2 (Dab2) is required for transforming growth factor beta-induced epithelial to mesenchymal transition (EMT). J. Biol. Chem. 280 (17), 17540–17548.

Shenouda, S.K., Alahari, S.K., 2009. MicroRNA function in cancer: oncogene or a tumor suppressor? Cancer Metastasis Rev. 28 (3–4), 369–378.

Tay, Y., Zhang, J., Thomson, A.M., Lim, B., Rigoutsos, I., 2008. MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature 455 (7216), 1124–1128.

Thiery, J.P., Sleeman, J.P., 2006. Complex networks orchestrate epithelial-mesenchymal transitions. Nat. Rev. Mol. Cell Biol. 7 (2), 131–142.

Tolkacheva, T., Boddapati, M., Sanfiz, A., Tsuchida, K., Kimmelman, A.C., Chan, A.M., 2001. Regulation of PTEN binding to MAGI-2 by two putative phosphorylation sites at threonine 382 and 383. Cancer Res. 61 (13), 4985–4989.

Valiente, M., Andres-Pons, A., Gomar, B., Torres, J., Gil, A., Tapparel, C., Antonarakis, S.E., Pulido, R., 2005. Binding of PTEN to specific PDZ domains contributes to PTEN protein stability and phosphorylation by microtubule-associated serine/threonine kinases. J. Biol. Chem. 280 (32), 28936–28943.

Whitsett, J., Haitchi, H., Maeda, Y., 2011. Intersections between pulmonary development and disease. Am. J. Respir. Crit. Care Med. 184 (4), 401–406.

Please cite this article as: Mirzadeh Azad, F., et al., Two lung development-related microRNAs, miR-134 and miR-187, are differentially expressed in lung tumors, Gene (2015), http://dx.doi.org/10.1016/j.gene.2015.11.040

Williams, A.E., Moschos, S.A., Perry, M.M., Barnes, P.J., Lindsay, M.A., 2007. Maternally imprinted microRNAs are differentially expressed during mouse and human lung development. Dev. Dyn. 236 (2), 572–580.

Yendamuri, S., Kuroki, T., Trapasso, F., Henry, A.C., Dumon, K.R., Huebner, K., Williams, N.N., Kaiser, L.R., Croce, C.M., 2003. WW domain containing oxidoreductase gene expression is altered in non-small cell lung cancer. Cancer Res. 63 (4), 878–881.

Zhang, X., Wang, H., Zhang, S., Song, J., Zhang, Y., Wei, X., Feng, Z., 2012. MiR-134 functions as a regulator of cell proliferation, apoptosis, and migration involving lung septation. In Vitro Cell. Dev. Biol. Anim. 48 (2), 131–136.

Zhang, Y., Yang, D., Weng, L., Wang, L., 2013. Early lung cancer diagnosis by biosensors. Int. J. Mol. Sci. 14 (8), 15479–15509.

Zhang, W., Wang, J., Wang, Y., Dong, F., Zhu, M., Wan, W., Li, H., Wu, F., Yan, X., Ke, X., 2015. B7-H3 silencing by RNAi inhibits tumor progression and enhances chemosensitivity in U937 cells. Onco. Targets Ther. 8, 1721–1733.

Zhao, J., Lei, T., Xu, C., Li, H., Ma, W., Yang, Y., Fan, S., Liu, Y., 2013. MicroRNA-187, down-regulated in clear cell renal cell carcinoma and associated with lower survival, inhibits cell growth and migration though targeting B7-H3. Biochem. Biophys. Res. Commun. 438 (2), 439–444.

Please cite this article as: Mirzadeh Azad, F., et al., Two lung development-related microRNAs, miR-134 and miR-187, are differentially expressed in lung tumors, Gene (2015), http://dx.doi.org/10.1016/j.gene.2015.11.040