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Restoration of tumour suppressor *hsa-miR-145* inhibits cancer cell growth in lung adenocarcinoma patients with epidermal growth factor receptor mutation

William C.S. Cho, Andrew S.C. Chow, Joseph S.K. Au*

Department of Clinical Oncology, Room 1305, 3/F, Block R, Queen Elizabeth Hospital, 30 Gascoigne Road, Kowloon, Hong Kong

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ABSTRACT

Background: In Hong Kong, about 30% of non-small cell lung cancer patients have never smoked tobacco. Among women, 83% are never-smokers and their histological type is invariably adenocarcinoma with 70% incidence of epidermal growth factor receptor (*EGFR*) mutation. The present study focuses on the microRNA (miRNA) expression profiles of this important subset of lung cancer.

Methods: Paired samples collected from the lung cancer tissue and adjacent normal lung parenchyma of 10 non-smoking patients with lung adenocarcinoma were profiled by miRNA microarray. Results were validated by quantitative reverse transcription polymerase chain reaction. Transfected cell viability assays were applied to determine the effects of candidate miRNAs on lung cancer cells.

Results: Comparing paired lung cancer tissue with adjacent normal lung parenchyma, *hsa-miR-126**, *hsa-miR-145*, *hsa-miR-21*, *hsa-miR-182*, *hsa-miR-183* and *hsa-miR-210* were found to be the most differentially expressed miRNAs. Most interestingly, an obvious inhibition of cell growth was observed in the *EGFR* mutant lung adenocarcinoma after transfection of *hsa-pre-miR-145*.

Conclusions: Our study is the first report to connect *miR-182* to lung cancer. Our results also show that restoration of tumour suppressor *hsa-miR-145* inhibits cancer cell growth in *EGFR* mutant lung adenocarcinoma. Further study on these specific differentially expressed miRNAs may provide important information on peculiar tumourigenetic pathways and may identify useful biomarkers.

1. Introduction

Non-smoking-related lung cancer has been prominently increasing worldwide even with the success of the antismoking campaign.[1] The incidences are particularly high in oriental women and they are almost always of adenocarcinoma histological subtype.[2,3] High incidences of epidermal growth factor receptor (*EGFR*) mutation, as well as distinct DNA and protein expression profiles have been identified.[4–7] However, none of the current biomarkers is sensitive and specific enough to be routinely used in the clinic as a diagnostic, therapeutic and prognostic tool.[8] Elucidating the molecular pathways of this important type of lung cancer may allow for better understanding of the carcinogenesis and new targeted strategies.

Recent evidences have shown that microRNAs (miRNAs) have diverse functions, including the regulation of cellular

* *Corresponding author:* Tel.: +852 2958 5441; fax: +852 2958 5455.
E-mail address: chocs@ha.org.hk (W.C.S. Cho).
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differentiation, proliferation and apoptosis.[9,10] They can function as tumour suppressors or oncogenes and thus are deemed to play a crucial role in the initiation and progression of human cancer.[11] This study aims to assess the feasibility of miRNA expression profiling of lung adenocarcinoma of non-smokers with or without *EGFR* mutation correlates with the characteristic expression pattern of miRNAs that may be the target of therapies for non-smoking patients with lung adenocarcinoma.

2. Materials and methods

2.1. Tissue collection

Pairs of primary lung adenocarcinoma tissues and adjacent non-tumourous lung parenchyma were collected from non-smoking patients ($n = 10$) undergoing lung cancer resection surgery at the Queen Elizabeth Hospital (Hong Kong). The collected tissue samples were prepared as previously described.[12] Voluntary informed consents to donate tissue samples for the present study were obtained from all research participants. Detailed backgrounds for each tissue donor, including age, sex and smoking history have been collected. All patients are genetically unrelated ethnic Chinese (nine females and one male) of ages ranging from 52 to 77 years.

2.2. RNA isolation

Total RNA was isolated from the collected tissues with Trizol reagent (Invitrogen Corporation, Carlsbad, CA) according to the manufacturer's instructions and quantified by spectrophotometer. To assess the purity of RNA, optical density (OD) was measured at 260 nm and 280 nm for determination of OD_{260}/OD_{280} ratio. The RNAs with >1.8 OD ratios were used in this study. Relative abundance and integrity of 18S and 28S ribosomal bands were assessed with agarose gel electrophoresis via the Bioanalyser 2100 system (Agilent Technologies, Santa Clara, CA). Those RNAs that exhibited intact 18S and 28S ribosomal bands with 1:1.5 relative abundance ratios were used in this study.

2.3. MicroRNA profiling

MicroRNA microarray profiling service was provided by Agilent Technologies using Eight-Multiplex Human MicroRNA Array v1.0. Design of the probes is based on Sanger miRBase 9.1. Each microarray contains 534 miRNA probes, among them 470 are unique human miRNAs and 64 are human-related viral miRNAs. High quality of RNA was confirmed by capillary electrophoresis. Each total RNA (100 ng) was directly labelled with Cyanine 3-pCp (T4 RNA ligase) and the labelled RNA was hybridised on the miRNA microarray. Pairs of tumour and non-tumourous tissues were profiled at the same time.

