Tumor Biol. (2012) 33:1327–1334
DOI 10.1007/s13277-012-0380-9

RESEARCH ARTICLE

The expression of heparanase and microRNA-1258 in human non-small cell lung cancer

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Received: 21 December 2011 / Accepted: 12 March 2012 / Published online: 10 April 2012

Abstract This study aims to discuss the correlation between miR-1258 and the expression of heparanase (HPSE) in the cancer cells of the patients with non-small cell lung cancer (NSCLC) and the inhibition mechanism of miR-1258 on the invasion of lung cancer cell. The expression level of miR-1258 was detected by TaqMan real-time PCR assay, the expression of HPSE was detected by immunohistochemistry, and the expression level of HPSE in the cancer tissue of each case was detected by western blot and in its adjacent tissue of 53 patients with NSCLC. The influence of miR-1258 on the invasion potential of the lung cancer cell line A549 was studied with lentivirus system including cloned miR-1258 fragments subsequently. The expression of HPSE and miR-1258 in NSCLC tissue was not obviously related to patient's gender, age, differentiation extent of cancer tissue, cancer types, etc., but also staging and lymph node metastasis, and the difference was significant. Further studies showed that the relationship between the expression level of miR-1258 and the expression of HPSE was closer. The relative expression level of miR-1258 was 0.58±0.07 in HPSE positive sample and 1.58±0.11 in HPSE negative sample, and the difference of which was notably significant ($P<0.0001$). Western blot showed that the expression level of HPSE was highly negatively related to the expression level of miR-1258. The invasion potential of A549 was notably lowered when transfected by miR-1258. The miR-1258 regulates the expression level of HPSE to influence the morbidity and metastasis of NSCLC. The miR-1258 is likely to become the key to the treatment of lung cancer metastasis.

Keywords Non-small cell lung cancer (NSCLC) · Acetyl-heparanase · miR-1258

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Background

Early in 1975, Höök et al. [1] found an endoglycosidase which could degrade side chains of heparan sulfate (HS) and characterized the activity of this enzyme for the first time. Hulett et al. [2] had cloned a human heparanase (HPSE) gene sequence since then, which pushed the relevant research into a new stage. Human HPSE gene is located at chromosome 4q21.3, the total length of cDNA is 1,758 bp, and the molecular weight of its encoded protein is 50 and 65 kD. HPSE expression is mainly restricted to the placenta and immune organs in normal tissues, whereas in a variety of malignant tumors, HPSE has high levels of expression. Researches indicated that HPSE expression level has a strong correlation with tumor cell invasion and metastatic potential. The extracellular matrix and basement membrane barriers must be broken through, in order to promote tumor cell invasion and metastasis. Extracellular matrix, a complex network system containing a variety of macromolecular, is composed primarily of collagen IV, laminin, heparan sulfate proteoglycans (comprise a protein core and a number of HS side chains), and others. Basement membrane is a special kind of extracellular matrix with a solid thin paper-like

structure. Tumor cells could secrete a variety of degrading enzyme to destruct extracellular matrix and basement membrane barriers, and some common examples are members from protease family like matrix metalloproteinase, serine protease, etc. HPSE is an endoglycosidase which could degrade HS in several sites and destroy the integrity of extracellular matrix and basement membrane, thus promoting tumor cell invasion and metastasis. Current research found that HPSE is the only enzyme with HS-degrading activity [3], which makes HPSE a new target for antimetastatic drugs.

The prevention of infiltration and metastasis, the important biological feature of a malignant cell, is one of the crucial ways to decrease tumor mortality. Heparanase contributes a lot in the processes of penetration through the vascular wall, infiltration, diffusion, and metastasis of infiltrative cell. It is confirmed that the levels and overall potency of multiple HS/heparin-binding growth and angiogenic factors, which are abundant in the extracellular matrix, can be regulated when released by heparanase, and the intervention of heparanase can cause a wide impact that not only alter the adjacent microenvironment, but also stimulate the growth and metastasis of tumor cell [4, 5]. Additionally, heparanase inhibitor can prevent the metastasis of tumor cells [6].

