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# Rs3842530 Polymorphism in MicroRNA-205 Host Gene in Lung and Breast Cancer Patients

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**Background:** The expression of miR-205 is closely related to the occurrence, development, and prognosis of lung cancer and breast cancer. However, studies show that it plays opposite roles in different tumor types. Because the expression and regulation of miR-205 are primarily confined to epigenetic areas, whether genetic variation of miR-205 is related to the occurrence or to the development of tumors has not been reported. The aim of this study was to screen genetic variation of miR-205 gene and to investigate its association with the risk and development of lung and breast cancer.





**Material/Methods:** Genomic DNA was extracted from cultured tumor cell lines and formalin-fixed and paraffin-embedded lung and breast tissue samples. Bisulfite Clone Sequencing (BCS) and qRT-PCR were employed to detect the DNA methylation status and gene expression of the miR-205 gene, respectively. Genetic variation of miR-205 and miR-205HG were genotyped with PCR-sequencing method. Immunohistochemical analysis for ER, PR, and HER2 was performed on breast tissue samples.

**Results:** A polymorphism, rs3842530, located downstream of the miR-205 gene and in the fourth exon of the miR-205 host gene (miR-205HG), was screened. rs3842530 had no correlation with the risk of breast cancer, but was associated with the risk of lung cancer ( $P < 0.05$ ).

**Conclusions:** These results indicate that the functional association of rs3842530 in miR-205HG and lung cancer might provide a possible explanation for the tissue-dependent function of miR-205 in different tumors.

**MeSH Keywords:** **Breast Neoplasms, Male • Lung Neoplasms • MicroRNAs • Polymorphism, Single Nucleotide**

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## Background

Widely found across species, microRNA (miRNA, miR) is a highly conserved non-coding RNA that is composed of 19~26 nucleotides [1]. Because miRNA can regulate the expression of nearly 1/3 of all protein coding genes in the human genome, it has become an important area of research in a variety of diseases, including tumors. The gene that contains the miRNA coding sequence is called the miRNA host gene (HG). Because miRNA originates from post-transcriptional splicing of its host gene and may affect its host gene's expression due to their innate complementary sequences, there is crosstalk between a miRNA and its host gene. Therefore, genetic and epigenetic association studies of miRNA and its host gene may provide a theoretical basis for revealing the relationship between them and may help explore the mechanism of tumor diseases.

Gene polymorphism refers to different sequences of 2 or more types in specific locations, or alleles, of a genome in a population. This polymorphism may not only occur in protein coding genes but can also occur in non-protein coding genes, such as the miRNA coding gene or its host gene [2]. For example, the miR-146a gene codes for 2 mature miRNAs: miR-146a-5p and miR-146a-3p. The polymorphism rs2910164 in the miR-146a-3p coding sequence has been associated with incidence of hepatocellular carcinoma [3]; similarly, the miR-423 gene contains the coding sequences for miR-423-5p and miR-423-3p, and the polymorphism rs6505162 at the 3' non-coding sequence of the miR-423 gene has been associated with bladder cancer, esophageal cancer, and breast cancer [4-6]. The miR-17 host gene (miR-17HG) is a gene cluster that codes 6 types of mature miRNAs: miR-17, miR-18a, miR-19a, miR-20a, miR-19b1, and miR-92a1. The polymorphism rs4284505 in its non-coding sequence has been correlated with the incidence of breast cancer [7].

The expression of miR-205 is closely related to the occurrence, development, and prognosis of both lung and breast cancer. However, studies show opposing roles for its involvement in different tumor types. Because the regulation of miR-205 in lung and breast cancer is primarily confined to epigenetic areas, such as gene methylation and histone modification, whether the genetic variation of miR-205 is related to the occurrence or developmental process of tumor growth has not been reported. Consequently, we screened 10 tumor cell lines for sequence variations of miR-205 and miR-205HG and analyzed the correlation between the polymorphism rs3842530 and the occurrence and development of lung and breast cancer to reveal the possible different regulation mechanisms of the miR-205 gene and its opposing functions in the different tumor types.

## Material and Methods

### Collection of clinical samples

Formalin-fixed and paraffin-embedded samples were obtained from the Jiangxi Provincial Chest Hospital and Jiangxi Provincial Tumor Hospital, comprising lung tissues from 286 patients, out of which 93 were benign, and 391 cases of breast tissues, out of which 197 were benign, with an age range of 15-90 years. All diagnoses were based on the WHO criteria [8]. These patients, with primary malignant tumors, were not pre-treated with radio- or chemotherapy.

### Microarray database

The SurvMicro database [9] (<http://bioinformatica.mty.itesm.mx:8080/Biomatec/Survmicro.jsp>) was used to analyze the correlation between the expression of miR-205 and the prevalence of lung cancer or breast cancer. The relevance between the expression of miR-205 and the survival rate of patients with lung cancer or breast cancer was evaluated by use of the PROGmiR database [10] (<http://www.compbio.iupui.edu/prog-mir>). The relationship between miR-205HG mutation and the survival rate of patients with lung cancer or breast cancer was assessed using the cBioPortal database [11,12] (<http://www.cbioportal.org/index.do>).

### Cell culture

The breast cancer cell lines T47D, MCF7, MDA-MB-231, and BT549, the lung cancer cell lines 95-D and A549, the gastric cancer cell lines AGS and MGC803, and the hepatocellular carcinoma cell lines HepG2 and SMMC7721 were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum. Cells were cultured at 37°C in a humidified incubator in 5% carbon dioxide.