2.4. Microarray data analysis

The miRNA profile data were analysed by GeneSpring GX 7.3.1 (Agilent Technologies) to identify up- or down-regulated expression signatures in each sample. For those with a signal <0.01, a fixed signal of 0.01 was set for data analysis. Per chip normalisation to 75th percentile was applied to the array results to eliminate systemic errors, such as pipetting, array batch and spectrophotometry. Per gene normalisation to median was then applied to allow comparison of the relative change in miRNA expression levels. After flag filtering, the flags of 256 miRNAs were presented. As a signal <64 correlates poorly with quantitative reverse transcription polymerase chain reaction (qRT-PCR), the mean total miRNA signal was filtered by expression data >64. There were 158 miRNAs with expression data >64. After data filtering, non-parametric test was used to analyse the filtered miRNAs. Supervised clustering, principal components analysis and Wilcoxon–Mann–Whitney test were used for comparison analysis to identify miRNAs that were differentially expressed. Paired miRNAs with $P > 0.05$ or <twofold change were filtered out.

2.5. Quantitative reverse transcription polymerase chain reaction analysis

To validate the expression of miRNAs identified by microarray profiling, quantification of miRNAs was performed with a two-step qRT-PCR procedure according to the manufacturer's instructions. In the RT step, complementary DNA (cDNA) was reverse transcribed from total RNA samples using reagents from the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). All RT reactions were carried out in a 15 μL volume, starting with 10 ng of total RNA. The reaction mixture was initially heated to 16 °C for 30 min, 42 °C for 30 min, 85 °C for 5 min and finally to 4 °C for 5 min. In the PCR step, PCR products were amplified from cDNA samples using specific miRNA primers from the TaqMan MicroRNA Assay together with the TaqMan Universal PCR Master Mix (Applied Biosystems). All assays were performed in triplicate on the ABI PRISM 7000 System (Applied Biosystems). The assay tubes were initially heated to 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. The expression levels of candidate miRNAs were evaluated by comparative C_T method and were normalised using RNU48 as the endogenous control. Relative quantitative expression levels of miRNAs were determined by the $2^{-\Delta\Delta C}{}_T$ method.

2.6. Sequencing of the epidermal growth factor receptor gene

Genomic DNA was extracted from the cancer specimens of the 10 recruited patients obtained at surgical resection. The cDNA-based PCR primers were used to amplify the N-lobe's and C-lobe's partial sequence of *EGFR* gene coding from the tyrosine kinase domain (exons 18–21). Primers were designed as: *EGFR*, forward 5′-CCAAGGGAGTTTGTGGAGAA-3′ and reverse 5′-TGGTACATATGGGGGCTGA-3′. All PCR assays were carried out in a 50 μL volume using 1.25 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems). The cDNA was amplified for 40 cycles at 95 °C for 30 s, 60 °C for 30 s and 72 °C for 80 s, followed by a 10-min extension at 72 °C. All PCR solutions were purified by ethanol precipitation and were then sequenced directly using the ABI PRISM Dye Terminator Cycle Sequencing Kit (Applied Biosystems). Sequence variants were

confirmed by sequencing the amplicons in both forward and reverse directions.

2.7. Cell culture and reagents

Normal human lung fibroblast cell line (CCD-11Lu), human lung adenocarcinoma cell line from a male patient (HCC4006) with *EGFR* mutation (del L747-E749 and A750P), human lung squamous cell line without *EGFR* mutation (NCI-H520), human lung adenocarcinoma cell line from a female patient (NCI-H1975) with *EGFR* mutation (L858R and T790M) and human lung adenocarcinoma cell line from a female patient without *EGFR* mutation (NCI-H1734) were obtained from American Type Culture Collection (Manassas, VA). All cell culture reagents were purchased from Invitrogen Corporation. Briefly, the cells were cultured at 37 °C in 5% CO_2 and RPMI-1640, supplemented with 10% foetal bovine serum, 2.98 g/L HEPES, 2 g/L $NaHCO_3$, 100 U/mL penicillin and 100 μg/mL streptomycin.

2.8. MicroRNA transfection

The human lung cell lines were seeded in a 24-well plate and cultured to 70% confluence. Anti-miR miRNA inhibitor and corresponding negative control, pre-miR miRNA precursor and corresponding negative control, as well as siPORT Amine transfection agent were purchased from Ambion Inc (Austin, TX). The miRNA inhibitors, precursors and negative controls were transfected into the human lung cell lines at a concentration of 100, 300 and 500 nM for 24, 48 and 72 h. After transfection, candidate miRNAs were reversely transcripted and quantified by TaqMan MicroRNA Assay (Applied Biosystems) according to the manufacturer's instructions.

2.9. Cell proliferation assay

Cell viability (%) was measured by Cell Proliferation Assay (Promega Corporation, Madison, WI) according to the manufacturer's instructions. Briefly, cells were incubated with MTS solution for 2 h and the absorbance was measured at 490 nm using an enzyme-linked immunosorbent assay reader. Absorbance of the mock transfected cells was defined as 100% survival. The results for treated miRNA inhibitors, treated miRNA precursors and negative controls were converted to ratios of the mock transfected cells. Morphological changes of the cells after transfection were evaluated by phase-contrast microscopy.

2.10. ELISA quantification of total EGFR

After 48-h transfection of *hsa-pre-miR-145*, total EGFR in the lung cell lysate was quantified using an ELISA kit (Cell Signaling Technology, Beverly, MA) according to the manufacturer's instructions. Briefly, 100 μL of each diluted cell lysate was incubated overnight at 4 °C. The well was subsequently washed and incubated with 100 μL of detection antibody, HRP-linked secondary antibody and TMB substrate, respectively. Absorbance was read at 450 nm within 30 min after adding stop solution.

2.11. Statistical analysis

The miRNA expression data were analysed with expression graphs and Wilcoxon–Mann–Whitney test, while the qRT-PCR data, transfected cell viability assay and transfection efficiency test were analysed with expression graphs and Mann–Whitney *U* test. $P < 0.05$ was considered statistically significant for comparisons testing.

