MicroRNA Expression Profiles and MiR-10a Target in Anti-benzo[a] pyrene-7, 8-diol-9, 10-epoxide-transformed Human 16HBE Cells[1]

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Objective To screen miRNA profiles of malignantly transformed human bronchial epithelial cells, 16HBE-T, induced by anti-benzo[a]pyrene-trans-7,8-diol-9,10-epoxide (anti-BPDE), and to analyze putative miR-10a targets in 16HBE-T. **Methods** A novel microarray platform was employed to screen miRNA profiles of 16HBE-T cells transformed by anti-BPDE. Microarray data for miR-10a and miR-320 were validated using quantitative real time polymerase chain reaction (QRT-PCR). The expression of a putative target for miR-10a, HOXA1, was analyzed by reverse transcription polymerase chain reaction (RT-PCR) and QRT-PCR. **Results** In comparison with the vehicle-treated cells (16HBE-N), 16HBE-T exhibited differential expression of 54 miRNAs, in which, 45 were over-expressed and 9 were down-regulated. The five most highly expressed miRNAs were miR-494, miR-320, miR-498, miR-129, and miR-106a. The lowest expressed miRNAs were miR-10a, miR-493-5p, and miR-363*. Three members of miR-17-92 cluster, miR-17-5p, miR-20a, and miR-92, showed significantly higher abundance in 16HBE-T as miR-21, miR-141, miR-27a, miR-27b, miR-16 and miRNAs of the let-7 family. The putative target for miR-10a, HOXA1 mRNA was up-regulated 3-9-fold in 16HBE-T, as compared with 16HBE-N. **Conclusion** The findings of the study provide information on differentially expressed miRNA in malignant 16HBE-T, and also suggest a potential role of these miRNAs in cell transformation induced by anti-BPDE. HOXA1 is similarly up-regulated, suggesting that miR-10a is associated with the process of HOXA1-mediated transformation.

Key words: MicroRNA; Anti-BPDE; Malignant transformation; 16HBE

INTRODUCTION

Benzo[a]pyrene (B[a]P) is a member of the polycyclic aromatic hydrocarbon (PAH) family, which includes ubiquitous environmental contaminants generated from industrial processes, vehicle exhaust and cigarette smoke. Human exposure to B[a]P is associated with increased risk of cancers, including lung cancer and uterine cervix cancer[1]. B[a]P undergoes metabolic activation after entering mammalian cells to produce highly toxic metabolic intermediates, which can irreversibly damage cellular macromolecules[2-3]. Anti-benzo[a]pyrene-trans-7, 8-diol-9, 10-epoxide (anti-BPDE) is the most important metabolite of B[a]P, and anti-BPDE perturbation of cell signaling is critical in the carcinogenic process of B[a]P[4]. However, the carcinogenic mechanism induced by B[a]P and anti-BPDE remains unknown .

MicroRNAs (miRNAs) are an abundant class of small (≈22 nucleotides), non-coding RNAs, processed sequentially from primary miRNAs (pri-miRNAs) and precursor transcripts (pre-miRNAs)[5-7]. Recent evidence has shown that miRNAs have diverse functions, including the regulation of cellular development, differentiation, proliferation and apoptosis[8-10]. MiRNAs regulate gene expression at the post-transcriptional level by base pairing to 3' untranslated region of target messenger RNAs (mRNAs), and work either by direct cleavage of the target mRNAs or by inhibition of protein synthesis[11]. The expression of miRNA is

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Abbreviations: anti-BPDE, anti-benzo[a]pyrene-trans-7,8-diol-9,10-epoxide; HBE, human bronchial epithelial cell line; miRNA, microRNA; mRNA, messenger ribonucleic acid; QRT-PCR, quantitative real time polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; 16HBE-N, control untransformed 16HBE cell; 16HBE-T, anti-BPDE-transformed 16HBE cell.

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highly specific for tissue or developmental stage. It is estimated that the vertebrate genome encodes up to 1 000 unique miRNAs, which are predicted to regulate expression of at least 30% of all genes. More than 500 miRNAs have been identified in humans. Emerging evidence suggests that altered regulation of miRNAs is potentially involved in the pathogenesis of a number of human cancers, including lung cancer[12-14], lymphocytic leukemia[15], and cholangiocarcinoma[16]. However, much remains to be learned about their precise cellular function as tumor suppressors or oncogenes[17].