### DNA extraction and genotyping

DNA extraction was carried out using a conventional phenol-chloroform method for cell lines and a salting-out method for formalin-fixed and paraffin-embedded samples [13]. Genotypes were analyzed with polymerase chain reaction (PCR) and sequencing. Primers were designed with the online tool Primer3 [14,15]. The primers, forward 5'-ACAGGCTGAGGTTGACATGC-3', reverse 5'-GAGTTACTCTTGCTGCTGCTG-3', were used to amplify a 247 bp fragment. All the amplified samples were sent to Shanghai Shenggong Bioengineering Company for sequencing.

### Bisulfite clone sequencing (BCS)

The unmethylated cytosines of the genomic DNA were converted to uridines by the addition of 5 M sodium bisulfite. The

Wizard DNA Clean-Up System Kit (Promega) was used to purify the bisulfite-treated DNA. Primers for bisulfite-converted DNA were designed with the online tool MethPrimer [16]. The forward primer: 5'-GATTGGTTATTTTGTGGATTTT-3' and reverse primer: 5'-ACCTTACACCTAAAACCTTAATCTC-3' were used to amplify a 218 bp sequence within miR-205 gene promoter. Fresh PCR products for each sample were cloned with the pEASY-T1 cloning kit (Transgen Biotech, Beijing, China), and clones were used as templates for a 468-bp fragment amplification with M13 primers, according to the manufacturer's instructions. Amplicons were sent to Shanghai Shengggong Bioengineering Company for sequencing.

### RNA extraction and quantitative real-time reverse transcription PCR

Total RNA was isolated from the cells with TRIzol reagent (Invitrogen Life Technologies), and reverse transcription was performed using a RevertAid First Stand cDNA Synthesis kit (Thermo, USA), following the manufacturer's instructions. Quantitative PCR was performed using SYBR Green Premix Ex Taq™ II (Takara Bio, Inc., Otsu, Japan) with ABI 7500 Real-time PCR System (Applied Biosystems, Carlsbad, U.S.A). All the primers were purchased from Guangzhou Ruiibo Company. Data were normalized to U6 small nuclear RNA (RNU6) levels. The miR-205 expression level was analyzed by the general formula  $\Delta Ct = Ct_{(U6)} - Ct_{(miR-205)}$ .

### Immunohistochemical analysis

IHC was performed using Max Vision techniques. Mouse anti-human HER2 monoclonal antibody (MAB-0198, Maixin, Fuzhou, China), rabbit anti-human PR antibody (Kit-0013, Maixin, Fuzhou, China), and rabbit anti-human ER antibody (Kit-0012, Maixin, Fuzhou, China) were used as primary antibodies. IHC detection results were independently judged by 2 pathologists. Nuclear stained particles were used for ER or PR positive detection and the amount of positive cell  $\leq 25\%$  for low expression level and  $\geq 25\%$  for high expression level [17]; cell membrane stained particles were for HER2 positive detection and color intensity was used to distinguish low and high expression.

### Statistical analysis

The genotype distribution in benign lesions and tumor groups were analyzed by chi-square test or corrected chi-square test. In all results,  $P \leq 0.05$  was regarded as statistically significant. All statistical analyses were performed using the SPSS19.0 software package (SPSS, Chicago, IL).

## Results

### Retrieval of high-throughput expression profiling databases

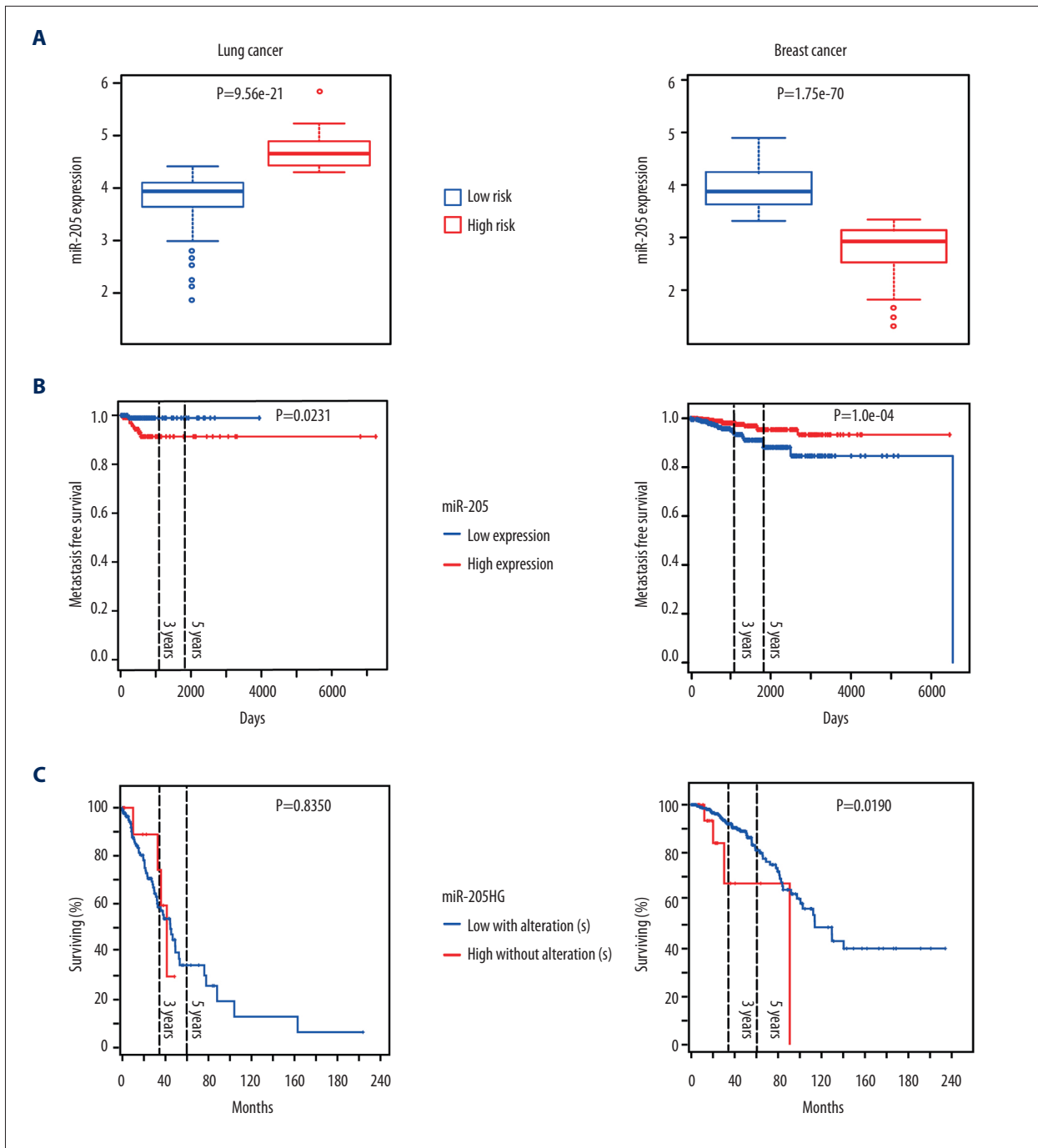
The SurvMicro database contains high-throughput microRNA expression profiling data, with 8 datasets each for lung and breast cancer. In the TCGA data for lung cancer, a higher expression of miR-205 correlates with a higher risk ( $P < 0.001$ ) (Figure 1A), whereas for breast cancer, a lower expression of miR-205 correlates with a higher risk ( $P < 0.001$ ) (Figure 1A). As the PROGmiR database shows (Figure 1B), the expression levels of miR-205 are closely related to the survival rates of patients with lung and breast cancer. A lower expression of miR-205 is correlated with a higher survival rate in patients with lung cancer ( $P = 0.0231$ ), whereas a higher expression of miR-205 is correlated with a higher survival rate in patients with breast cancer ( $P = 0.0001$ ). As the cBioPortal database shows (Figure 1C), for lung cancer patients with mutations in miR-205HG, the survival rate is similar to that for those who do not have mutations in this gene ( $P = 0.835$ ). In contrast, for breast cancer patients, the survival rate of patients with mutations at miR-205HG is significantly higher than for those without mutations ( $P = 0.019$ ).