3. Results

3.1. MicroRNA expression pattern in lung adenocarcinoma tissues of non-smokers

Supervised clustering and principal components analysis of miRNA expression data revealed that miRNA profiles of cancer specimens were distinctly different from non-tumour profiles (Fig. 1). Univariate analysis was then employed to identify those miRNAs that were responsible for this unique miRNA expression pattern. Of the 55 independent miRNAs that were found to be differentially expressed in cancer ($P < 0.05$ with >twofold change), 37 miRNAs were identified at lower expression levels in cancer whereas 18 miRNAs were identified at higher expression levels in cancer.

3.2. Expression of distinguished candidate microRNAs in lung adenocarcinoma tissues of non-smokers

In pairwise comparisons ($n = 10$) of lung adenocarcinoma tissues of non-smoking patients *versus* adjacent non-tumourous lung parenchyma, *hsa-miR-126** and *hsa-miR-145* were found to be the most differentially hypo-expressed miRNAs in cancer whereas *hsa-miR-21*, *hsa-miR-182*, *hsa-miR-183* and *hsa-miR-210* were found to be the most differentially hyper-expressed miRNAs in cancer. The list of these six most differentially expressed miRNAs is presented in Table 1.

3.3. Validation of the microRNA microarray expression data by quantitative reverse transcription polymerase chain reaction analysis

The miRNA microarray results were validated by qRT-PCR analysis of pairwise comparisons ($n = 10$) of lung adenocarcinoma tissues of non-smoking patients *versus* adjacent non-tumourous lung parenchyma. Both *hsa-miR-126** and *hsa-miR-145* were significantly expressed at lower levels in cancer. In contrast, *hsa-miR-21*, *hsa-miR-182*, *hsa-miR-183* and *hsa-miR-210* were all significantly expressed at higher levels in cancer (Table 1). Quantitative RT-PCR analysis of these six candidate miRNAs demonstrated correlated expression patterns with the results of miRNA microarray profiling (Fig. 2).

3.4. EGFR mutation

Among the 10 cancer specimens, only three specimens did not have *EGFR* mutation. There were five specimens that contained one commonly identified *EGFR* mutation (L858R) while another *EGFR* mutation (T790M) was identified in one specimen. One specimen contained two *EGFR* mutations (L858R and T790M).