There are more and more reports indicating that microRNAs (miRNA) affect the tumor progression as oncogenes or tumor suppressor genes [7–11]. MiRNAs are single-stranded, non-coding RNA molecules consisting of approximately 22 nucleotides that result from the sequential processing of putative miRNA transcripts by the RNaseIII enzymes, Drosha and Dicer [7]. It is followed by Zhang et al. [12] who find that miR-1258, a specific miRNA, could aim at heparanase gene and influence heparanase gene expression and, thus, has profound effects on the function of this molecule in brain metastatic breast cancer (BMBC) cell invasion and metastatic profiles. At regulatory level, miR-1258 inhibited the expression and activity of heparanase in BMBC cells; however, modulating heparanase blocked the phenotypic effects of miR-1258. At functional level, stable expression of miR-1258 in BMBC cells inhibited heparanase in vitro cell invasion and experimental brain metastasis.

Heparanase gene miRNA, which does not express in the nonimmune tissues of the heart, lung, liver, pancreas, etc., can be detected in the immune tissues of the spleen, lymph node, thymus, placenta, etc. and is ubiquitous in metastatic malignant cell [13]. Previous studies have found that the expression levels of heparanase in various tumor tissues of esophageal cancer [14], breast cancer [15], gastric cancer [16], colon cancer, pancreatic cancer [17], bladder cancer [18], etc. are obviously higher than its originated normal tissues. So, is the regulation of miR-1258 on heparanase exists only in breast cancer or all kinds of tumor cells with features of infiltration and metastasis? This study investigated the mechanism of influences of miR-1258 on lung cancer through the experiment of the relationship between heparanase and miR-1258 in lung cancer cell line with lung cancer clinical samples.

Patients and materials

Fifty-three cases of non-small cell lung cancer (NSCLC) tissue were obtained from the patients received by the Shanghai Pulmonary Hospital from September 2009 to June 2011. All the patients were operated and pathologically diagnosed with NSCLC without receiving preoperative chemotherapy and radiotherapy. All tissues were obtained from cancer and its peripheral cancer tissue (normal lung tissue 5 cm away from cancer focus), and the swelling lymph nodes were obtained at the same time in the case of lymph node metastasis. Fifty-three patients (37 males, 16 females) ranged in age from 37 to 79 with a mean of 58. According to WHO's *Histological Types of Lung Tumor* (1981), these patients can be classified by the anatomical parts into two types: 31 patients of squamous carcinoma and 22 patients of adenocarcinoma, or by histological differentiation into three types: 18 patients of poor-differentiated carcinoma, 23 patients of middle-differentiated carcinoma, and 12 patients of high-differentiated carcinoma. According to UICC's *The Lung Cancer TNM Staging System* (1997), these patients can be classified into five types: 6 patients of phase I, 17 patients of phase II, 26 patients of phase III, 4 patients of phase IV, and 40 patients of lymph node metastasis. Each sample was divided into two parts, one was preserved in liquid nitrogen for extraction of RNA and protein, and the other was fixed in 4 % paraformaldehyde and embedded in paraffin for immunohistochemistry staining.

Lung cancer cell line A549 was purchased from the Cell Bank of Chinese Academy of Sciences (China). RPMI 1640, DMEM medium, and trypsin were purchased from Gibco (USA). The monoclonal mouse anti-HPSE antibody, which could recognize inactive (65 kDa) as well as active (50 kDa) forms of HPSE, was purchased from Cedarlane Labs. The mouse antihuman beta-actin monoclonal antibody, HRP-labeled goat anti-mouse IgG antibody, and goat anti-rabbit IgG antibody were purchased from Santa Cruz. Easy RT-PCR kit was purchased from Beijing TransGen Biotech Co., Ltd. (China). SYBR Premix Ex TaqTM II kit was purchased from TaKaRa. The lentivirus vector systems (PHR-SIN-CSGW-H1a, psPAX2, and PMD2.G) were obtained from Tronolab. DMEM, restriction enzyme, and ligase were purchased from New England Biolabs Co., Ltd. (NEB). Olympus BX51TR 32FB3F01 was used as fluorescence microscope.