### Bisulfite clone sequencing (BCS) analysis of the promoter of the miR-205 gene in 10 tumor cell lines

After sodium bisulfite modification and PCR for the miR-205 promoter in DNA from 10 tumor cell lines, 218 bp amplicons were TA cloned (Figure 2A). Overall, 468 bp of PCR products from positive clones was sent for subsequent sequencing (Figure 2A). Although 7 CpG sites were found in the CpG island site of the miR-205 gene, there were only 5 CpG sites consistent with methylation status (Figure 2B). The methylation frequency for the 5 CpG sites was not uniform in the 10 different cell lines (Figure 3A): the methylation frequency was low in the MCF7 (11.67%) and T47D (0.00%) cell lines and high (between 93.85–100%) in the MDA-MB-231, BT549, A549, AGS, MGC803, HepG2 and SMMC7721 cell lines, with only 1 exception for the 95-D cell line, where the methylation frequency was 62.50%.

### Expression analysis of miR-205 in 10 tumor cell lines

As the qRT-PCR results for the 10 tumor cell lines show (Figure 3A), the expression level of miR-205 was lowest in the MDA-MB-231 and 95-D cell lines and highest in the T47D and MCF7 cell lines.

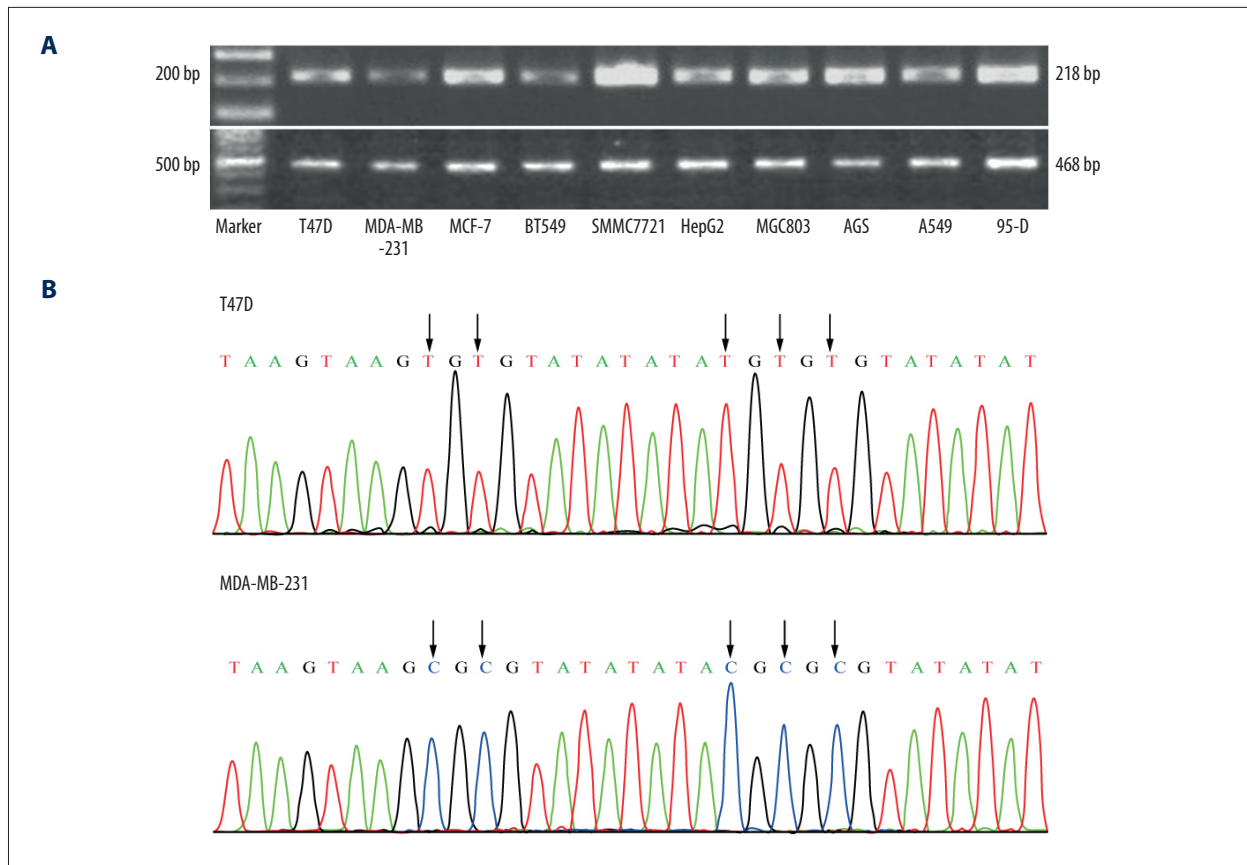


**Figure 1.** Retrieval of high-throughput expression profiling databases. **(A)** A schematic showing the association between the expression levels of miR-205 and the prevalence risk of lung and breast cancer; **(B)** Kaplan-Meier curve demonstrating overall survival for patients according to the expression of miR-205; **(C)** Kaplan-Meier curve indicating overall survival for patients according to the mutation of miR-205HG.

**Gene polymorphism analysis of miR-205 and miR-205HG in 10 tumor cell lines**

The 110 bp miR-205 coding gene as well as the 38 bp upstream and 99 bp downstream sequences were all covered by