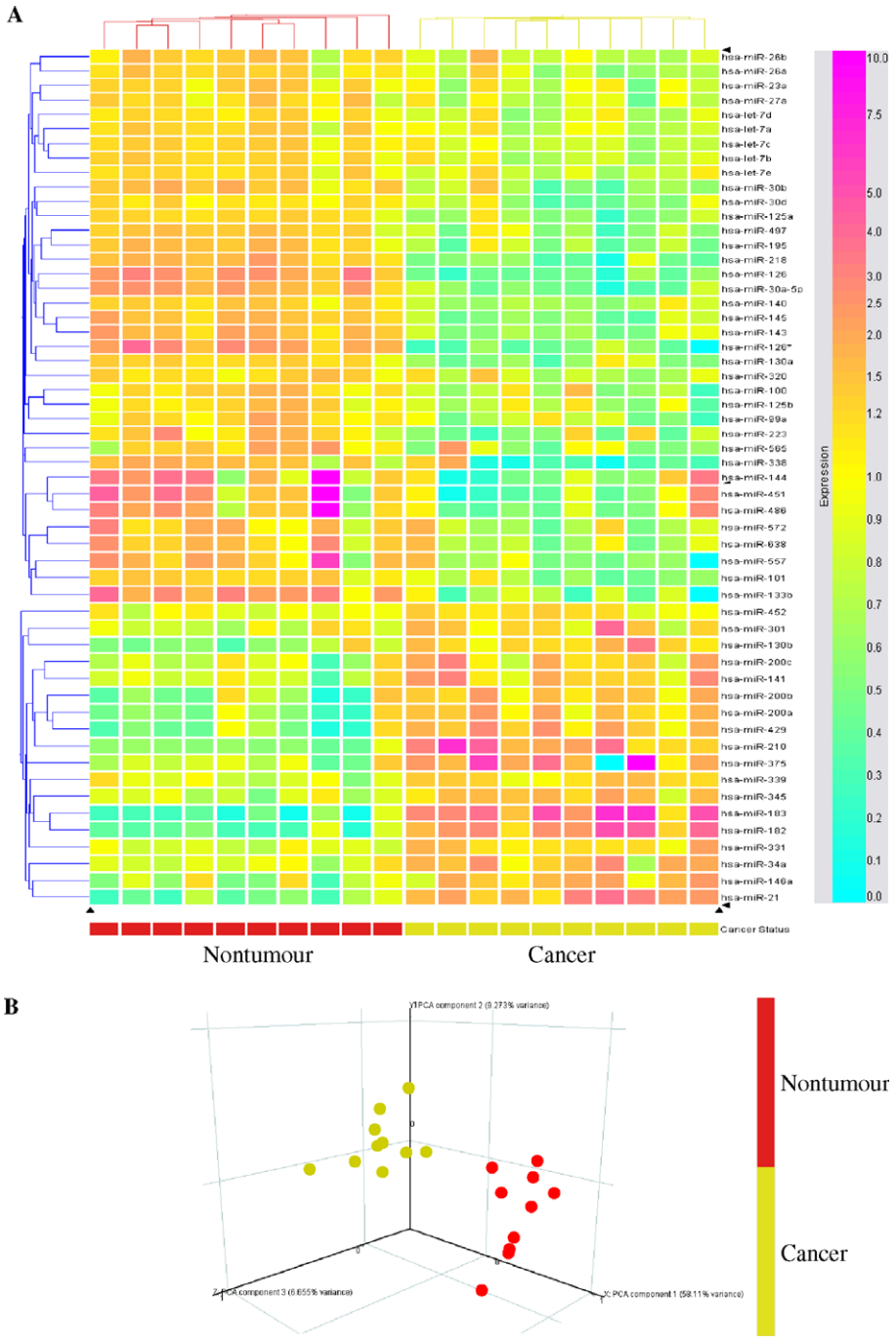

Fig. 1 – Multivariate analysis revealing that microRNA (miRNA) profiles of lung cancer tissue samples (green) are distinctly different from adjacent non-tumourous lung parenchyma (red) profiles. (A) Supervised clustering for miRNA expressions of cancer specimens compared to non-tumour specimens. (B) Three-dimensional principal components analysis for miRNA expressions of cancer specimens compared to non-tumour specimens.

3.5. *Transfected cell viability (%) and transfection efficiency of hsa-pre-miR-145*

To determine the expression patterns of the four most up- or down-regulated miRNAs *hsa-miR-126**, *hsa-miR-145*, *hsa-miR-182* and *hsa-miR-183*, miRNA transfection assays were performed in a panel of three human lung tissue cells. The experiment revealed that transfection of 300 nM *hsa-pre-miR-145* significantly down-regulated 45% of cell viability in human lung adenocarcinoma cells with *EGFR* mutation ($P < 0.05$), but had no obvious effect on normal human lung fibroblast cells and human lung squamous carcinoma cells. In contrast, it had not been able to detect such obvious differential expression with transfection of *hsa-pre-miR-126**, *hsa-anti-miR-182* and *hsa-anti-miR-183* in any of the three human lung tissue cells (Fig. 3A).

Table 1 – List of microRNAs most differentially expressed in lung adenocarcinoma tissues of non-smokers relative to the controls.

MicroRNA	Comparison	Expression	Fold change	P-value	
				Microarray	qRT-PCR
*Hsa-miR-126**	Cancer *versus* non-tumour	Lower in cancer	5.3	1.08E–5	4.33E–5
Hsa-miR-145	Cancer *versus* non-tumour	Lower in cancer	2.7	1.08E–5	4.33E–5
Hsa-miR-21	Cancer *versus* non-tumour	Higher in cancer	4.7	1.08E–5	2.88E–3
Hsa-miR-182	Cancer *versus* non-tumour	Higher in cancer	6.3	1.08E–5	2.06E–4
Hsa-miR-183	Cancer *versus* non-tumour	Higher in cancer	11.2	1.08E–5	1.08E–5
Hsa-miR-210	Cancer *versus* non-tumour	Higher in cancer	4.8	1.08E–5	3.89E–3

qRT-PCR: quantitative reverse transcription polymerase chain reaction.

