Original Article

4-(methylnitrosamino)-1-(3-pyridyl) -1-butanone Induces Circulating MicroRNA Deregulation in Early Lung Carcinogenesis*

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Abstract

Objective To study the alteration of circulating microRNAs in 4-(methylnitrosamino)-1-(3-pyridyl) -1-butanone (NNK)-induced early stage lung carcinogenesis.

Methods A lung cancer model of male F344 rats was induced with systemic NNK and levels of 8 lung cancer-associated miRNAs in whole blood and serum of rats were measured by quantitative RT-PCR of each at weeks 1, 5, 10, and 20 following NNK treatment.

Results No lung cancer was detected in control group and NNK treatment group at week 20 following NNK treatment. The levels of some circulating miRNAs were significantly higher in NNK treatment group than in control group. The miR-210 was down-regulated and the miR-206 was up-regulated in NNK treatment group. The expression level of circulating miRNAs changed from week 1 to week 20 following NNK treatment.

Conclusion The expression level of circulating miRNAs is related to NNK-induced early stage lung carcinogenesis in rats and can therefore serve as its potential indicator.

Key words: NNK; Circulating microRNA; Early stage; Lung carcinogenesis

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INTRODUCTION

Lung cancer is one of the most common types of cancer and a leading cause of cancer death in the world. Although great efforts have been made to improve its treatment, the 5-year survival rate of lung cancer patients still remains low over the past 30 years. Many studies indicate that the 5-year survival rate of lung cancer patients strongly correlates with diagnosis time. Unfortunately, owing to lack of valid biomarkers or indicators are not available for the diagnosis of early

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lung cancer, most of lung cancer patients are diagnosed at the advanced stage. It is, therefore, important to explore effective biomarkers or indicators for timely diagnosis of early lung cancer.

MicroRNAs (miRNAs) are a class of small endogenous noncoding RNAs (approximately 19-25 nt) that post-transcriptionally and negatively regulate the expression of messenger RNAs (mRNAs) in general. A large number of studies demonstrated that miRNAs are involved in a variety of biological processes, including initiation and progression of cancer, and cell growth, proliferation, apoptosis, etc[1-3]. It was reported that expression of miRNAs is dysregulated in lung cancer patients[4-6] and many studies indicate that circulating miRNAs may serve as a stable, non-invasive marker for cancer[7-9]. A recent study showed that chemical carcinogens induce alteration of miRNAs[10]. Dysregulation of certain miRNAs is associated with initiation and other events in carcinogenesis[11-14]. To date, the alteration of circulating miRNA expression induced by environmental carcinogens, such as NNK, still remains largely unknown.

NNK is a tobacco-specific N-nitrosamine and a potent lung carcinogen in rats, mice, and hamsters. NNK-induced lung tumor exhibits remarkable organ specificity. The systemic administration of NNK in rats is a reproducible and robust method to induce lung tumors. Male F344 rats are highly sensitive to NNK-induced lung cancer[15]. In the present study, a lung cancer model of male F344 rats was established by systemic administration of NNK. Lung cancer-associated candidate miRNAs in whole blood and serum of F344 rats were analyzed by quantitative RT-PCR, candidate circulating microRNAs were identified in each animal, and dynamic circulating microRNA levels were measured at different time points during NNK administratio, indicating that NNK induces alteration of circulating miRNAs in early lung cancer with dynamic change of circulating miRNAs, and circulating miR-210 and miR-206 can serve as a potential indicator of NNK-induced early stage lung carcinogenesis.

MATERIALS AND METHODS

Animal Treatment

NNK >99% was purchased from International Laboratory (San Francisco, CA, USA). Sixteen 5-week old male F344 rats, purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd., were raised at the specific pathogen free (SPF) animal house of China State Key Laboratory of Respiratory Disease and acclimated to the laboratory environment for 2 weeks prior to administration of NNK. The animals were then randomly assigned to control group and NNK treatment group (8 in each group), and maintained in a 12/12 hour light-dark cycle at a constant temperature of 25±1 °C with a humidity of 50%-70%. They received a basal diet, and their body weight was measured every week. No significant difference was observed in the body weight between control group and NNK treatment group before NNK treatment. A rat lung cancer model was established as previously described[16]. Briefly, 0.3 mL saline and NNK (0.0055 mmol/kg) containing 0.3 mL saline (newly prepared prior to each administration) were subcutaneously injected into the rats of control group and NNK treatment group, 3 times a week for 20 weeks. Two rats randomly selected from control group and NNK treatment group at week 20 were sacrificed to detect whether lung tumor was induced. The animals were then maintained in a SPF animal house until sacrificed. The study was approved by Guangzhou Medical University Institutional Review Board and Institution Animal Care and Use Committee.