To better understand the molecular mechanisms of cell transformation induced by anti-BPDE, this study investigated differentially expressed miRNAs in anti-BPDE-transformed cells. A high-throughput miRNA microarray approach was employed to screen miRNA profiles in anti-BPDE-transformed human bronchial epithelial cells, 16HBE-T. Microarray data were confirmed using quantitative real time polymerase chain reaction (QRT-PCR).

The RNA target of miR-10a was predicted by computational methods[18] and verified in humans[19]. It locates within the HOXB cluster on 17q21, and is associated with risk of human megakaryocytopoiesis and adult acute myeloid leukaemia[20]. In our study, we used bioinformatics to identify HOXA1 as a target for miR-10a. We further examined HOXA1 mRNA expression by employing reverse transcription polymerase chain reaction (RT-PCR) and QRT-PCR, and found that the expression levels of both miR-10a and HOXA1 genes were elevated, suggesting that these miRNAs may play an important role in chemical carcinogenesis processes. The study may also provide insights regarding therapeutic targets for prevention and treatment of human lung cancer.

MATERIALS AND METHODS

Cell Lines and RNA Isolation

A malignant transformed cell line derived from human bronchial epithelial cell line 16HBE induced by anti-BPDE (16HBE-T) and a control untransformed cell line (16HBE-N) were prepared as previously described[21]. The 16HBE cell line was kindly provided by Professor Jun XU (Guangzhou Institute of Respiratory Diseases, Guangzhou, Guangdong, China). Briefly, 16HBE cells were treated 4 times with anti-BPDE (98.3% purity, NCI Chemical Carcinogen Reference Standard Repository, Midwest Research Institute, Kansas City, MO, USA) at a concentration of 2.0 μmol/L and had 30 passages. The malignant features of the cells were assessed by soft agar culture and tumorigenesis in nude mice[21]. In contrast, 16HBE-N cells were treated with dimethylsulphoxide (DMSO; Sigma, Louis, MO, USA) solvent at a concentration equivalent to that used for creating 16HBE-T cells. Both cell lines were grown in MEM with 10% new-born calf serum, penicillin and streptomycin in a humidified atmosphere containing 5% CO_2 at 37℃. The total RNA samples of 16HBE-T and 16HBE-N of the thirtieth passages were isolated using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacture's instructions. RNA integrity was monitored using a 1% denaturing agarose gel.

Microarray Analysis

The miRNA microarray screening procedure has been described elsewhere[22-23]. Briefly, 5 μg total RNA sample from 16HBE-T or 16HBE-N cells was enriched for small RNAs using a RNeasy mini kit (Qiagen, Hilden, Germany), tailed using a miRCURY^{TM} array labeling kit (Exiqon, Vedbaek, Denmark), and labeled with hy3 fluorescent dyes (Exiqon, Vedbaek, Denmark). RNA samples of 16HBE-T and 16HBE-N cells were mixed and incubated for 16 h with miRCURY™ LNA arrays (Exiqon, Vedbaek, Denmark). Slides (Exiqon, Vedbaek, Denmark) were prepared in quadruplicate using robotics for spotting of 327 capture probes, perfectly matched probes for all human miRNAs as registered and annotated in the miRBase release 8.0 at the Welcome Trust Sanger Institute. The capture probes were oligonucleotides enhanced by locked nucleic acid (LNA™). By varying the LNA™ content and the length of capture probes, the probes were Tm-normalized to hybridize optimally under conditions of the product protocol. Spike-in synthetic controls were used to assure optimal labeling, hybridization, normalization and discrimination. U6 snRNA was used as a positive control, and a non-target capture probe carrying hy3 fluorescent label was used for slide orientations (landing lights). After hybridization, microarray was scanned with a Genepix 4000B scanner (Axon, Foster City, CA, USA) and raw data were imported into Genepix pro 6.0 software (Axon, Foster City, CA, USA). Calculations were done and expression maps were generated with a significance analysis of microarrays (SAM) for Excel.