TaqMan miRNA assays

The miR-1258 levels were assessed by quantitative real-time PCR with TaqMan real-time PCR assay (Applied Biosystems, Inc. (ABI)). PCR-based detection of mature miR-1258 was achieved using specific probes for hsa-miR-1258 (ID no. 4427975, ABI) and endogenous control RNU44 (ID no. 4373384, ABI). A mirVana miRNA detection kit (Ambion) was used for real-time reverse transcription (RT)-PCR-based detection. Generally, there are two steps in the assay: stem-loop RT and real-time PCR. The mirVana miRNA kit (Ambion) was used for the isolation of the microRNAs of patient's lung cancer tissue, its adjacent tissue, and the tissue transfected by A549 cell line of lentivirus. When binding to stem-loop RT primers, the miRNA molecules are transcribed by reverse transcriptase using a kit (cat no. 4366596, ABI). Then the product was quantified using the conventional TaqMan PCR that contained a miR-1258 specific forward primer: (5′-CTGCGAGTCCCTG GAGTTAG-3′), reverse primer (5′-CGGTCCCCTA-ACT ACCCATT-3′), and SYBR-labeled TaqMan probes for miR-1258 and RNU44 control levels. The I-Cycler detector (Bio-Rad Laboratories, Inc.) was used under the conditions of 48 °C for 30 min, 95 °C for 10 min, 40 cycles at 95 °C for 15 s, and 60 °C for 60 s to perform PCR assays, and each of them was performed in triplicate. PCR results were recorded as threshold cycle numbers (Ct), normalized against RNU44, and expressed as fold change.

Immunohistochemistry

Immunohistochemistry was used to detect HPSE expression in formalin-fixed, paraffin-embedded human lung cancer tissues. Anti-HPSE monoclonal antibody (Cedarlane) was diluted at 1:3,000, incubated at 4 °C for 16 h (overnight), and then at room temperature (25 °C) for 30 min with biotinylated anti-mouse IgG (H+L). Parallel staining was performed using a Vectastain ABC kit (Vector Laboratories) per manufacturer's instructions, followed by review of stained and coded sections by pathologists blinded to study groups. Staining of HPSE was distributed through a 0 to 3+ intensity scoring scale: 0 corresponded to background negative staining; 1+, weak staining; 2+, moderate staining; and 3+, strongest staining. Pathologically, assessment showed that cases with an intensity score of 1 or more are HPSE-positive.

Western blot

Parts of the tissue preserved in liquid nitrogen were obtained using a scalpel, and then protein was extracted following the operating instructions of the protein extraction kit. For lentivirus-transfected A549, cells of each group were obtained exactly 48 h after transfection, rinsed with PBS for three times, and immediately added to 100 μl/well cell lysis solution (Beyotime Co., Ltd.), then followed by the extraction of the total protein after placed on ice for 30 min. BCA protein assay kit (Beyotime Co., Ltd.) was used for the assay of protein extracted from tissues preserved in liquid nitrogen or lentivirus-transfected cells. Total cellular protein (15 μg) was obtained for conventional SDS-PAGE electrophoresis with constant voltage of 110 V, in which the concentration of stacking gel and separating gel is 5 and 10 %, respectively. Then the protein was transferred onto PVDF membrane, enclosed with 0.5 % BSA, orderly combined with the primary antibody (i.e., the monoclonal mouse anti-HPSE antibody) and secondary antibody (i.e., the AP-labeled goat anti-mouse IgG) and, finally, X-ray scanned after X-ray exposure, developing and fixing in the darkroom using CDP-Star (Roche) as the chemiluminescent substrate of alkaline phosphatase. The protein expression of β-actin was used as a control.

Construction of PHRI-miRNA-1258 lentivirus vector

Primers were designed according to the primer design program of the exact endpoints of the DNA sequence. Restriction enzyme sites of Mlu I and Cla I were introduced, respectively, into the 5′ end of upstream primer, pSuper-miR-1258 forward: 5′-GATCCCCttccacgacctaatcc taactTTCAAGAGAagttaggattaggtcgtggaaTTTTTA-3′, and downstream primer, pSuper-miR-1258 reverse: 5′-AGCT TAAAAAttccacgacctaatcctaactTCTCTTGAAagttaggat taggtcgtggaaGGG-3′. The two primers were, respectively, dissolved with ddH_2O to 50 uM, and 10 ul of each was obtained to mix and boil for 5 min before nature cooling to room temperature. Then the two samples were linked with PHR-SIN-CSGW-H1a plasmid which was recycled by gel after restriction enzyme digestion by Mlu I and Cla I and transformed into *Escherichia coli* DH5α competent cells, among which positive recombinants are picked out to clone for DNA sequencing.