the 247 bp miR-205HG PCR amplicons (Figure 4A). The electrophoresis speed of these PCR products was slightly different; the PCR amplicons from the T47D, MCF7, BT549, and AGS cell lines moved slightly faster than those from the other 6 cell lines (Figure 3B). The sequencing results showed that in the



**Figure 2.** Bisulfite clone sequencing (BCS) analysis of the promoter of the miR-205 gene. **(A)** Gel electrophoresis of 218 bp amplicons of the promoter of the miR-205 gene and 468 bp cloning products from 10 tumor cell lines; **(B)** DNA chromatograms illustrating the 5 CG sites in the promoter of miR-205 gene, with unmethylated CGs being converted to TG in T47D cell line and methylated CGs remained to be CG in MDA-MB-231 cell line.

10 tumor cell lines, no gene polymorphism was found within the miR-205 coding sequence. At 50 bp downstream of the miR-205 gene, in the fourth exon of miR-205HG, a repetition number variation of repeated AGC sequences was discovered (Figure 4B). In addition to the wild-type sequence (AGC)<sub>13</sub>, which designates a 13 triple-nucleotide of AGC, there are 2 polymorphisms: rs3842530 (AGC)<sub>9</sub>, and rs781155012 (AGC)<sub>7</sub>. The MDA-MB-231 cell line has a homozygous type of 13/13, whereas the 95-D, A549, HepG2, MGC803, and SMMC7721 cell lines have the heterozygous type of 9/13; the AGS, BT549, MCF7 cell lines have the homozygous type of 9/9, and the T47D cell line has the heterozygous type of 7/9.

#### Gene polymorphism analysis of miR-205 and miR-205HG in lung and breast tissue samples

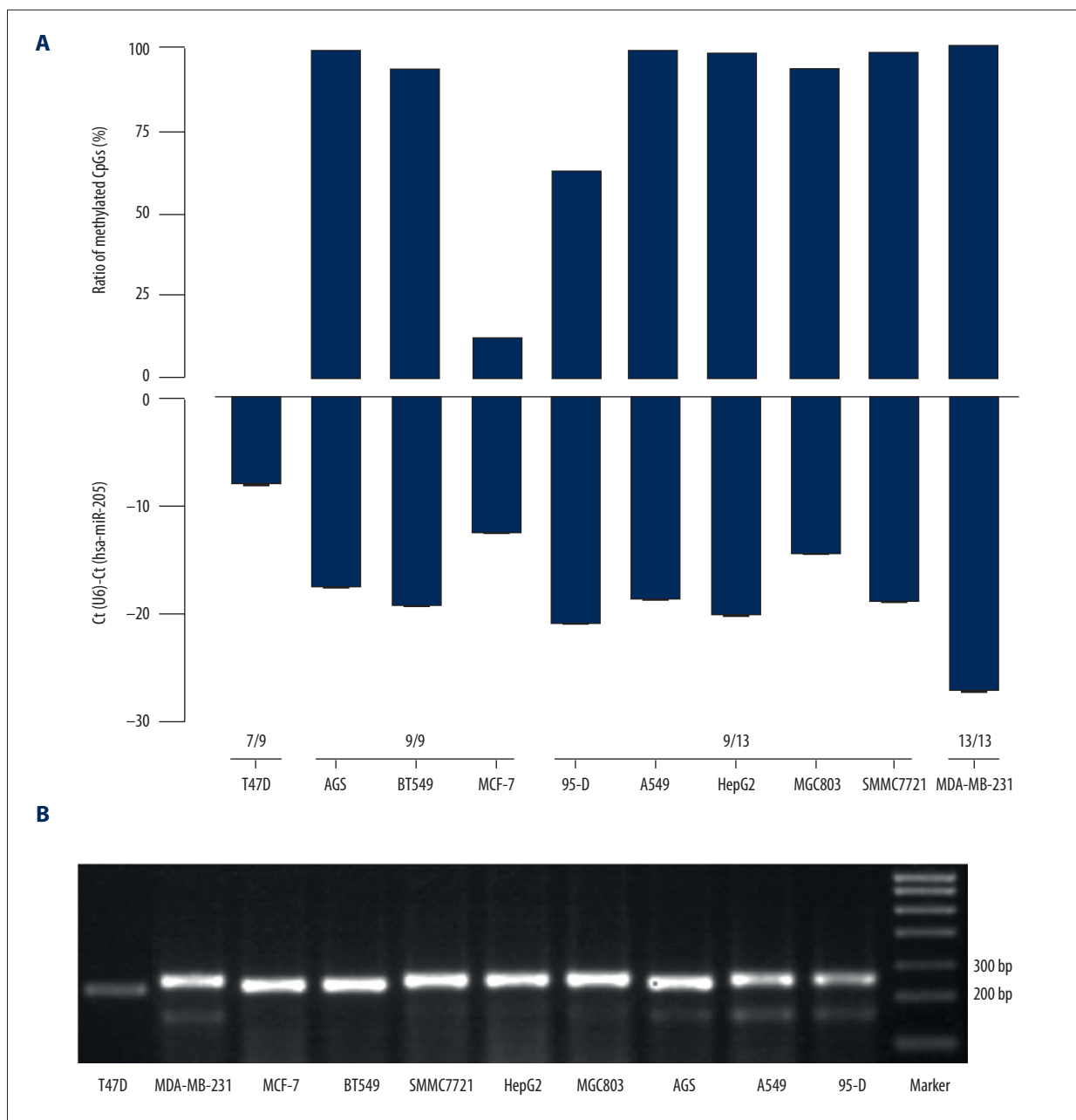
The sequencing results of PCR products from lung and breast tissues revealed no polymorphism in the miR-205 coding gene. However, downstream of the miR-205 gene, at the fourth exon of miR-205HG, some reported SNP *loci* were discovered (Figure 4B), including rs563793291 5 bp downstream,