Relative QRT-PCR for MiR-10a and MiR-320

As compared with the control 16HBE-N cells, miR-10a and miR-320 were up- and down-regulated in 16HBE-T cells, respectively. MiRNA microarray data were validated for miR-10a and miR-320 using normalized relative QRT-PCR. Total RNA from 16HBE-T or 16HBE-N cells was exposed to RNase-free DNase I (Takara, Dalian, China) before

reverse transcription. The miRNA sequence-specific stem-loop reverse transcription primers for miR-10a, miR-320 and endogenous control U6 snRNA were purchased from GenePHarma Company (Shanghai, China). Reverse transcriptase reaction containing 2 μg purified total RNA, 50 nmol/L stem–loop RT primer, 1×RT buffer, 0.25 mmol/L each of dNTPs, 3.33 U/mL MultiScribe reverse transcriptase and 0.25 U/mL RNase inhibitor, was carried out according to the manufacture's instructions using the SuperScript™ III first-strand synthesis SuperMix for QRT-PCR (Invitrogen, Carlsbad, CA, USA). The 25 μL reaction was incubated in a Tgradient thermocycler (Biometra, Gottingen, Germany), in a 96-well plate for 30 min at 16 ℃, for 30 min at 42 ℃, for 5 min at 85 ℃ and then held at 4 ℃. All reverse transcriptase reactions, including no-template controls and reverse transcription minus controls, were run in duplicate.

Relative QRT-PCR analysis was done on the Mx3000P instrument (Stratagene, Cedar Creek, TX, USA) using a hairpin-it^{TM} miRNAs real-time PCR quantitation kit (GenePHarma, Shanghai, China). The 20 μL PCR included 2 μL reverse transcriptase reaction product, 4 μmol/L miRNA specific primer set, 1× real-time PCR Master Mix, 1U Tag DNA polymerase, 1 mmol/L reference dye. The reaction was incubated in a 96-well plate at 94 ℃ for 3 min, followed by 45 cycles at 94 ℃ for 22 s and at 50 ℃ for 25 s. All reactions were run in triplicate and included no template and no reverse transcription controls for each gene. The threshold cycle (Ct) was defined as the fractional cycle number at which the fluorescence passed the fixed threshold. Template-negative control was also run in order to test for DNA-contaminated primers. The expression of U6 snRNA was used for normalization prior to comparison. Expression levels of miR-10a and miR-320 were assessed as previously described[24].

Absolute QRT-PCR for MiR-10a

MiR-10a was selected for absolute QRT-PCR detection to further verify the results of microarray and relative QRT-PCR tests. QRT-PCR reaction was prepared as described in the above section of *Relative QRT-PCR for MiR-10a and MiR-320*. The standard curve of miR-10a was plotted using serial dilutions of miR-10a product as templates. Total RNA was isolated from 16HBE-T and 16HBE-N cells and normalized before reactions. The absolute copy number of miR-10a was determined for the two cell lines using target-specific standard curves.

Bioinformatics Analysis for MiR-10a Target

Target analysis was performed for miR-10a as reports have shown its correlation with human cancer, and one of its predicted targets, HOXA1, might be involved in oncogenicity. MiR-10a targets were predicted using Targetscan (http://genes.mit.edu/targetscan), Pictar (http://pictar.bio.nyu.edu) and Miranda (www.microrna.org/miranda_new.html) softwares. Results from these three target databases were compared, and only common targets for miR-10a were evaluated. Among the predicted targets, we found one target which is directly related to the p44/42 MAP kinase pathway[25], HOXA1. Gain-of-function of HOXA1 protein has been found to be involved in human differential megakaryocytopoiesis[10]. MiR-10a has been shown to serve as a negative feedback regulator of HOXA1 action, and is considered important in the promotion of human mammary carcinoma[25]. We therefore examined HOXA1 expression in 16HBE-T cells.