Virus package

293 T cells were cultivated in 1× DMEM culture solution (containing 10 % fetal bovine serum, gentamicin 100 U/ml, and kanamycin 50 U/ml) under the conditions of 37 °C and 5 % CO_2. The cells were seeded 24 h before transfection in 100-mm Petri dishes ($2–3\times10^6$ cells per dish, about 40–50 % of density) at 37 °C and 5 % CO_2. Standard calcium phosphate coprecipitation method was adopted for DNA transient transfection, 2–4 h before which the culture solution was updated. Forty-one micrograms plasmid DNA (20 ug PHRI-miRNA-1258+15 ug psPAX2+6 ugPMD2.G) were obtained and diluted with ddH_2O to a total volume of

436 μl, then the dilution was mixed, added with 64 μl 2 M $CaCl_2$, and mixed gently again. Five hundred microliters of 2× HBS (280 mM NaCl, 10 mM KCl, 1.5 mM Na_2HPO_4, 12 mM glucose, 50 mM HEPES, pH 7.05± 0.05) were slowly trickled into the solution, which was then placed for 20–25 min at room temperature before 1 ml suspension was dropped slowly into the 100-mm Petri dishes and mixed gently when opaque appeared in the solution. After cultivating for 8 h under the conditions of 37 °C and 5 % CO_2, 10 ml fresh culture solution was replaced and kept cultivating for 24–36 h before gaining the virus. Virus supernatant (100 ul) which was obtained 48 h after transfection, together with polybrene (1,000:1), were added to 35-mm dish of 293 T cells, and the culture solution was replaced 8 h after infection. The virus titer was measured by quantitative PCR, and the sample was concentrated and preserved at −80 °C.

A549 cell culture and infection experiments

A549 cells (1×10^5) were seeded in a 24-well plate 12 h before infection. Lentivirus supernatant, together with polybrene (1,000:1) infectious accelerant to promote infection, were added, and then the culture solution was replaced with fresh ones 12 h after infection and kept cultivating for 48 h. The infection efficiency was observed under fluorescence microscope to confirm positive cells for further expanding culture. Experiments were divided into the following three groups: (1) untreated group, (2) scrambled group (only lentivirus without miR-1258 fragment was inserted when transfection was performed), and (3) miR-1258 group. Each group repeated six times, and then the cells were tested by western blot.

Cell invasion experiment in vitro

Serum-free DMEM culture medium (500 μl) was added inside and outside of the invasion chamber (BD BioCoat™ Matrigel™ Invasion Chamber, packaged with Matrigel), which was then hydrated for 2–3 h in a 37 °C, 5 % CO_2 incubator. The three groups of A549 cells were digested by trypsin, resuspended in a serum-free medium, and counted before adding 1.0×10^5 cells of each group into the cabin, respectively. DMEM medium (700 μl) (containing 0.1 mg/ml fibronectin and 10 % FBS) were added in the subcabin and generally cultivated for 48 h in a 37 °C and 5 % CO_2 incubator, then the cabin was taken out with up-cabin liquid and abandoned, and crystal violet staining was performed at room temperature. Cells on the cabin membrane surface, which had not penetrated the membrane, should be wiped with cotton swabs, then the invasion cells in five fields of vision was counted using a light microscope, the average number was calculated, and each group repeated three times.

Statistical analyses

All data were analyzed using ANOVA or Student's *t* test and represented the mean ± SD, and SPSS statistical software (version 18) was used for statistical tests. *P* values below 0.05 were considered to be statistically significant.

Results

The expression of miR-1258 and HPSE in the lung cancer and peripheral cancer tissue of patients

The expression of HPSE in lung cancer and adjacent normal tissue is shown in Table 1. After hematoxylin and eosin (HE) staining, brown-yellow to brown-black granular material appeared in the cancer cell membrane and/or the cytoplasm of lung cancer tissue of lung adenocarcinoma (Fig. 1a) or lung squamous carcinoma (Fig. 1b), which is particularly evident in the membrane, and the staining intensity was obviously higher at the edge of cancer nests and in the basement membrane. The expression of HPSE was also positive in lymph node metastasis cancer and cancer cells with infiltration to the capillaries. The positive rate of heparanase in the lung cancer tissue of 53 NSCLC cases was 74.2 %. The expression level of heparanase was not related to patients' gender, age, and the histologic type and differentiation degree of cancer cell ($P>0.05$), but to TMN staging ($P=0.005$) and lymph node metastasis ($P=0.005$). The positive rate of heparanase in the group with lymph node metastasis was verified as 85.0 % after operation, while without lymph node metastasis, was 46.2 %, and the difference had statistics significance when two groups were compared ($P=0.005$).

According to TaqMan real-time PCR assay, the expression level of miR-1258 in the adjacent tissue (1.64±0.39) was obviously higher than that in the corresponding lung cancer tissue (0.82±0.52). This was opposite to the expression level of heparanase in the lung cancer tissue and its adjacent tissue. What consisted with the expression of heparanase was the expression of miR-1258 in the lung cancer tissue. A *P* value less than 0.01 was obtained when the cases were grouped by TNM stage and lymph node metastasis, which meant a significant difference. If the cases were grouped by HPSE expression, then a *P* value far less than 0.0001 was obtained, which meant a notably significant difference (see Fig. 2). It suggested that differences in the expression level of miR-1258 between groups divided according to lymph node metastasis and groups divided according to TNM staging reflected essentially the differences in the expression level of HPSE.