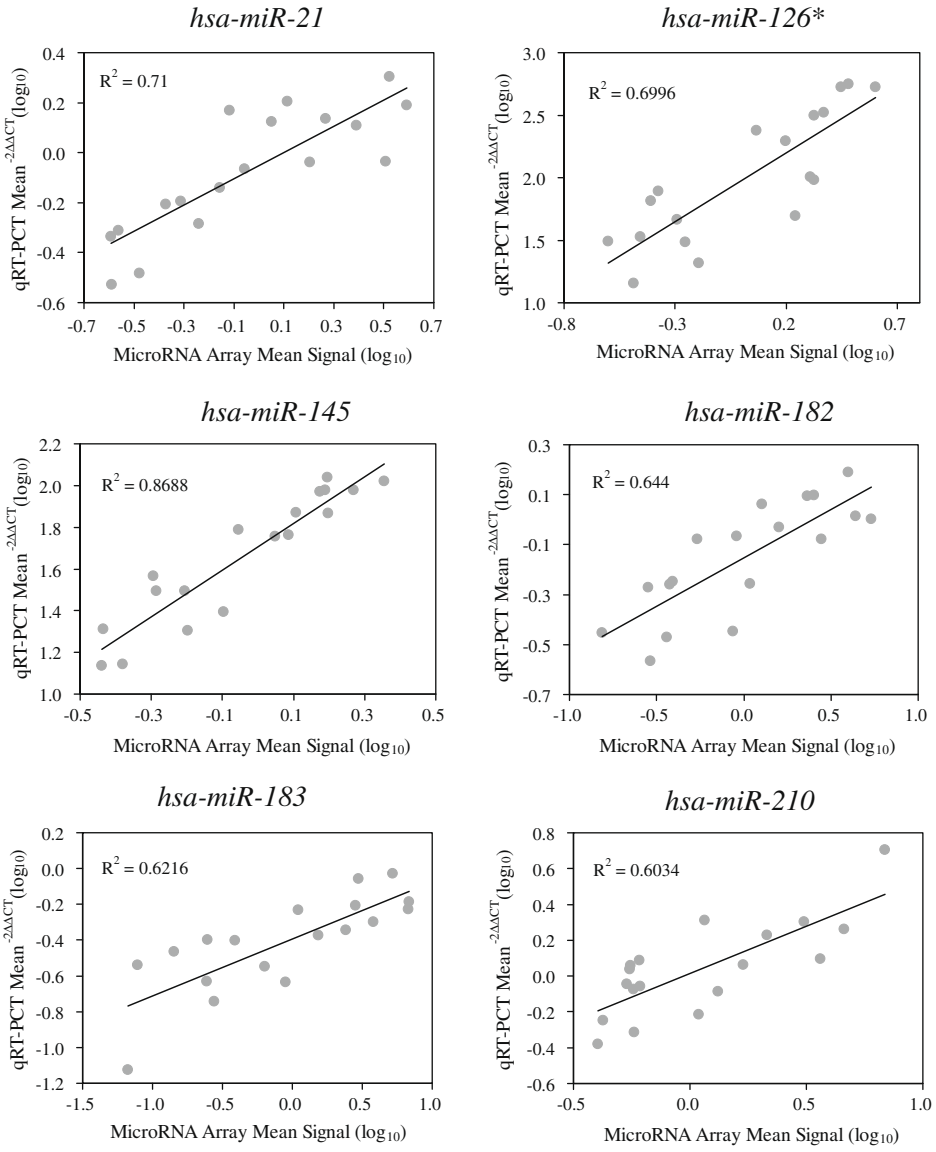

Fig. 2 – MicroRNA (miRNA) microarray profiling of the six candidate miRNAs demonstrating relative expression with the results of quantitative reverse transcription polymerase chain reaction (qRT-PCR). Mean signals and C_T values from the reactions of miRNA microarray (X-axis) and qRT-PCR (Y-axis) are plotted. Results demonstrate correlated expression patterns between both methods.

Transfection efficiency test by qRT-PCR showed that transfection of 300 nM *hsa-pre-miR-145* effectively increased the relative expressions of *hsa-miR-145* in all the three lung tissue cells as compared to the controls (Fig. 3B).

3.6. Cell morphology of human lung adenocarcinoma cells with EGFR mutation after candidate microRNAs transfection

Transfection of *hsa-pre-miR-145* for 72 h obviously induced apoptosis of cancer cells in human lung adenocarcinoma with *EGFR* mutation as compared to the controls, but no obvious effect was observed with transfection of *hsa-pre-miR-126**, *hsa-anti-miR-182* and *hsa-anti-miR-183*.

3.7. Expression of hsa-miR-145 in patients with or without EGFR mutation

Decreased expression of *hsa-miR-145* ($P = 5.83E{-}4$) was observed in pairwise comparisons ($n = 7$) of lung cancer tissue of non-smoking patients with *EGFR* mutation *versus* adjacent non-tumourous lung parenchyma, but no significant difference in the expression of *hsa-miR-145* ($P = 1.00E{-}1$) was observed in pairwise comparisons ($n = 3$) of lung cancer tissue of non-smoking patients without *EGFR* mutation *versus* adjacent non-tumourous lung parenchyma.

3.8. Hsa-pre-miR-145 induced growth inhibition of human lung adenocarcinoma, especially for cells with EGFR mutation

To determine whether *hsa-miR-145* could be an effective therapeutic agent for lung adenocarcinoma, the effect of transfected *hsa-pre-miR-145* on cell growth of the normal human lung fibroblast cell line and the human lung adenocarcinoma cell lines was examined. It was observed that *hsa-pre-miR-145* inhibited growth of HCC4006, NCI-H1975 and NCI-H1734 cells, revealing 44%, 61% and 16% inhibition at a dose of 500 nM after a 48-h transfection (Fig. 4A). The dose response (100, 300 and 500 nM) and time course (24, 48, and 72 h) transfection showed that in the presence of *hsa-pre-miR-145*, the human lung adenocarcinoma cell growth decreased gradually with a maximum occurring at a dose of 500 nM after 48-h transfection.

The qRT-PCR analysis showed that transfection of *hsa-pre-miR-145* for 48 h effectively increased the relative expressions of *hsa-pre-miR-145* in all the four lung tissue cells as compared to the mock transfected controls (Fig. 4B) with their endogenous controls (RNU48) similarly expressed in all the cell lines analysed.

3.9. Cell morphology of human lung adenocarcinoma cells with EGFR mutation after transfection of hsa-pre-miR-145 for 48 h

Transfection of *hsa-pre-miR-145* for 48 h obviously induced apoptosis of cancer cells in human lung adenocarcinoma with *EGFR* mutation as compared to the mock transfected controls, but no obvious effect was observed with transfection of *hsa-pre-miR-145* in normal human lung fibroblast cells and human lung adenocarcinoma cells from patients without *EGFR* mutation (Fig. 5).

3.10. Decrease in EGFR protein expression after transfection of hsa-pre-miR-145

Transfection of *hsa-pre-miR-145* for 48 h down-regulated (from 0.458 to 0.232) the EGFR protein expression in *EGFR* mutant lung adenocarcinoma cells from non-smoking patients (NCI-H1975) by 49%. In contrast, transfection of *hsa-pre-miR-145* only caused an 18% decrease (from 0.186 to 0.153) in the EGFR protein expression in cell lines without *EGFR* mutation (NCI-H1734).

4. Discussion

Emerging evidences have suggested that non-smoking-related lung cancer may correlate with a characteristic pattern of gene expression. One study has shown that 45 genes are differentially expressed between smokers and non-smokers.[13] Another study has demonstrated that the number of genes that are expressed differently between paired lung cancer tissues and adjacent non-tumourous lung parenchyma is fourfold higher in never-smokers than in smokers.[14] It has also been reported that alveolar macrophages exhibit differential expression of 75 genes in smokers and non-smokers.[15] The *EGFR* mutation has been found to be strongly associated with several cancers, including those of the colorectum,[16] prostate[17] and breast.[18] The *EGFR* gene is also amplified or over-expressed in non-small cell lung cancer.[19,20] Most of the non-smoking lung cancer patients are women and the commonest histological type is adenocarcinoma with high incidences of *EGFR* mutation.[21] Despite extensive studies, the mechanism leading to this important subset of lung cancer remains unclear.