RT-PCR for HOXA1

The total RNA of 16HBE-T or 16HBE-N cells of 2 μg was reverse transcribed to cDNA using random hexamer and thermoscript RT (Invitrogen, Carlsbad, CA, USA). The reverse transcriptase reactions were incubated in a T Gradient thermocycler (Biometra, Gottingen, Germany), in a 96-well plate for 10 min at 25 ℃, for 30 min at 48 ℃, for 5 min at 95 ℃ and then held at 4 ℃. All Reverse transcriptase reactions, including no-template controls and reverse transcription minus controls, were run in duplicate. The sequences of primer sets used to generate specific fragments were: HOXA1 (GenBank: S79910) forward primer 5'-TCC TGG AAT ACC CCA TAC TTA GCA-3' and reverse primer 5'-GCC GCC GCA ACT GTT G-3' (122bp), GAPDH (GenBank: NM_002046) forward primer 5'-TCA CCA GGG CTG CTT TTA ACT C-3' and reverse primer 5'-GGA ATC ATA TTG GAA CAT GTA AAC CAT-3' (101 bp). GAPDH was used for normalization. All primers were synthesized in Saibaisheng Gentech (Beijing, China). RT-PCR was performed with PCR mix (Promega, Madison, MI, USA) at 95 ℃ for 15 min, followed by 33 cycles at 95 ℃ for 15 s, at 60 ℃ for 30 s, at 72 ℃ for 1 min. End-point reaction products were analyzed on a 2% agarose gel (Takara, Dalian, China) stained with ethidium bromide to discriminate the correct amplification products from the potential primer dimers and densitometry results were analyzed using Tannon Gis software (Tannon, Shanghai, China).

QRT-PCR for HOXA1

cDNA and primer sets were prepared as described in 2.6. QRT-PCR was performed with

Brilliant® SYBR® green QPCR master mix (Stratagene, Cedar Creek, TX, USA) and conditions used were as follows: at 95 ℃ for 15 min, followed by 40 cycles at 95 ℃ for 15 s, at 60 ℃ for 30 s, at 72 ℃ for 1 min. Melting curve analysis (at 95 ℃ for 15 s, at 60 ℃ for 15 s, and at 60 ℃ for 15 s) was performed at the end of 40 cycles to verify the PCR product identity. All reactions were run in triplicate on a Mx3000P thermocycler (Stratagene, Cedar Creek, TX, USA). Data were analyzed using Mxpro software (Stratagene, Cedar Creek, TX, USA).

RESULTS

MiRNA Expression Profiles

The pattern of miRNA expression in 16HBE-T cells was markedly different from that in 16HBE-N cells. When relative expression of miRNAs was compared, 54 miRNAs were differentially expressed, of which, 45 miRNAs were up-regulated (＞2.00-fold) while 9 miRNAs were down-regulated (＜0.50-fold) in 16HBE-T cells (Table 1). The five most highly expressed miRNAs in 16HBE-T cells were miR-494, miR-320, miR-498, miR-129, and miR-106a (22.04-, 17.78-, 16.23-, 12.12-, and 11.20-fold change, respectively). The lowest expression of miRNAs was observed in miR-10a, miR-493-5p, and miR-363* (0.37-, 0.32-, 0.17-fold change, respectively). Three members of the miR-17-92 cluster, miR-20a, miR-17-5p, and miR-92, showed relatively higher abundance (8.70-, 6.37-, and 3.32-fold change, respectively. Members of the let-7 family, MiR-21, miR-141, miR-27a, miR-27b, and miR-16 showed a range of expression levels in 16HBE-T ranging from 2.70- to 5.71-fold change.