Collection of Whole Blood, Serum, and Lung Tissues of Rats

Approximately 1.0 mL whole blood was collected from each rat by orbital bleeding at weeks 1, 5, 10, and 20, respectively, following NNK treatment. A volume of 250 μL whole blood was added into a 2-mL microcentrifuge tube containing 650 μL RNAlater solution (Ambion, Austin, TX, USA), and mixed thoroughly by inverting the tube. The samples were then incubated in RNAlater solution overnight at 4 °C and kept at -20 °C until use. The remaining whole blood was utilized for serum isolation. Blood samples were kept at room temperature for at least 30 min, and then centrifuged at 1 600 × g for 15 min at 4 °C. Serum was put into a 1.5-mL microcentrifuge tube for further centrifugation at 16 000 × g for 15 min at 4 °C, then transferred into a new microcentrifuge tube and stored at -80 °C until RNA extraction. All rats were anesthetized with CO_2 and sacrificed. Their normal lung tissues and lung tumor tissues were isolated and placed into sterile freezing vials, and stored immediately in liquid nitrogen for further use.

Cell Culture

A transformed malignant human bronchial epithelial cell line 16HBE-T, established in our previous study[17], was used. Human bronchial epithelial cell line 16HBE was selected as normal control cells which were maintained in a minimum essential medium (MEM; Invitrogen, Carlsbad, CA) containing 10% heat-inactivated fetal bovine serum (FBS, Sijiqing, Hangzhou, China) at 37 °C in a humidified atmosphere containing 5% CO_2. Lung adenocarcinoma A549 cells were purchased from the Cell Bank of Experimental Animal Center of Sun Yat-sen University (Guangzhou, China). The cell lines were maintained in RPMI1640 (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated FBS at 37 °C in a humidified atmosphere containing 5% CO_2.

RNA Isolation from Whole Blood, Serum of Rats and Cell Lines

Total RNA was extracted respectively from the whole blood of rats using a RiboPure™-blood kit (Ambion, Austin, TX, USA) according to manufacturer's instructions and from serum samples using a miRNeasy Mini kit (Qiagen, Hilden, Germany) according to the Qiagen supplementary protocol, and from lung cancer cell lines using Trizol reagent (Invitrogen, USA) according to its manufacturer's instructions. The quality and quantity of RNAs were assessed on A260/A280 nm reader using a NanoDrop1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). RNA integrity was determined by running RNA samples on a denaturing agarose gel stained with ethidium bromide. The ratio of 28S ribosomal RNA to 18S ribosomal RNA was 2:1, indicating that the RNA samples are intact.

Quantitative RT-PCR Analysis of MicroRNA

MiRNAs were analyzed by quantitative RT-PCR following the protocol of TaqMan microRNA assay (Applied Biosystems, Foster City, CA, USA). RNA was reversely transcribed using a TaqMan microRNA reverse transcription kit (Applied Biosystems), total RNA (10 ng) was utilized in RT reactions (at 16 °C for 30 min, at 42 °C for 30 min, at 85 °C for 5 min, and then at 4 °C), followed by specific TaqMan microRNA assay using the Biosystem Prism 7 500 RT-PCR System (Applied Biosystems). Mature miRNAs were detected and quantified. The reaction mixture containing 1.00 μL TaqMan® small RNA assay (20×), 1.33 μL RT product, 10 μL TaqMan® universal PCR master mix II (2×) and 7.67 μL nuclease-free water was incubated at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 15 s and an extension at 60 °C for 1 min. The relative expression of miRNAs was detected using a $2^{-\Delta Ct}$ method. MiR-16 was used as an internal standard gene to normalize the data in whole blood or serum samples ($\Delta Ct=Ct_{interested\ miRNA}-Ct_{miR-16}$). Relative expression of miRNAs in cell samples was detected using a $2^{-\Delta\Delta Ct}$ method and normalized with RNU6B endogenous control. ΔCt was calculated by subtracting the Ct value of RNUB6 from the Ct value of the interested miRNA and then ΔΔCt was calculated by subtracting the mean ΔCt of 16HBE cells from the ΔCt of lung cancer cells. All experiments were repeatedly performed at least 3 times.