rs186821678 14 bp downstream, rs568300824 23 bp downstream, rs776814851 47 bp downstream, rs112301138 48 bp downstream, and rs3842530, rs781155012, rs766738965, and rs565985624, all of which were 50 bp downstream of the miR-205 coding gene. The repetition number variation of the AGC repeated sequence 50 bp downstream of the miR-205 coding gene is common and is mostly due to the rs3842530 polymorphism, which can be divided into the 13/13 type, 9/9 type, and heterozygous type with different repetitions. Unreported polymorphic *loci* were not found in this amplified region.

#### Correlation analysis between the polymorphism rs3842530 in miR-205HG and the risk and clinical pathological characteristics of lung cancer and breast cancer

In the lung cancer group, compared with the other 2 genotypes (9/9 type or 9/13 type), the 13/13 genotype at the rs3842530 site was correlated with a lower risk in lung cancer ( $P < 0.05$ ) compared to the benign lung lesion group. In the breast cancer group, the distribution of the rs3842530 polymorphism showed no





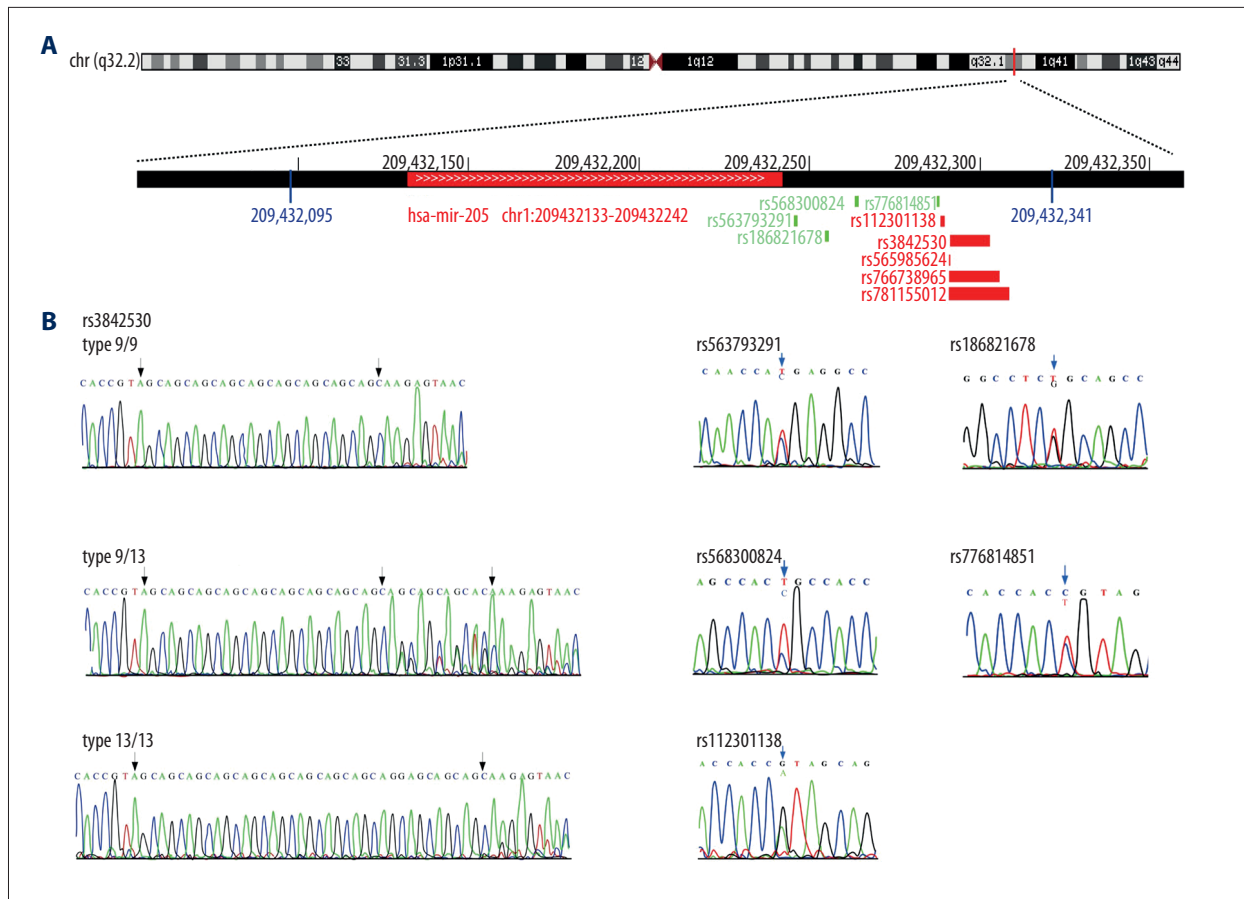
**Figure 3.** Methylation status, expression and sequence variation analysis of miR-205 gene in 10 tumor cell lines. **(A)** BCS and qRT-PCR were employed to detect the methylation status of the promoter of miR-205 gene and the expression of miR-205 in 10 tumor cell lines; **(B)** Agarose gel electrophoresis for 247 bp amplicons of miR-205 gene in 10 tumor cell lines.