TABLE 1

Relative Expressions of 54 miRNAs in 16HBE-T Cells *versus* 16HBE-N Cells

miRNA	16HBE-T Cells Certified Value	16HBE-N Cells Certified Value	Fold Changes (16HBE-T/16 HBE-N)	miRNA	16HBE-T Cells Certified Value	16HBE-N Cells Certified Value	Fold Changes (16HBE-T/16 HBE-N)
miR-494	13.00	0.59	22.04	miR-324-3p	3.76	1.22	3.08
miR-320	45.14	2.54	17.78	miR-133a-133b	3.29	1.08	3.05
miR-498	23.93	1.47	16.23	let-7c	3.80	1.29	2.93
miR-129	14.61	1.21	12.12	miR-451	3.03	1.04	2.92
miR-106a	13.21	1.18	11.20	miR-205	4.70	1.63	2.88
miR-20a	8.93	1.03	8.70	miR-518c*	3.72	1.29	2.87
miR-106b	9.01	1.04	8.67	let-7b	2.78	1.03	2.71
miR-22	13.97	1.69	8.25	miR-21	3.36	1.24	2.70
miR-492	8.63	1.21	7.16	miR-93	2.38	0.91	2.61
miR-24	16.02	2.41	6.65	miR-18b	2.70	1.05	2.56
miR-373*	7.84	1.18	6.64	miR-99b	2.42	0.99	2.45
miR-17-5p	7.02	1.10	6.37	miR-30a-5p	1.75	0.77	2.27
miR-503	7.81	1.23	6.34	let-7g	2.03	0.90	2.26
miR-16	6.04	0.96	6.28	miR-222	4.08	1.81	2.26
miR-200c	4.57	0.78	5.85	miR-29b	1.63	0.74	2.20
miR-18a	5.71	0.99	5.78	miR-370	2.57	1.18	2.18
miR-141	6.73	1.18	5.71	miR-193b	4.72	2.17	2.18
miR-513	5.60	1.10	5.07	miR-185	2.35	1.13	2.08
let-7d	6.73	1.63	4.13	miR-137	0.48	0.99	0.48
miR-100	7.09	1.79	3.95	miR-452*	0.50	1.05	0.47
miR-27b	3.71	1.00	3.71	miR-506	0.48	1.09	0.44
miR-27a	4.93	1.36	3.63	miR-542-3p	0.31	0.72	0.43
miR-151	2.94	0.83	3.53	miR-422a	0.58	1.36	0.43
let-7a	6.54	1.94	3.38	miR-142-3p	0.47	1.15	0.40
miR-92	3.36	1.01	3.32	miR-10a	0.43	1.14	0.37
miR-125b	4.53	1.41	3.21	miR-493-5p	0.69	2.14	0.32
miR-30c	2.70	0.85	3.19	miR-363*	0.96	5.71	0.17

Expressions of MiR-10a and MiR-320 by QRT-PCR

We used relative QRT-PCR to confirm the microarray data for miR-10a and miR-320. The relative expression was determined using U6 control snRNA and the $2^{-\triangle\triangle Ct}$ method[26], where $\triangle Ct = Ct_{(miRNA)} - Ct_{(U6\ snRNA)}$, $\triangle\triangle Ct = \triangle Ct_{(16HBE\text{-}T)} - \triangle Ct_{(16HBE\text{-}N)}$. After normalization with U6 snRNA control, the expression values were determined as the ratio of miRNA in 16HBE-T and 16HBE-N cells. The expression of miR-10a in 16HBE-T was 0.01 time that of 16HBE-N, while the expression of miR-320 was 8.06 times greater. The expression of miR-10a and miR-320 by relative QRT-PCR was consistent with the miRNA microarray findings.

MiR-10a transcript was further analyzed by absolute QRT-PCR. A standard curve was plotted using serial dilutions of miR-10a amplicons with a known concentration. Using this approach, 6.40×10^6 and 4.36×10^8 copies of miR-10a were detected in 16HBE-T and 16HBE-N cells, respectively. The results of absolute QRT-PCR for MiR-10a in 16HBE-T and 16-HBE-N were identical to those obtained by relative QRT-PCR. When the template concentrations were equalized at 80 ng before amplification, the MiR-10a expression in 16HBE-T was 0.01-fold higher than that in 16HBE-N cells.

Expression of HOXA1 by RT-PCR and QRT-PCR

RT-PCR and QRT-PCR results demonstrated that HOXA1 mRNA expression were 3.12-fold (Fig. 1) and 8.75-fold higher than that in 16HBE-N. Melting curve analysis of the QRT-PCR reactions consistently produced single peaks for HOXA1 and GAPDH amplification products (Fig. 2), demonstrating the presence of only one product in the reaction, which was further verified by gel electrophoresis (data not shown).

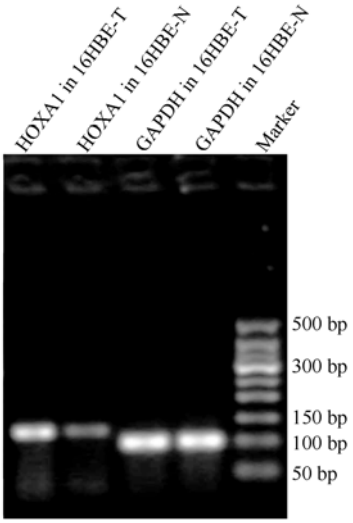

FIG. 1. Amplification of HOXA1 and GAPDH by RT-PCR.
Total RNA was extracted from cultured cells. Reverse transcription polymerase chain reactions with primers for the HOXA1 and GAPDH were performed. After 33 cycles of amplification, samples were electrophoresed and stained with ethidium bromide.