The relative expression levels of HPSE and miR-1258 in the cancer tissue of each case were obtained by western blot

Table 1 Relationship between heparanase and miR-1258 expression and clinicopathological characteristics in NSCLC

Category	Heparanase					miR-1258 (cancer tissue)			miR-1258 (peripheral cancer tissue)		
	Positive	Negative	Positive (%)	x^2	*P* value	Mean	Variance	*P* value	Mean	Variance	*P* value
Gender				1.79	0.181			0.367			0.389
Male	26	11	70.3			0.87	0.29		1.67	0.13	
Female	14	2	87.5			0.72	0.20		1.57	0.19	
Age (year)				0.82	0.366			0.645			0.658
≤60	22	9	71.0			0.85	0.30		1.66	0.17	
>60	18	4	81.8			0.78	0.23		1.61	0.12	
Phase				7.88	0.005			0.013			0.165
I, II	13	10	56.5			1.05	0.28		1.72	0.18	
III, IV	27	3	90.0			0.65	0.19		1.57	0.12	
Differentiation extent								0.580[a]			0.160[a]
Higher	10	2	83.3			0.84	0.31		1.77	0.15	
Middle	16	7	69.6			0.91	0.28		1.57	0.15	
Lower	14	4	77.8			0.64	0.12		1.57	0.11	
Lymph node metastasis				8.00	0.005			0.000			0.330
No	6	7	46.2			1.31	0.33		1.73	0.12	
Yes	34	6	85.0			0.67	0.15		1.61	0.16	
Cancer types				0.82	0.366			0.810			0.785
Squamous cell carcinoma	23	8	74.2			0.84	0.29		1.65	0.16	
Glandular cancer	17	5	77.3			0.80	0.24		1.62	0.14	

[a] *P* value was compared between well differentiation group and poor differentiation group; groups were set by differentiation extent, and the differences of miR-1258 expression were not significant among each group

experiments and miR-1258 quantitative PCR tests, respectively. We found that the expression level of HPSE and miR-1258 had an obvious negative correlation when combined together for analysis. The expression levels of miR-1258 and HPSE in the cancer tissue from each case of the 53 lung cancer patients were shown in the scatter plot of Fig. 3. In samples with a relatively high expression of miR-1258, the HPSE expression was relatively low, whereas in samples with a relatively low expression of miR-1258, the HPSE expression was relatively high. The correlation coefficient was −0.739. Thus, we inferred that miR-1258 could reduce the expression of HPSE.

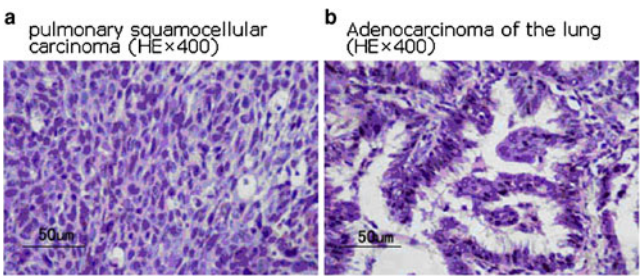

Fig. 1 The expression of acetyl-HPSE protein in the slice of NSCLC tissue. **a** HE staining of the pulmonary squamocellular carcinoma (HE×400), **b** HE staining of adenocarcinoma of the lung (HE×400). Immunohistochemical staining showed that heparanase protein was mainly located in the cytoplasm and cytomembrane. The expression levels in different tissues were different. No expression was seen in the lung cancer adjacent tissue (not shown in Fig. 1)