In the present study, the expressions of miRNAs in human lung tissues and cells have been investigated. Our microarray data show that non-smokers with lung adenocarcinoma are characterised by a specific expression pattern of miRNAs. Significant differences in the expressions of a selective number of miRNAs in lung adenocarcinoma of non-smoking patients were identified. Using qRT-PCR, the expressions of six most differentially expressed miRNAs *hsa-miR-21*, *hsa-miR-126**, *hsa-miR-145*, *hsa-miR-182*, *hsa-miR-183* and *hsa-miR-210* in paired lung adenocarcinoma tissues of non-smokers *versus* adjacent non-tumourous lung parenchyma were confirmed.

Some of these miRNAs have been found to be up- or down-regulated in several cancers. Previous studies reported that *miR-21* was highly expressed in breast cancer,[22] cholangiocarcinoma,[23] head and neck cancer,[24] leukaemia[25] and lung cancer.[26] Musiyenko and colleagues showed that the expression of *miR-126** was ectopically low in prostate cancer.[27] Other studies found that *miR-145* was down-regulated in breast cancer,[22] colorectal cancer[28] and cervical cancer.[29] Bandres and colleagues also identified the over-expression of *miR-183* in colorectal cancer.[28] Some studies indicated the elevated expression of *miR-210* in breast cancer,[30] ovarian cancer[31] and lymphoma.[32] Our study is the first report to connect *miR-182* to lung cancer. Further investigation of these miRNAs may contribute to a better understanding of lung cancer and possibly other cancers.

On the other hand, decreased expression of *hsa-miR-145* in lung adenocarcinoma was demonstrated in this study. *Hsa-miR-145* is a tumour suppressor located on chromosome 5 that has been shown to play an important role in inhibiting tumour cell proliferation.[33] Previous studies have also reported

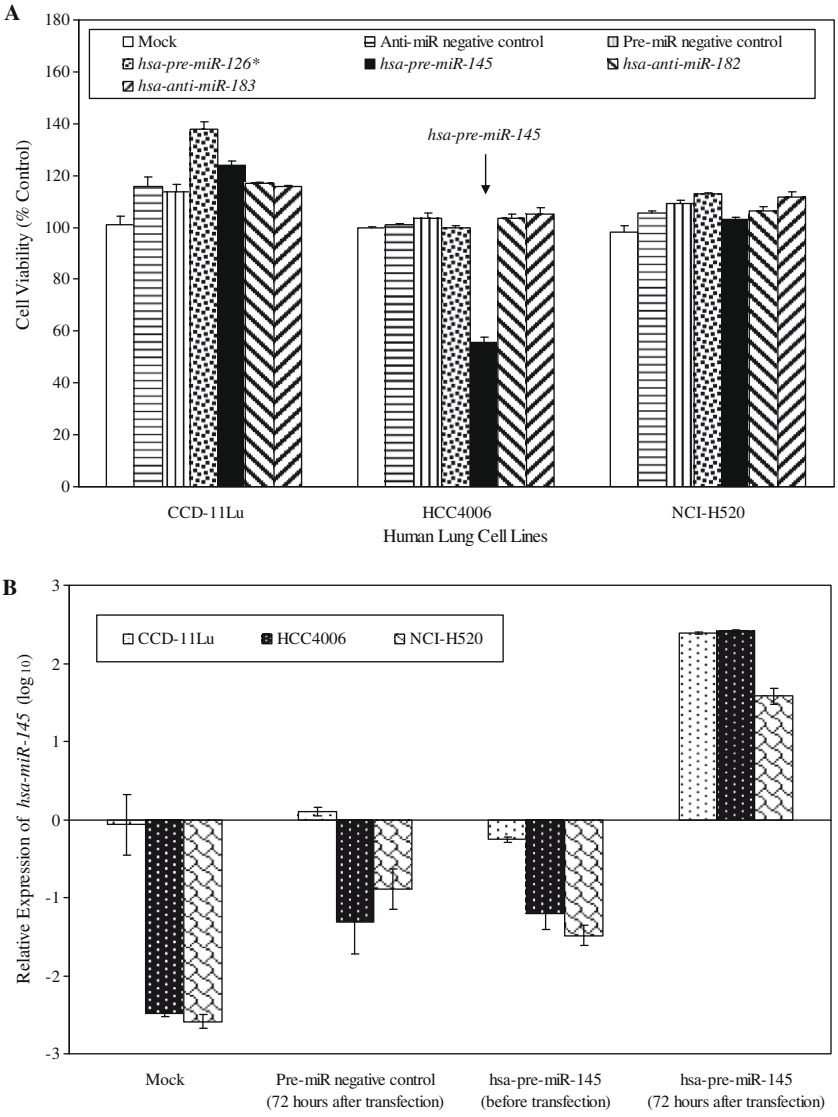

Fig. 3 – Transfected cell viability (%) assay and transfection efficiency test of the four most differentially expressed microRNAs in normal human lung fibroblast cell line (CCD-11Lu), human lung adenocarcinoma cell line with *EGFR* mutation (HCC4006) and human lung squamous cell line (NCI-H520). (A) Transfection of 300 nM *hsa-pre-miR-145* for 72 h significantly down-regulated 45% of cell viability in HCC4006 ($P < 0.05$), but not in CCD-11Lu and NCI-H520. In contrast, transfection of *hsa-pre-miR-126, *hsa-anti-miR-182* and *hsa-anti-miR-183* had no obvious effect on any of the three human lung tissue cells. Data presented are the mean % ± SE relative to mock transfected controls from independent transfection experiments each done in triplicate. (B) Transfection of 300 nM *hsa-pre-miR-145* for 72 h effectively increased the relative expression of *hsa-miR-145* in all the three lung tissue cells as compared to the controls. Data presented are the mean$^{-2\Delta\Delta CT}$($\log_{10}$) ± SE from independent qRT-PCR analyses each done in triplicate.**