significant difference ( $P>0.05$ ) (Table 1). Lung and breast cancer patients with detailed clinical pathological characteristics were divided into 2 groups according to the rs3842530 polymorphism: a homozygous group with genotype 9/9, and a mixed group with genotypes 9/13 and 13/13. The comparative analysis of clinical pathological features showed that the rs3842530 genotype had no statistical correlation with the lung cancer patients' sex, age, tumor location, tumor size, clinical staging, or lymph node metastasis. The distribution of the rs3842530 genotype had no

significant correlation with the breast cancer patients' sex, age, tumor location, tumor size, clinical staging, lymph node metastasis, or ER, PR, and HER2 expression levels (Table 2).

### Discussion

As an important regulator for carcinogenesis, much research has focused on miR-205. However, miR-205 acting as a promoter



**Figure 4.** Gene polymorphism analysis of miR-205 and miR-205HG in lung and breast tissue samples. (A) SNPs loci of miR-205HG discovered in lung and breast tissue samples; A 247 bp PCR products covering miR-205 and the fourth exon of miR-205HG from 209,432,095 to 209,432,341 were amplified; (B) DNA chromatograms illustrating the genomic sequence variation of miR-205HG.

**Table 1.** Association analysis between rs3842530 genotypes and the risk of lung cancer or breast cancer (P-value: compared with type 13/13 respectively).

Genotype	Lung		P	Breast		P
	Benign lesion	Cancer		Benign lesion	Cancer	
9/9	68 (51.13%)	65 (48.87%)	0.032	119 (52.65%)	107 (47.35%)	0.657
9/13	69 (52.27%)	63 (47.73%)	0.040	65 (47.10%)	73 (52.90%)	0.921
13/13	16 (76.19%)	5 (23.81%)		13 (44.44%)	14 (55.56%)	

or suppressor in the occurrence and development of tumors is tissue-dependent [18]. miR-205 can suppress the metastasis of prostate cancer cells by inhibiting the proliferation of tumor cells [19]. However, miR-205 can be an oncogene by promoting the proliferation and spreading of endometrial cancer cells *in vitro* [20]. Similarly, the role of miR-205 is different in the pathogenesis of lung and breast cancer. miR-205 has been shown to stimulate the occurrence and development of lung cancer. In squamous cell lung cancer, the expression level of miR-205 in

cancer tissues was significantly higher than that of para-carcinoma tissues [21,22]. However, in breast cancer, the expression level of miR-205 is mostly reduced. Studies have shown that the elevated expression of miR-205 can suppress breast cancer development by acting on EerbB3 and VEGF-A [23,24]. By analyzing the SurvMicro and PROGMiR databases and the literature, the expression levels of miR-205 in lung and breast tissues were retrieved in this study. We showed that although the expression of miR-205 was closely associated with the

**Table 2.** Association analysis between rs3842530 genotypes and the clinical characteristics of patients with lung cancer or breast cancer.

Variables	Lung cancer			P	Breast cancer			P
	9/9	9/9 and 9/13			9/9	9/9 and 9/13		
Gender	Male	37 (61.67%)	23 (38.33%)	0.423	3 (60.00%)	2 (40.00%)	0.781	
	Female	7 (50.00%)	7 (50.00%)		103 (55.98%)	81 (44.02%)		
Age	<45	2 (66.67%)	1 (33.33%)	0.765	33 (55.93%)	26 (44.07%)	0.943	
	≥45	40 (56.96%)	29 (43.04%)		74 (56.49%)	57 (43.51%)		
Tumor site	Left	15 (48.39%)	16 (51.61%)	0.100	57 (54.81%)	47 (45.19%)	0.696	
	Right	29 (67.44%)	14 (32.56%)		49 (57.65%)	36 (42.35%)		
Tumor size	≤3	11 (50.00%)	11 (50.00%)	0.200	22 (56.41%)	17 (43.59%)	0.990	
	>3	33 (66.00%)	17 (34.00%)		85 (56.29%)	66 (43.71%)		
Clinical stage	I+II	23 (63.89%)	13 (36.11%)	0.471	ND	ND	ND	
	III+IV	20 (55.56%)	16 (44.44%)		ND	ND		
Lymphatic metastasis	No	22 (52.38%)	20 (47.62%)	0.178	39 (53.42%)	34 (46.58%)	0.427	
	Yes	23 (67.65%)	11 (32.35%)		49 (59.76%)	33 (40.24%)		
ER expression	Low	ND	ND	ND	60 (52.17%)	55 (47.83%)	0.074	
	High	ND	ND		41 (66.13%)	21 (33.87%)		
PR expression	Low	ND	ND	ND	61 (54.95%)	50 (45.05%)	0.463	
	High	ND	ND		40 (60.61%)	26 (39.39%)		
HER-2 expression	Low	ND	ND	ND	57 (54.81%)	47 (45.19%)	0.402	
	High	ND	ND		47 (61.04%)	30 (38.96%)		

ND – not determined.

risk and progression of lung cancer and breast cancer, its effect is distinctly opposite in these 2 types of cancer, suggesting that the regulation mechanism for miR-205 in these 2 tumors may not be the same.