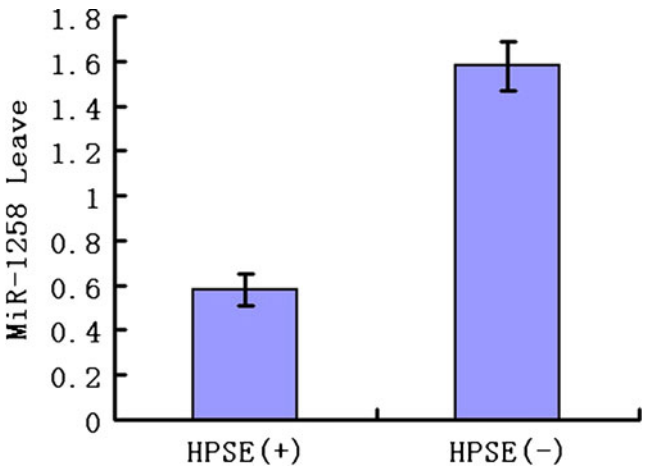

Fig. 2 The miR-1258 expression levels in HPSE-positive and HPSE-negative groups of lung cancer patients. The lung cancer tissues of lung cancer patients were divided into two groups according to the existence of the expression of HPSE, and the level of miR-1258 in the cells of these two groups was detected respectively. The result showed that the difference of the expression level between these two groups was significant ($p<0.001$). HPSE-positive and HPSE-negative samples, the difference of miR-1258 expression levels was significant ($p<0.0001$)

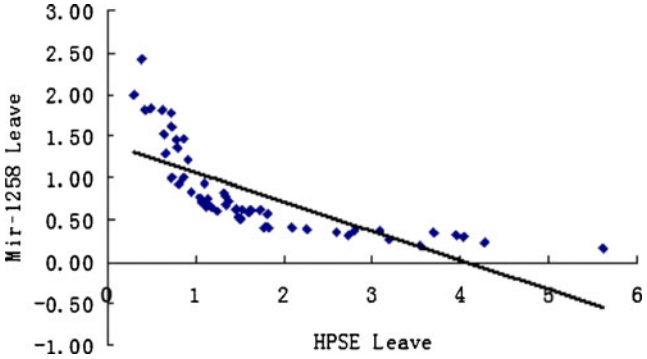

Fig. 3 Correlation analysis of miR-1258 and HPSE expression level in patients' lung cancer tissue. Scatter diagram was drawn with data of lung cancer patients with the expression level of miR-1258 as ordinate and the expression level of HPSE as abscissas, and the result of which showed that there was an obvious negative correlation between both levels. The *straight line* was a trend line

Results of western blot showed that miR-1258 inhibited the expression of HPSE

To verify the deduction from our tests on patients that miR-1258 could lead to reduce the expression level of HPSE, we cloned the segment of miR-1258 into lentivirus carrier and then transfected into A549 cell line. Green fluorescent A549 cell could be identified with fluorescence microscope after transfection by miR-1258 lentivirus, and the efficiency of infection was more than 90 %. A549 cell culture was divided into three groups: (1) untreated group, (2) scrambled group (only lentivirus without miR-1258 fragment was inserted when transfection was performed), and (3) miR-1258 group. The expression of HPSE in cells of the three groups was obtained by western blot detection (see Fig. 4), and real-time PCR was used to detect the expression level of miR-1258 after the cells of three groups were being treated. The result showed that HPSE expression was lower in miR-1258 group compared with other groups, whereas the expression level of untreated and scrambled groups was higher than that of the miR-1258 group, and the difference between the former two groups was not big. Accordingly, the expression levels of miR-1258 in the cells of three groups were 0.20±0.06, 0.22±0.07, and 1.81±0.16, respectively, and transfection status was met with the expected result. It indicated that miR-1258 had an obvious inhibition on the expression of HPSE, and this inhibition was determined by the sequence specificity of miR-1258.

The inhibition of miR-1258 on invasion ability of A549 cell

A549 cell was infected by lentivirus supernatant, and the fluorescence would be observed 48 h after infection as shown in Fig. 5. The efficiency of infection was more than 90 %.

We examined the effects of miR-1258 on cell invasion in order to evaluate miR-1258 functionality. Downregulation