Statistical Analysis

The data from the present study were analyzed by ANOVA using the SPSS software (version 13.0, IL, USA). All p-values are two-sided and $P<0.05$ was considered statistically significant. Non-parameters were compared by Mann-Whitney test.

RESULTS

NNK-induced Early Stage Lung Carcinogenesis Model in Rat

Ninety-five weeks after the early lung cancer model of rats was induced by NNK as previously described[16], a lung adenocarcinoma was detected at histopathological examination and the carcinoma is consistent with the findings in previous study[16]. Fourteen lung tumors were detected in total in NNK treatment group but not in control group. The incidence of lung cancer was significantly higher in NNK treatment group than in control group (100% *vs* 0%, $P<0.01$). No lung tumor was detected in control group and NNK treatment group at week 20 after NNK treatment, showing that NNK can induce early stage lung carcinogenesis model in rats.

Selection of Lung Cancer-associated Candidate MiRNAs

Papers on lung cancer-associated miRNAs covered in PubMed were analyzed and the significantly up-regulated or down-regulated miRNAs in lung cancer tissues were selected as candidate

miRNAs for further study. The levels of MiR-21, miR-195, miR-29b, miR-210, miR-20a, and miR-17-5p were significantly higher in lung cancer tissues than in normal lung tissues, which are consistent with the findings in previous studies[2,4-6,18-19], whereas the levels of miR-206 and miR-30a were significantly lower in lung cancer tissues than in normal lung tissues[5,20-21], which are also consistent with the findings in previous studies, indicating that they are associated with lung cancer. The 8 lung cancer-associated miRNAs were selected as candidate miRNAs for further study (Table 1). In the present study, the expression of these lung miRNAs in rats with early lung cancer were measured after NNK treatment.

Individual Expression of Candidate miRNAs in Circulation by quantitative RT-PCR

To investigate whether circulating miRNAs can serve as an indicator for NNK-induced early lung cancer, the expression of candidate miRNAs in rats were measured by quantitative RT-PCR at week 20 following NNK treatment. The miR-16 is one of the most stably expressed miRNAs in human serum or in whole blood as previously reported[22-23]. However, no significant difference was found in the expression level of miR-16 between control group and NNK treatment group in the present study (Figures 1A and 1B) and miR-16 was then used in this study as an endogenous control gene to normalize the data.

Table 1. Expression of Candidate Circulating MiRNAs in Rats after NNK treatment

MiRNA of Interest	MiRNA Expression in Lung Cancer	References
miR-21	Up regulation	[4];[5];[18]
miR-195	Up regulation	[6]
miR-29b	Up regulation	[6]
miR-206	Down regulation	[20]
miR-210	Up regulation	[4];[20]
miR-20a	Up regulation	[2];[6]
miR-30a	Down regulation	[5];[20];[21]
miR-17-5p	Up regulation	[19]

The expression level of miR-210 was significantly lower whereas that of miR-206 was significantly higher in rat whole blood of NNK treatment group than in control group (P=0.0379; P=0.0011, Figure 2A and 2B). However, no significant difference was observed in the expression analysis of miR-21, miR-20a, miR-30a, miR-29b, miR-195, and miR-17-5p between control group and NNK treatment group (data not shown). In addition, the expression level of miR-210 was significantly lower and that of miR-206 was significantly higher in rat serum of NNK treatment group than in control group (Figures 2C and 2D), indicating that the expression levels of lung cancer-associated miRNAs are similar in rat whole blood and serum at early stage lung carcinogenesis.

Dynamic Changes of Circulating miRNA Expression in Rats with NNK-Induced Early Lung Cancer

No significant difference was found in the base line levels of miR-210 and miR-206 between control group and NNK treatment group before NNK treatment (data not shown). The level of miR-210 was significantly lower in rat whole blood and serum of NNK treatment than in control group at weeks 1, 5, 10, and 20 respectively (Figure 3A and 3C). In addition, the level of miR-206 was significantly higher in rat whole blood and serum of NNK treatment than in control group (Figure 3B and 3D). The expressions of miR-210 and miR-206 in rat whole blood were consistent with that in serum of the animals (Figure 3), indicating that the expression level of circulating miRNAs is different in rats with NNK-induced early lung cancer.