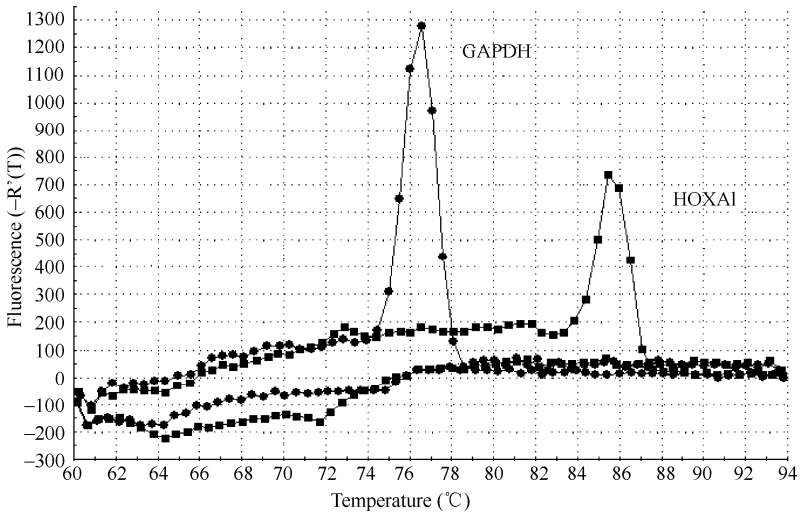

FIG. 2. Heat Dissociation Protocol.
The entire series of cDNA were amplified by 40 cycles of QRT-PCR using primers for HOXA1 and GAPDH (SYBR green detection). Following the final QRT-PCR cycles, samples were subjected to a heat dissociation protocol over the indicated temperature range.

DISCUSSION

Events leading to cell transformation are complex and involve a variety of changes in gene expression and structure. Protein-coding genes are traditionally viewed as the principal effectors and regulators of tumorigenesis. More recent advances suggest that non-protein-coding miRNA may play a significant role in regulating gene expression in cancer. It was reported that expression of miRNAs is altered in lung and breast cancer[12], B-cell lymphoma[27] and glioblastomas[8]. It has been recently reported that the pattern of miRNA expression varies dramatically across cancer types and that miRNA profiles reflect the developmental lineage and differentiation state of cancer[28]. However, the global pattern of cellular functions and pathways affected by miRNAs in carcinogenesis remains elusive.

The present study employed a miRNA microarray system to examine the differential profiles of miRNA expression between 16HBE-T and 16HBE-N cells. The miRNA microarray data were validated by QRT-PCR with two selected miRNAs, miR-10a and miR-320. The detected expressions of miR-10a and miR-320 by relative QRT-PCR and absolute QRT-PCR were consistent with those by miRNA microarray. To our knowledge, among the five most highly differently expressed miRNAs and the three lowest differently expressed miRNAs, miR-494, miR-320, miR-498, miR-129, miR-493-5p, or miR-363* have not been reported to be involved in cancers to date. Altered expressions of the other two miRNAs, miR-106a and miR-10a have been found in some cancers. Volinia *et al.*[29] reported that miR-106a is one of the characterized miRNAs in solid colon, pancreas, and prostate cancers. It was also recently found that miR-106a is up-regulated in primary neuroblastomas[30]. MiR-10a was predicted with computational methods and lately has been verified in humans[18-19]. MiR-10a, located within the HOXB cluster on 17q21, is correlated with human megakaryocytopoiesis and adult acute myeloid leukaemia[20]. Our results show that miR-10a was down-regulated in 16HBE-T cells compared to 16HBE-N cells, which is in agreement with other studies[10, 20]. Its predicted target was discussed below.