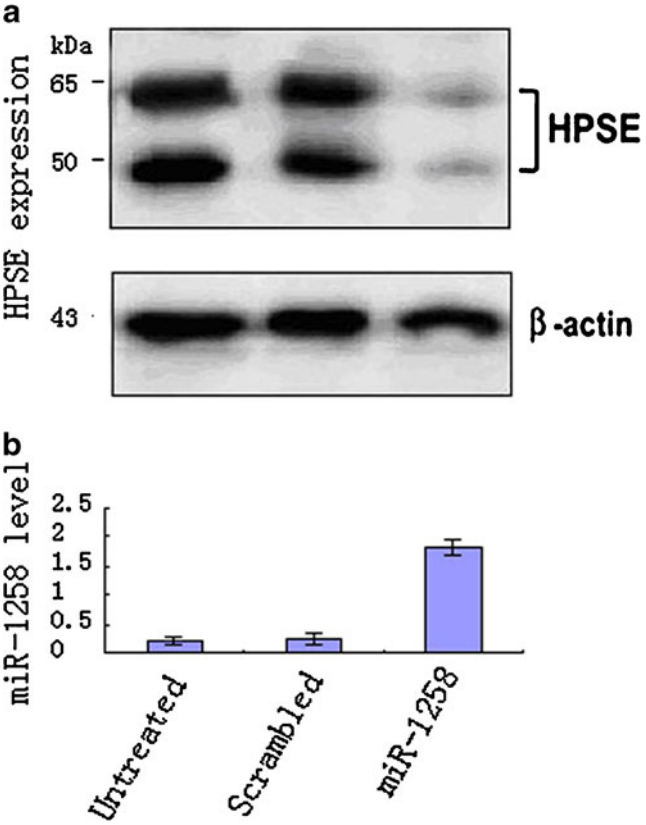

Fig. 4 The inhibition of miR-1258 on HPSE expression in A549 cell. **a** Untreated group, **b** scrambled group, **c** miR-1258 group. The expression level of HPSE in untreated and scrambled groups was higher, whereas in miR-1258, it was lower when transfection with lentivirus was performed. Accordingly, the expression level of miR-1258 in miR-1258 group after transfection was much higher than the other two groups

of heparanase expression was reflected by a corresponding modulation of cell invasion miR-1258. The counting under a microscope showed that the number of invasive cells in untreated group had no obvious difference ($P>0.05$) compared

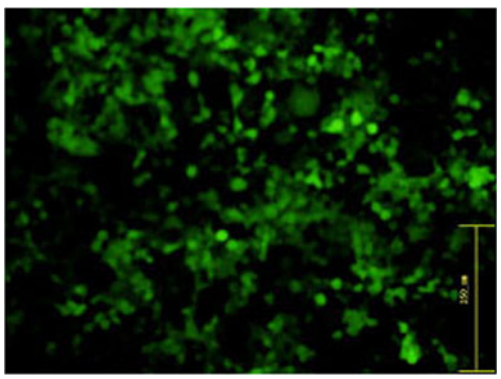

Fig. 5 The fluorescence of miR-1258 lentivirus in A549 cell (amplification factor, ×100). Green fluorescent A549 cell could be identified with fluorescence microscope after transfection with miR-1258 lentivirus was performed, and the efficiency of infection was more than 90 %

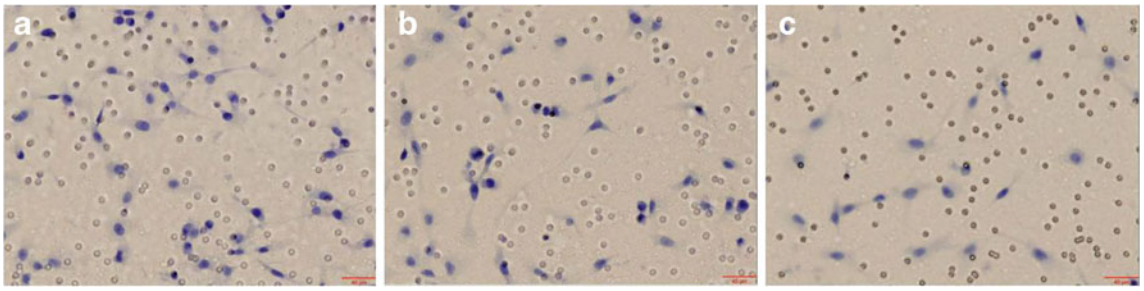

Fig. 6 Number of invasive cell penetrating the basement membrane in A549 cell of each group (HE, ×400). **a** Untreated group, **b** scrambled group, **c** miR-1258 group. Number of invasive cell in miR-1258 group was less than that in untreated and scrambled groups

with scrambled group, whereas there was a significant difference between the miR-1258 group and untreated group. Invasive cell number in the three groups was 118.5±4.6, 116.7±6.8, and 53.2±3.9, respectively, (see Figs. 6 and 7).

Discussion

Many studies have explored the influence of heparanase antisense oligonucleotides on the cell adhesion, invasion, and apoptosis of various types of cancer cell lines. There is a general conclusion of these researches: HPSE antisense RNA infection could downregulate HPSE protein expression in cells, inhibit cell adhesion and invasion significantly, and thereby induce cell apoptosis [4–6]. The reason why maintaining the functional concentrations of the siRNA or antisense in vivo is difficult is that the cells would try to recover the HPSE status. So, it is critical and of potential therapeutic value to develop miRNA-based approaches regulating heparanase.