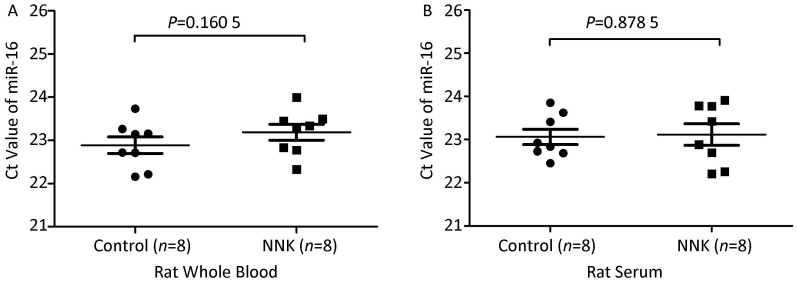

Figure 1. Scattered Ct values of miR-16 in control group (n=8) and NNK treatment group (n=8). The data were analyzed by the Mann-whitney test and expressed as mean±SEM.

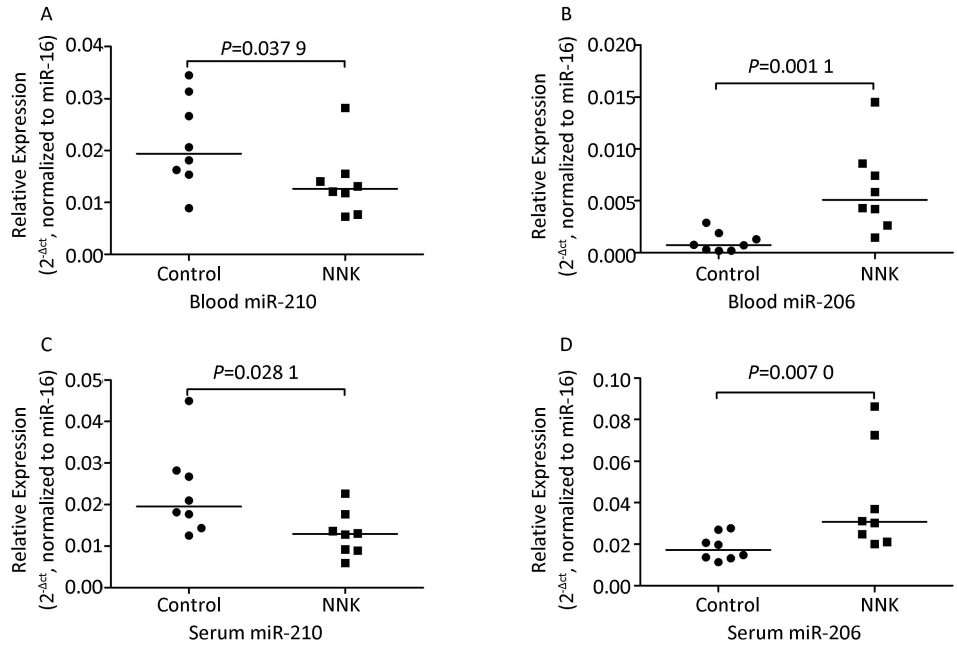

Figure 2. Expression of circulating miR-210 (A), miR-206 (B), and serum miR-210 (C), and serum miR-206 (D) in rats with early lung caner at week 20 following NNK treatment.