Studies on the regulation of the miR-205 gene have revealed its distinct roles in different types of tumors. Although the relationship between miR-205 expression and tumor growth has been widely reported, studies on miR-205 gene regulation are still insufficient. To date, this field of research is mainly limited to DNA methylation and histone modification in its promoter region. Reduced polycomb protein Me1-18 levels can decrease the expression of miR-205 by promoting methylation of the miR-205 gene, thus contributing to the occurrence of breast cancer[25]. Sun s found that a long non-coding RNA, HOX transcript antisense RNA (HOTAIR), can control miR-205 gene expression, and Guan et al. reported that HOTAIR can affect the histone modification status of H3K4me3 and H3K27me3 in its promoter region [26]. To determine whether the expression level of miR-205 is correlated with its methylation status

in its promoter region, the methylation status and expression level of miR-205 in 10 strains of cancer cells were detected by BCS and qRT-PCR, respectively. Except for the lung adenoma cell line 95-D (total methylation frequency is 62.50%), the expression of miR-205 and total methylation frequency was negatively correlated within the 9 other cancer cell strains. This result suggests that the expression level of miR-205 is mainly affected by the methylation status in its promoter region, but methylation regulation in the promoter region may not be the only way to regulate the expression of the miR-205 gene.

Not only closely related to DNA methylation and other epigenetic regulation, the expression process of a gene is also associated with its sequence structure. The human miR-205 gene is located within the miR-205HG (LINC00510) region at chromosome location 1q32.2, which includes a 110-bp sequence and encodes 2 mature miRNAs: miR-205-5p and miR-205-3p. The fourth exon of miR-205HG shares a 39-bp sequence with the miR-205 coding gene, in which relatively intensive polymorphism loci exist. By searching the cBioPortal database, we



found that in breast cancer, the survival rate of patients with miR-205HG mutations was significantly higher than that of those without mutations ( $P=0.019$ ), which suggests that sequence variation in miR-205HG may affect the development of cancer. Therefore, by designing primers that target the miR-205 gene and the fourth exon of miR-205HG, PCR product sequencing for 10 tumor cell lines can help determine whether polymorphism *loci* exist in this region. With agarose gel electrophoresis, the speed of PCR products from 10 tumor cell lines was slightly different, indicating the presence of potential deletion or insertion sequences in this region. The sequencing results of the PCR products revealed a deletion/insertion variation in the AGC repetitive sequences of the fourth exon of miR-205HG. Because type 13 is 12 bp longer than type 9, it can be seen that the electrophoresis speeds of cell lines of type 7/9 or type 9/9 (T47D, AGS, BT549, and MCF7) were significantly faster than the other 6 cell lines of either type 13/13 or 9/13. Combined with the expression of miR-205 in the 10 cell lines, it can be seen that the repetition number variation of AGC sequences in the miR-205HG may affect the expression of miR-205. With an increasing number of AGC repeats, the expression of miR-205 shows a gradual decreasing trend.

Studies show that miRNA coding sequences occur in spacer regions, exons, or introns of genes, and in the gene containing the miRNA coding sequence, called a host gene. The host gene can be either a protein-coding gene or a non-protein coding gene, such as the long non-coding RNA gene. Because the transcription and maturation of miRNA is closely linked to its host gene expression, they often present a co-expression and co-regulation pattern during the occurrence and development of diseases. Guan et al. found that in thyroid carcinoma, the expression of both miR-218-2 and its host gene SLIT3 was reduced, and they regulated the proliferation and mobility of thyroid cancer cells in a coordinated manner [27]. Meanwhile, due to the complementary pairing relationship, mature miRNA and its host gene can be mutually regulated. Truscott found that miR-11 is co-expressed with its host gene, dE2F1, and can suppress the accumulation of the dE2F1 transcription activator to inhibit dE2F1-dependent apoptosis [28]. Moreover, the

polymorphism within the miRNA host gene can regulate the expression of miRNA. The miR-126 gene lies in the intron of its host gene, EGFL7. The polymorphism rs4636297, which is 12 bp downstream of the miR-126 gene, can influence the development of diabetic retinopathy by regulating the expression of miR-126 [29]. While there have been many clinical studies on lung cancer [30], whether the miR-205HG variation affects tumor disease progression and whether this variation impacts different effects on lung cancer and breast cancer remain unknown. Therefore, we detected rs3842530 of miR-205HG in lung and breast tissue samples. The results show that rs3842530 is not correlated with the risk of breast cancer or the clinical pathological features of patients with lung and breast cancer ( $P>0.05$ ), but is associated with the risk of lung cancer ( $P<0.05$ ). Compared with types 9/9 and 9/13, the population that carries type 13/13 has a lower risk of lung cancer. This may be related to the increased expression of miR-205 in populations with genotype 9/9 or 9/13, which are more prone to lung cancer.

In summary, combining DNA methylation and the expression analysis of the miR-205 gene, this study screened rs3842530, a polymorphism located downstream of the miR-205 gene and in the fourth exon of miR-205HG, and analyzed the correlation between the polymorphism and both the risk and clinical pathological features of lung and breast cancer. We speculate that the rs3842530 polymorphism may be related to the risk of lung cancer, which may provide a possible explanation for the tissue-dependent function of miR-205 in different tumors.