dysregulated expression of *miR-145* in lung cancer cells.[34,35] After transfection of *hsa-pre-miR-145*, a dose (500 nM) and time (48-h transfection) for maximum cytotoxicity of lung adenocarcinoma cells were obtained.

Our result provides the first evidence that 45% apoptosis of *hsa-pre-miR-145*-transfected cancer cells may be correlated with *EGFR* mutation. It is tempting to speculate that *hsa-miR-145* may play an important role in the anti-proliferation of lung adenocarcinoma cells with *EGFR* mutation. Consistent with this hypothesis, the present study has found that forced expression of *hsa-pre-miR-145* transfection led to 44–61% apoptosis in *EGFR* mutant lung adenocarcinoma epitheliums, but induced just 16% apoptosis in lung adenocarcinoma cell without *EGFR* mutation. ELISA data also confirmed that transfection of *hsa-pre-miR-145* significantly inhibited EGFR protein expression in *EGFR* mutant cells, but not in the cell line without *EGFR* mutation.

Our results suggest that *hsa-miR-145* is a key player in protecting a cell against *EGFR* mutation. This implies that loss or silence of this tumour suppressor *hsa-miR-145* may be one of the hidden components of the *EGFR* pathway. Restoration of *hsa-miR-145* successfully inhibits growth in lung