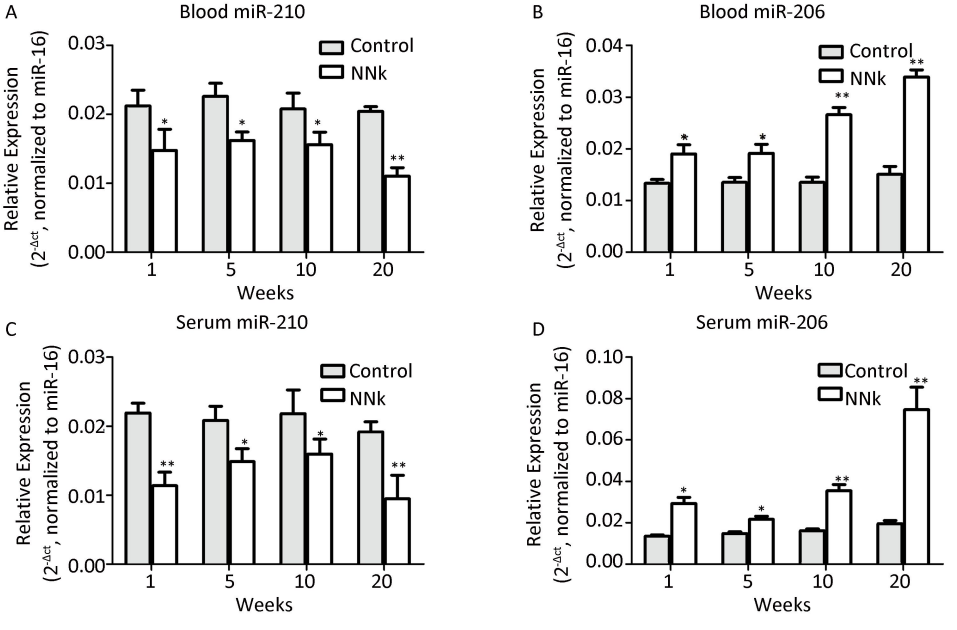

Figure 3. Expression of circulating miR-210 (A) and miR-206 (B), serum miR-210 (C) and miR-206 (D) at different time points following NNK treatment. Data are presented as mean±SEM and analyzed by Mann-Whitney test. $^{*}P<0.05$; $^{**}P<0.01$.

Expression of miR-210 and miR-206 in Lung Adenocarcinoma Cell Lines

Because lung adenocarcinoma can be induced by NNK, the expression levels of miR-210 and miR-206 were measured in transformed malignant human bronchial epithelial cell line 16HBE-T and lung adenocarcinoma cell line A549 also. The expression level of miR-210 was significantly higher whereas that of miR-206 was significantly lower in these two cell lines 16HBE-T and A549 than in the normal human bronchial epithelial cell line 16HBE (Figure 4A and 4B).

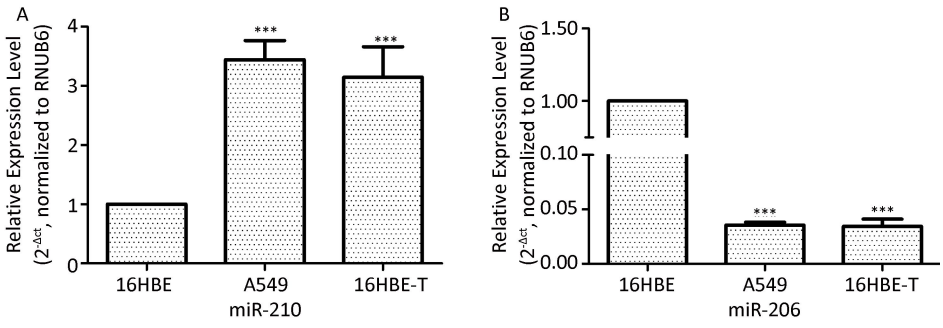

Figure 4. Quantitative RT-PCR showing expression of miR-210 (A) and miR-206 (B) in lung cancer cell lines. Data are presented as mean±SEM and analyzed by ANOVA. ***, $P<0.001$.

DISCUSSION

There is evidence that miRNAs are associated with lung cancer initiation and progression. It was reported that circulating miRNAs are a potentially stable biomarker or indicator for this carcinogenesis. However, the reports on this are few. In the present study, a lung cancer model of rats was successfully established by administration of NNK as previously described[16] and the alteration of lung cancer-associated circulating miRNAs was observed.

Chen T et al.[24] reported that the expression of 38 miRNAs are significantly higher than those of 9 miRNAs in rat liver tissue. Juhász K et al.[25] revealed that 7, 12-dimethylbenz(α)anthracene (DMBA) can significantly up-regulate the expression of let-7, miR-21, and miR-146a in CBA/CA mice. We previously reported that the miR-106a can potentially function as an oncogene in NNK–induced transformation[26] and NNK can induce alteration of lung cancer-associated circulating miRNAs in rats. The expression levels of miR-210 and miR-206 were significantly lower at week 20 following NNK treatment. To date, few reports are available on the alteration of circulating miR-210 and miR-206 in animals with lung cancer. Miyachi et al.[27] reported that circulating miR-206 is a potential diagnostic marker for rhabdomyosarcoma, indicating that chemical carcinogens potentially induce alteration of circulating miRNA and changes of certain lung cancer-associated miRNAs occur in rats with NNK-induced early lung cancer.