## Conclusions

These results indicate that the functional association of rs3842530 in miR-205HG and lung cancer might provide a possible explanation for the tissue-dependent function of miR-205 in different tumors.

## Conflict of interest

The authors declare that they have no conflict of interest.

## References:

1. Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell*, 2004; 116: 281–97
2. Flego V, Ristic S, Devic Pavlic S et al: Tumor necrosis factor-alpha gene promoter -308 and -238 polymorphisms in patients with lung cancer as a second primary tumor. *Med Sci Monit*, 2013; 19: 846–51
3. Xu T, Zhu Y, Wei QK et al: A functional polymorphism in the miR-146a gene is associated with the risk for hepatocellular carcinoma. *Carcinogenesis*, 2008; 29: 2126–31
4. Yang H, Dinney CP, Ye Y, Zhu Y et al: Evaluation of genetic variants in microRNA-related genes and risk of bladder cancer. *Cancer Res*, 2008; 68: 2530–37
5. Ye Y, Wang KK, Gu J et al: Genetic variations in microRNA-related genes are novel susceptibility *loci* for esophageal cancer risk. *Cancer Prev Res*, 2008; 1: 460–69
6. Kontorovich T, Levy A, Korostishevsky M et al: Single nucleotide polymorphisms in miRNA binding sites and miRNA genes as breast/ovarian cancer risk modifiers in Jewish high-risk women. *Int J Cancer*, 2010; 127: 589–97
7. Chacon-Cortes D, Smith RA, Lea RA et al: Association of microRNA 17-92 cluster host gene (MIR17HG) polymorphisms with breast cancer. *Tumour Biol*, 2015; 36: 5369–76
8. Sobin LH, Gospodarowicz MK, Wittekind C: TNM classification of malignant tumours. New York, Wiley-Blackwell, 2009

9. Aguirre-Gamboa R, Trevino V: SurvMicro: Assessment of miRNA-based prognostic signatures for cancer clinical outcomes by multivariate survival analysis. *Bioinformatics*, 2014; 30: 1630–32
10. Goswami CP, Nakshatri H: PROGmiR: A tool for identifying prognostic miRNA biomarkers in multiple cancers using publicly available data. *J Clin Bioinforma*, 2012; 2: 23
11. Cerami E, Gao J, Dogrusoz U et al: The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discov*, 2012; 2: 401–4
12. Gao J, Aksoy BA, Dogrusoz U et al: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*, 2013; 6: p1
13. Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*, 1988; 16: 1215
14. Koressaar T, Remm M: Enhancements and modifications of primer design program Primer3. *Bioinformatics*, 2007; 23: 1289–91
15. Untergasser A, Cutcutache I, Koressaar T et al: Primer3 – new capabilities and interfaces. *Nucleic Acids Res*, 2012; 40: e115
16. Li LC, Dahiya R: MethPrimer: Designing primers for methylation PCRs. *Bioinformatics*, 2002; 18: 1427–31
17. Bulut G, Kosem M, Bulut MD et al: Is immunohistochemical sex hormone binding globulin expression important in the differential diagnosis of adenocarcinomas? *Asian Pac J Cancer Prev*, 2016; 16: 8203–10
18. Qin AY, Zhang XW, Liu L et al: MiR-205 in cancer: An angel or a devil? *Eur J Cell Biol*, 2013; 92: 54–60
19. Gandellini P, Profumo V, Casamichele A et al: miR-205 regulates basement membrane deposition in human prostate: implications for cancer development. *Cell Death Differ*, 2012; 19: 1750–60
20. Xie H, Zhao Y, Caramuta S et al: miR-205 expression promotes cell proliferation and migration of human cervical cancer cells. *PLoS One*, 2012; 7: e46990
21. Lebanony D, Benjamin H, Gilad S et al: Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma. *J Clin Oncol*, 2009; 27: 2030–37
22. Fassina A CR, Fassin M: Classification of non-small cell lung carcinoma in transthoracic needle specimens using microRNA expression profiling. *Chest*, 2011; 140: 1305–11
23. Wu H, Zhu S, Mo YY: Suppression of cell growth and invasion by miR-205 in breast cancer. *Cell Res*, 2009; 19: 439–48
24. Wu H MY: Targeting miR-205 in breast cancer. *Expert Opin Ther Targets*, 2009; 13: 1439–48
25. Lee JY, Park MK, Park JH et al: Loss of the polycomb protein Mel-18 enhances the epithelial-mesenchymal transition by ZEB1 and ZEB2 expression through the downregulation of miR-205 in breast cancer. *Oncogene*, 2014; 33: 1325–35
26. Sun X, Du P, Yuan W et al: Long non-coding RNA HOTAIR regulates cyclin J via inhibition of microRNA-205 expression in bladder cancer. *Cell Death Dis*, 2015; 6: e1907
27. Guan H, Wei G, Wu J et al: Down-regulation of miR-218-2 and its host gene SLIT3 cooperate to promote invasion and progression of thyroid cancer. *J Clin Endocrinol Metab*, 2013; 98: E1334–44
28. Truscott M, Islam AB, Lopez-Bigas N, Frolov MV: mir-11 limits the proapoptotic function of its host gene, dE2f1. *Gen Dev*, 2011; 25: 1820–34
29. McAuley AK, Dirani M, Wang JJ et al: A genetic variant regulating miR-126 is associated with sight threatening diabetic retinopathy. *Diab Vasc Dis Res*, 2015; 12: 133–38
30. Brunese L, Greco B, Setola FR et al: Studio comparativo con esame TC contrastografico e con PET-TC del tumore polmonare non a piccole cellule. *Med Sci Monit*, 2013; 19: 95–101