Some of the other up-regulated miRNAs are members of the miR-17-92 cluster which resides in intron 3 of the C13orf25 gene at 13q31.3, and comprises seven miRNAs (miR-17-3p, miR-17-5p, miR-18, miR-19a, miR-19b-1, miR-20a, and miR-92) and can be amplified in lymphomas[31]. A recently discovered connection between the miR-17-92 cluster and the c-Myc oncogene[32] is of special note because members of the *myc* gene family have been shown to be frequently amplified and/or over expressed in lung cancers. MiR-17-92 cluster could enhance the growth of lung cancer cells and is directly regulated by c-Myc[33]. Hayashita *et al.*[12] found that the miR-17-92 cluster is markedly over expressed in lung cancers, especially in most aggressive small-cell lung cancer. Furthermore, there is evidence that introduction of miR-17-92 could enhance lung cancer cell growth[12]. Transgenic over-expression of miR-17-92 cluster could promote proliferation and inhibit differentiation of lung epithelial progenitor cells[34]. In our study, three members of miR-17-92 cluster (miR-17-5p, miR-20a, and miR-92), had relatively high abundance in anti-BPDE-transformed 16BHE-T cells, implicating that this cluster is active and involved in the transformation induced by anti-BPDE. These findings clearly suggest that marked over expression of the miR-17-92 cluster promotes tumorigenesis, acts as a potential oncogene, and plays an important role in the development of cancer.

MiR-21, miR-141, miR-27a, miR-27b, and miR-16 are the other over expressed miRNAs in transformed 16HBE-T cells, showing an increase by 2.70-, 5.71-, 3.63-, 3.71-, 6.28-fold, respectively. MiR-21 has been described as an antiapoptotic factor in human glioblastoma cell lines[8] while other authors reported that miR-21 suppression increases the growth of Hela cells without affecting their apoptosis[35]. MiR-141 has been identified to enhance proliferation of cholangiocarcinoma cell lines[16]. It was reported that miR-27a and miR-27b regulate 2BTB10/RIN2F (SP1 transcription suppressor) and RYBP/DEDAF (apoptotic factor)[36]. Let-7 family was a special note of miRNAs in the present study. The gene family has 12 let-7 homologs in the human genome, mapping to fragile sites associated with lung, breast, urothelial, and cervical cancers[37]. Takamizawa *et al.*[13] and Yanaihara *et al.*[38] showed that transcripts of certain let-7 homologs are significantly down regulated in human lung cancer and that low levels of let-7 correlate with a poor prognosis of cancer. Moreover, it has been shown that transient expression of miRNAs of the let-7 family in cell lines derived from lung adenocarcinomas inhibits colony formation, suggesting that these miRNAs have growth-suppressing properties. Nevertheless, our study showed unexpected results. All the analyzed members of let-7 family (let-7a, let-7b, let-7c, let-7d, and let-7g) were over expressed in transformed 16HBE-T cells. In contrast to previous findings, miRNAs of the let-7 family did not act as a tumor suppressor in anti-BPDE-transformed 16BHE-T cells. This could be an example of cell- and

tissue-specific miRNA activation. The expression of miRNA is highly specific for cell, tissue or developmental stage. MiRNA expression patterns suggest differences in biological effects.

We also performed a predicted target analysis for miR-10a, which was chosen because it is associated with human cancer and one of its predicted targets, HOXA1, might be involved in oncogenicity. Our study showed that HOXA1 mRNA was up-regulated 3-9-fold in 16HBE-T compared with 16HBE-N cells. It has been found that forced expression of HOXA1 dramatically increases anchorage-independent proliferation of immortalized human mammary epithelial cells[39]. One mechanism by which HOXA1 mediates oncogenic transformation is to modulate the P44/42 MAP kinase pathway[25]. The expression of HOXA1 is up-regulated in human non-small cell lung cancer and affects the survival and death of tumor cells[40]. The overall outcome might increase cell proliferation, invasiveness or angiogenesis, and decrease apoptosis or dedifferentiation, ultimately leading to tumor formation[41]. The down-regulated miR-10a and its up-regulated predicted target HOXA1 were expressed reciprocally in 16HBE-T, suggesting that miR-10a is potentially involved in the 16HBE transformation, and might act as a candidate tumor suppressor by intervening HOXA1.

The present study provided general miRNA profiles of transformed 16HBE-T cells induced by anti-BPDE. The differences in expression of miRNAs between 16HBE-T and 16HBE-N could point to the potential role of these miRNAs in cell transformation. Changes in miRNA expression may play an important role in the genesis and evolution of 16HBE malignancies. MiR-10a is a candidate tumor suppressor. The results of this study may have important implications for the role of miRNAs in the anti-BPDE carcinogenesis process. The study may also provide insights regarding therapeutic molecular targets for prevention and treatment of human lung cancer.

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