In the present study, the expression of miR-210 was significantly lower in NNK treatment group than in control group at week 1 following NNK treatment whereas that of circulating miR-206 was significantly higher in NNK treatment group than in control group from week 1 to week 20 after NNK treatment. These demonstrate that alteration of circulating miRNA expression occurs at week 1 after NNK treatment. Li et al.[28] reported that expression of miRNAs in mouse liver tissue are correlated with lenght of time following *N*-ethyl-*N*-nitrosourea treatment. Similarly, dynamic changes of circulating miR-210 and miR-206 were observed in NNK-induced early lung cancer tissues at differing time points.

The expression of MiR-210 was significantly higher whereas that of miR-206 was significantly lower in lung adenocarcinoma cell lines in this study. To our knowledge, the miR-210 and miR-206 are usually over-expressed or poorly-expressed in lung cancer tissues[4-5,20]. In the present study, the expression of circulating miR-210 was significantly lower whereas that of miR-206 was significantly higher in rats with NNK-induced early lung cancer. Chen D et al.[29] reported that the miR-34a expression level is higher on day 1 following N-ethyl-N-nitrosourea exposure. However, the miR-34a expression level is usually lower in human tumor tissues[30-31] and higher in tissues chronically or sub-chronically exposed to carcinogens[32-33]. Tanaka et al.[34] reported that the miR-92 is poorly expressed in acute leukemia and over-expressed in acute leukemia tumor cells. The mechanism underlying the different in expressions of miRNAs in circulation and tumor cell lines is still unclear. It was reported that miRNAs are packaged inside exosomes which can be specifically digested by or released from cancer cells. Although the mechanism and function of circulating miRNAs in tumor biology remain unclear, findings in the present study indicated that circulating miRNAs are a potential indicator for chemical carcinogens –induced early lung cancer.

Sukata et al.[36] demonstrated that chemical carcinogen-induced liver cancer can aberrantly fluctuate the circulating miRNAs in a rat model and circulating miRNAs and is associated with human

prostate cancer. To the best of our knowledge, few research reports are available on circulating miRNAs in early lung cancer.

In summary, NNK can induce the alteration of circulating miRNAs in early lung cancer in rats. Circulating miR-210 and miR-206 may be a potential indicator for NNK-induced early lung cancer.