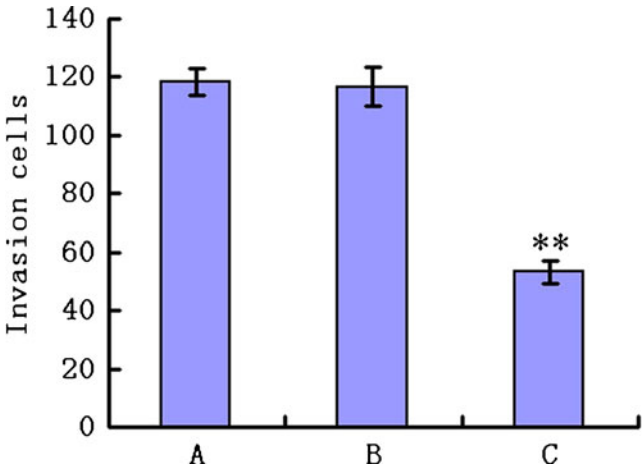

Fig. 7 Statistical number of invasive cell penetrating the basement membrane in A549 cell of each group. Cell invasion ability of A549 was reduced after transfection with miR-1258 was performed, while there was no substantial difference between the untreated group and scrambled group in comparison

In 2011, Zhang et al. [12] found that miR-1258, a candidate microRNA, may directly target HPSE and suppress BMBC. As a support of their hypothesis, they found that miR-1258 expression level had a negative correlation with HPSE expression, enzymatic activity, and cancer cell metastatic propensities; they also found that miR-1258 expression level was at the lowest in highly aggressive BMBC cell variants compared with either nontumorigenic or nonmetastatic human mammary epithelial cell. Analyses of miR-1258 and HPSE content in paired clinical specimens, normal mammary gland versus invasive ductal carcinoma and primary breast cancer versus BMBC, were performed to confirm these findings. In regulatory experiments, the expression and activity of HPSE in BMBC cells was inhibited by miR-1258, whereas miR-1258 was blocked by modulating HPSE. In functional experiments, cell invasion of HPSE in vitro and experimental brain metastasis was inhibited by miR-1258 in BMBC cells. What is more, their findings also illustrate how microRNA mechanisms are linked to brain metastatic breast cancer through HPSE control, which could offer a strong rationale to develop HPSE-based therapeutics for treatment of cancer patients with brain metastases, especially for BMBC.

Is the finding only applicable to breast cancer, or to all kinds of cancer with metastasis? Since HPSE mRNA commonly exists in metastatic malignant tumor cells, we believed that miR-1258 has universal significance for HPSE, and this is why we studied the relationship between miR-1258 and HPSE expression in lung cancer patients. Our findings confirmed an obvious negative correlation between miR-1258 and HPSE expression in lung cancer tissues.

Our experiments indicate that the expression levels of HPSE and miR-1258 in NSCLC tissue are closely related. In HPSE-positive samples, the expression levels of miR-1258 were relatively low; in HPSE-negative samples, the expression levels of miR-1258 were relatively high. The difference was extremely significant. This conclusion is accordant with the finding of Zhang et al. in breast cancer. It also shows that the inhibition of miR-1258 on HPSE was not just limited to breast cancer, but was also applicable to lung cancer. As for other cancers, new research data were needed to be supplemented for the correlation between HPSE and miR-1258 expression level.

The miR-1258 transfection experiment in A549 cell showed that the transfection of miR-1258 could directly reduce the expression of HPSE and inhibit cell invasion, suggesting that miR-1258 could directly inhibit or degrade the expression of HPSE. The correlation found between miR-1258 and HPSE expression level in lung cancer tissue was probably due to the continuous existence of miR-1258 in vivo, which inhibited HPSE expression. We have just studied the relationship between miR-1258 and the expression level of HPSE and the relationship between miR-1258 and the invasive ability in vitro of A549 not in more cell lines, but A549, the only one kind of lung cancer cell line, consequently, whether the conclusion suitable for other sell lines remained to be proved.

Since miR-1258 could downregulate the expression of HPSE protein in lung cancer cells as well as inhibiting cell invasion, then could miR-1258 possibly be a new method for lung cancer treatment? As far as we are concerned, this will come true until problems, such as the further clarification of the specific mechanism and the correlation on the pathways, etc., are solved.

Acknowledgments This study was supported by a grant from Shanghai Natural Science Fund of China (09ZR1425900).

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