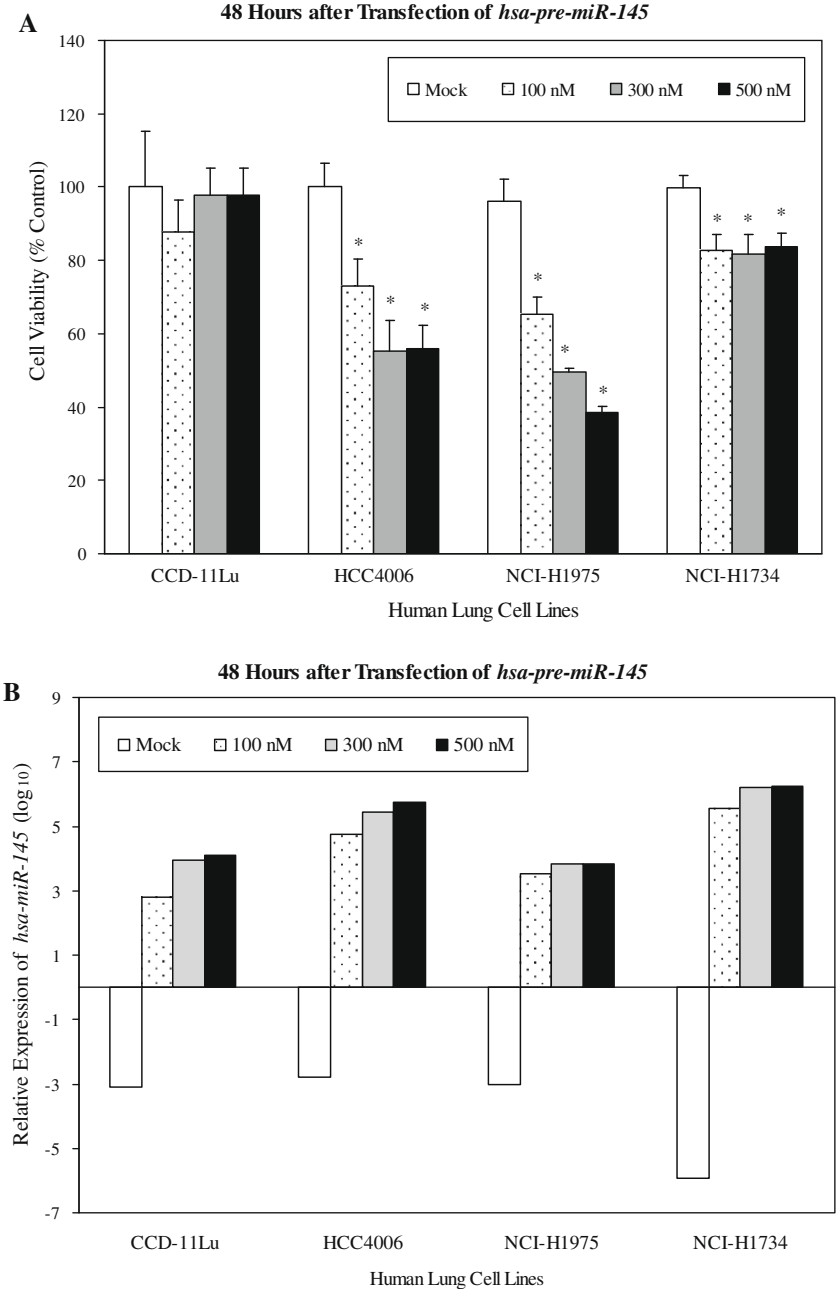

Fig. 4 – Transfected cell viability (%) assay and transfection efficiency test by quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis of *hsa-miR-145* in normal human lung fibroblast cell line (CCD-11Lu), human lung adenocarcinoma cell line from a male patient with *EGFR* mutation (HCC4006), human lung adenocarcinoma cell line from a female patient with *EGFR* mutation (NCI-H1975) and human lung adenocarcinoma cell line from a female patient without *EGFR* mutation (NCI-H1734). (A) Transfection of *hsa-pre-miR-145* for 48 h effectively inhibited growth in lung adenocarcinoma cells, especially for those with *EGFR* mutation. Data presented are the mean % ± SE relative to mock transfected controls from independent transfection experiments each done in triplicate. *$P < 0.05$ compared with the mock transfected controls. (B) Transfection of *hsa-pre-miR-145* for 48 h effectively increased the relative expression of *hsa-miR-145* in all the four lung tissue cells in a dose response manner as compared to the mock transfected controls. Data presented are the mean$^{-2\Delta\Delta CT}$($\log_{10}$) value from independent qRT-PCR analyses each done in duplicate.

adenocarcinoma cells with *EGFR* mutation. These are the first reported data on the potential of *hsa-miR-145* as a therapeutic target for *EGFR* mutant lung adenocarcinoma. Further study on this specific differentially expressed miRNA may provide important information on peculiar tumourigenetic pathways and may identify a useful biomarker for diagnosis or therapy for this important subset of lung cancer.

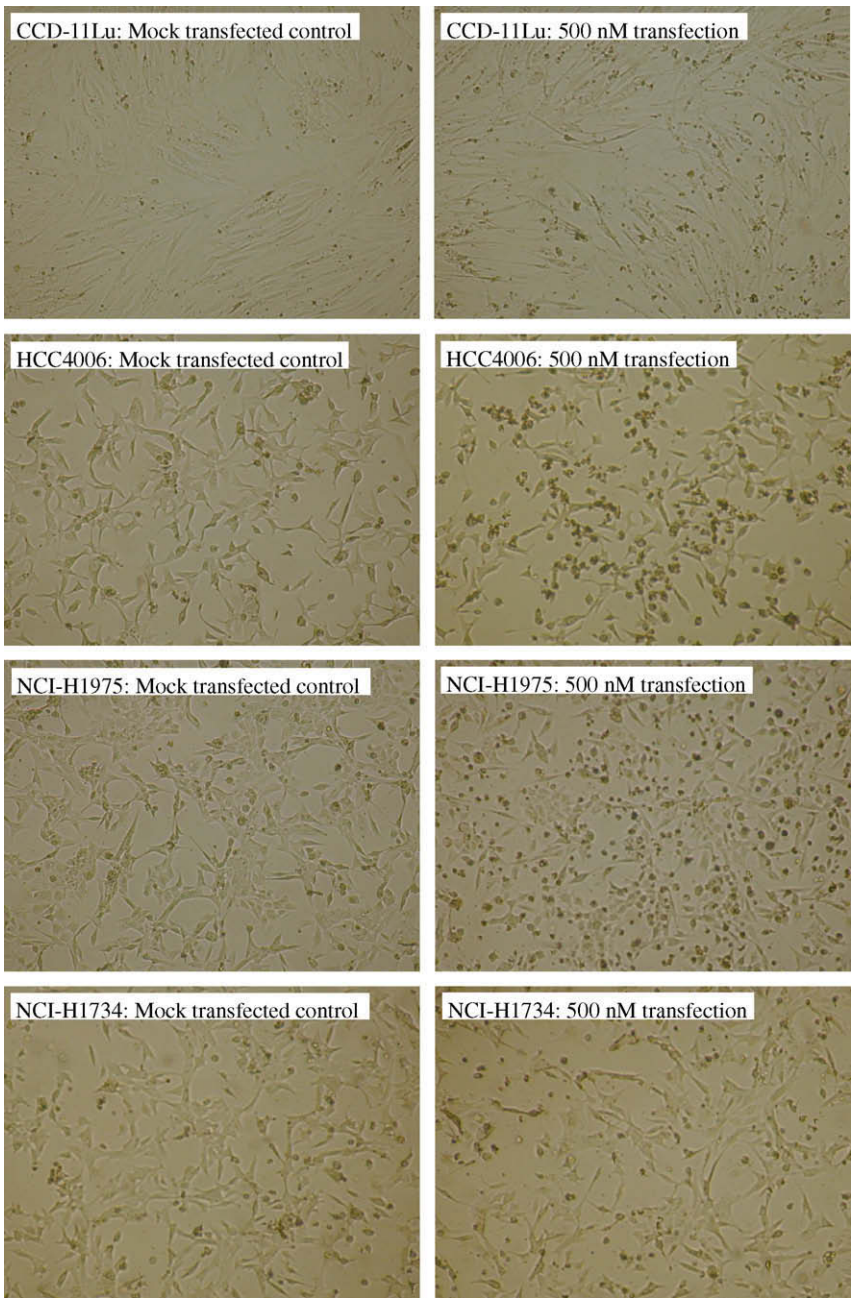

Fig. 5 – Cell morphology demonstrating that transfection of 500 nM *hsa-pre-miR-145* for 48 h obviously induced apoptosis of cancer cells in human lung adenocarcinoma with *EGFR* mutation (HCC4006 and NCI-H1975) as compared to the mock transfected controls, but no obvious effect was observed with transfection of *hsa-pre-miR-145* in human lung fibroblast cells (CCD-11Lu) and human lung adenocarcinoma cells from a patient without *EGFR* mutation (NCI-H1734).

Conflict of interest statement

None declared.

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