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REFERENCES

1. Imam JS, Buddavarapu K, Lee-Chang JS, et al. MicroRNA-185 suppresses tumor growth and progression by targeting the Six1 oncogene in human cancers. Oncogene, 2010; 29, 4971-9.
2. Hayashita Y, Osada H, Tatematsu Y, et al. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res, 2005; 65, 9628-32.
3. Cimmino A, Calin GA, Fabbri M, et al. MiR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci USA, 2005; 102, 13944-9.
4. Yanaihara N, Caplen N, Bowman E, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell, 2006; 9, 189-98.
5. Seike M, Goto A, Okano T, et al. MiR-21 is an EGFR-regulated anti-apoptotic factor in lung cancer in never-smokers. Proc Natl Acad Sci U S A, 2009; 106, 12085-90.
6. Landi MT, Zhao Y, Rotunno M, et al. MicroRNA expression differentiates histology and predicts survival of lung cancer. Clin Cancer Res, 2010; 16, 430-41.
7. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A, 2008; 105, 10513-8.
8. Chen X, Ba Y, Ma L, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res, 2008; 18, 997-1006.
9. Moussay E, Wang K, Cho JH, et al. MicroRNA as biomarkers and regulators in B-cell chronic lymphocytic leukemia. Proc Natl Acad Sci USA, 2011; 108, 6573-8.
10. Chen T. The role of MicroRNA in chemical carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 2010; 28, 89-124.
11. Bhatti I, Lee A, Lund J, et al. Small RNA: a large contributor to carcinogenesis? J Gastrointest Surg, 2009; 13, 1379-88.
12. Datta J, Kutay H, Nasser MW, et al. Methylation mediated silencing of MicroRNA-1 gene and its role in hepatocellular carcinogenesis. Cancer Res, 2008; 68, 5049-58.
13. Hagan JP, Croce CM. MicroRNAs in carcinogenesis. Cytogenet Genome Res, 2007; 118, 252-9.
14. Osada H, Takahashi T. MicroRNAs in biological processes and carcinogenesis. Carcinogenesis, 2007; 28, 2-12.
15. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst, 1999; 91, 1194-210.
16. Hecht SS, Trushin N, Castonguay A, et al. Comparative tumorigenicity and DNA methylation in F344 rats by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and N-nitroso-dimethylamine. Cancer Res, 1986; 46, 498-502.
17. Zhou L, Jiang Y, Tan A, et al. Silencing of N-Ras gene expression using shRNA decreases transformation efficiency and tumor growth in transformed cells induced by anti-BPDE. Toxicol Sci, 2008; 105, 286-94.
18. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA, 2006; 103, 2257-61.
19. Raponi M, Dossey L, Jatkoe T, et al. MicroRNA classifiers for predicting prognosis of squamous cell lung cancer. Cancer Res, 2009; 69, 5776-83.
20. Võsa U, Vooder T, Kolde R, et al. Identification of miR-374a as a prognostic marker for survival in patients with early-stage nonsmall cell lung cancer. Genes Chromosomes Cancer, 2011; 50, 812-22.
21. Yang Y, Li X, Yang Q, et al. The role of microRNA in human lung squamous cell carcinoma. Cancer Genet Cytogenet, 2010; 200, 127-33.
22. Ng EK, Chong WW, Jin H, et al. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. Gut, 2009; 58, 1375-81.
23. Heneghan HM, Miller N, Lowery AJ, et al. Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg, 2010; 251, 499-505.
24. Chen T, Li Z, Yan J, et al. MicroRNA expression profiles distinguish the carcinogenic effects of riddelliine in rat liver. Mutagenesis, 2012; 27, 59-66.
25. Juhász K, Gombos K, Szirmai M, et al. DMBA induces deregulation of miRNA expression of let-7, miR-21 and miR-146a in CBA/CA mice. *In Vivo*, 2012; 26, 113-7.
26. Jiang Y, Wu Y, Greenlee AR, et al. miR-106a-mediated malignant transformation of cells induced by anti-benzo[a]pyrene-trans-7,8-diol-9,10-epoxide. Toxicol Sci, 2011; 119, 50-60.
27. Miyachi M, Tsuchiya K, Yoshida H, et al. Circulating muscle-speci?c microRNA, miR-206, as a potential diagnostic marker for rhabdomyosarcoma, Biochem. Biophys Res Commun, 2010; 400, 89-93.
28. Li Z, Branham WS, Dial SL, et al. Genomic analysis of microRNA time-course expression in liver of mice treated with genotoxic carcinogen N-ethyl-N-nitrosourea. BMC Genomics, 2010; 11, 609; doi: 10.1186/1471-2164-11-609.
29. Chen D, Li Z, Chen T. Increased expression of miR-34a in mouse spleen one day after exposure to N-ethyl-N-nitrosourea. J Appl Toxicol, 2011; 31, 496-8.
30. Chang TC, Wentzel EA, Kent OA, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell, 2007; 26, 745-52.
31. Corney DC, Hwang CI, Matoso A, et al. Frequent downregulation of miR-34 family in human ovarian cancers. Clin Cancer Res, 2010; 16, 1119-28.
32. Rokhlin OW, Scheinker VS, Taghiyev AF, et al. MicroRNA-34 mediates AR-dependent p53-induced apoptosis in prostate cancer. Cancer Biol Ther, 2008; 7, 1288-96.
33. Yamakuchi M, Lowenstein CJ. MiR-34, SIRT1 and p53: the feedback loop. Cell Cycle, 2009; 8, 712-5.
34. Tanaka M, Oikawa K, Takanashi M, et al. Down-regulation of miR-92 in human plasma is a novel marker for acute leukemia patients. PLoS One, 2009; 4, e5532.
35. Sukata T, Sumida K, Kushida M, et al. Circulating microRNAs, possible indicators of progress of rat hepatocarcinogenesis from early stages. Toxicol Lett, 2011; 200, 46-52.
36. Selth LA, Townley S, Gillis JL, et al. Discovery of circulating microRNAs associated with human prostate cancer using a mouse model of disease.Int J Cancer, 2011; doi: 10.1002/ijc.26405.