Tumor Biology

MicroRNA-183 promotes migration and invasion of CD133+/CD326+ lung adenocarcinoma initiating cells via PTPN4 inhibition
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Abstract: Non-small cell lung cancer (NSCLC) is the most common cancers worldwide and is a leading cause of lung cancer mortality due to early stage metastases. Cancer stem-like cells (CSLCs) or tumor initiating cells (TICs) are a rare subpopulation cells that are responsible for maintaining tumor growth and invasion leading to recurrence and metastasis. Previous studies revealed that miR-183 can mediate the invasiveness and growth of NSCLC. However, the exact role of miR-183 in regulating the biological behavior of CSLCs in NSCLC remains unclear.

In the present study, we explored the regulation of protein tyrosine phosphatase nonreceptor type 4 (PTPN4) by miR-183 in vitro using luciferase-reporter assays, and we further analyzed the effects of miR-183 on the invasiveness of CSLCs in vitro and in vivo using transwell and bioluminescence assays.

Following our finding that miR-183 binds to PTPN4 mRNA to prevent its translation.
through the 3′untranslated region (UTR), we found that overexpression of miR-183 in CSLCs decreased PTPN4 protein levels while inhibition of miR-183 increased PTPN4 protein levels. The suppression of PTPN4 levels in CSLCs by miR-183 paralleled with a significant promotion in their motility in vitro and in vivo, while antisense miR-183 increased PTPN4 levels in CSLCs, which paralleled with a significant decrease in their invasiveness. Furthermore, correlation analysis between miR-183 and PTPN4 in clinical samples demonstrated a statistically significant inverse correlation between PTPN4 mRNA levels and miR-183.

In brief, our data indicate that miR-183 plays a proinvasive role by inverse regulation of PTPN4 and this axis may be a new therapeutic target for suppressing the metastatic capability of CSLCs in NSCLC.

Response to Reviewers:

Dear Reviewers,

Thank you very much for your comments concerning our Ms. entitled MicroRNA-183 promotes migration and invasion of CD133+/CD326+ lung adenocarcinoma initiating cells via PTPN4 inhibition (TUBI-D-15-03413). We have studied the comments carefully and have made corrections which we hope meet with approval. Revised portion are marked in bold face.

Reviewer #2

You describe that you based on TargetScan, PicTar and miRanda selected PTNPN4 as the potential target gene of mir-183. Could you describe more why you selected PTNPN4, what made you decide that PTNPN4 could be the potential target? After delineated by biology prediction, we selected several potential target genes to perform the pre-experiment. The experimental results indicated that the expression of PTPN4 and miR-183 had an inversed relationship. Based on the existing researches on the function of PTPN4, we speculated it might be a potential target of miR-183 and selected it as the target gene for follow-up study.

Have you thought of using other cell lines as well? Please address this. The reviewer is right. Beside the A549 cell line, there are another cell lines (such as CALU1, LC12, LC31 and LC52) possess the subpopulation which has the character of stemness in Non-small cell lung cancer (NSCLC) (Eur J Cardiothorac Surg. 2009 Sep; 36(3):446-53.). Tirino et al found that Cancer stem-like cells (CSLCs) can be also induced from CALU1, LC12, LC31 and LC52 cell lines by non-adherent culture condition. Our project is based on this research and choose the most common cell line A549 to study.

In the material and method section Quantitative RT-PCR, what is the sequence of Beta-actin? We have added the sequence of Beta-actin in the revised version of manuscript.

In the material and method section, what is the catalogue numbers of the antibodies used? We have added the catalogue numbers of the antibodies in the revised version.

In the material and method section for total protein extraction, what buffer did you use? We used the RIPA Lysis Buffer for total protein extraction.

The pictures are blurry, please make them clearer. Taking reviewer's suggestion, we have made the pictures more clearer in the revised version.

For the western blot in Figure 3C., it would be good if you can show quantification data. Taking reviewer's suggestion, we have show the quantification data for the western blot in Figure 3C.

Special thanks for your kind suggestions.
MicroRNA-183 promotes migration and invasion of CD133+/CD326+ lung adenocarcinoma initiating cells via PTPN4 inhibition

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Short Title: MicroRNA-183 promotes motility of CD133+/CD326+ CSLCs through PTPN4 repression

Abstract

Non-small cell lung cancer (NSCLC) is the most common cancers worldwide and is a leading cause of lung cancer mortality due to early stage metastases. Cancer stem-like cells (CSLCs) or tumor initiating cells (TICs) are a rare subpopulation cells that are responsible for maintaining tumor growth and invasion leading to recurrence and metastasis. Previous studies revealed that miR-183 can mediate the invasiveness and growth of NSCLC. However, the exact role of miR-183 in regulating the biological behavior of CSLCs in NSCLC remains unclear.

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Following our finding that miR-183 binds to PTPN4 mRNA to prevent its translation through the 3’untranslated region (UTR), we found that overexpression of miR-183 in CSLCs decreased PTPN4 protein levels while inhibition of miR-183 increased PTPN4 protein levels. The suppression of PTPN4
levels in CSLCs by miR-183 paralleled with a significant promotion in their motility in vitro and in vivo, while antisense miR-183 increased PTPN4 levels in CSLCs, which paralleled with a significant decrease in their invasiveness. Furthermore, correlation analysis between miR-183 and PTPN4 in clinical samples demonstrated a statistically significant inverse correlation between PTPN4 mRNA levels and miR-183.

In brief, our data indicate that miR-183 plays a proinvasive role by inverse regulation of PTPN4 and this axis may be a new therapeutic target for suppressing the metastatic capability of CSLCs in NSCLC.

**Key words:** miR-183, PTPN4, CD133/CD326, Cancer stem-like cells, Tumor initiating cells

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China (NSFC81201682).

**Introduction**

Non-small cell lung cancer (NSCLC) is one of the most common cancers worldwide and ranks as a leading cause of lung cancer mortality partly due to distant metastasis even in the early stages. An increasing number of studies have revealed that primary tumors contain a rare subpopulation of cells, termed cancer stem-like cells (CSLCs) or tumor initiating cells (TICs) that possess stronger invasiveness to form metastases in remote organs [1-5].

MicroRNAs (miRNAs) are a class of small, endogenous, non-coding RNAs that negatively regulate gene expression by partial or entire complementary binding to 3′ untranslated region (UTR) of mRNAs. Emerging evidence reveals that abnormal miRNA expression is relevant to the dysregulation of CSLCs in various cancers such as miR-181 and hepatic CSLCs [6], miR34 and pancreatic CSLCs [7], miR-340 and glioma CSLCs [8], miR-125a and breast CSLCs [9], miR-17 and ovarian CSLCs [10], miR-146a and colorectal CSLCs [11], and miR-320 and prostate CSLCs [12]. However, the function of miRNAs in regulating lung CSLCs has been rarely investigated and remains to be elucidated. In a previous study [13], we identified a subpopulation of cells marked by CD133+/CD326+ that could represent TICs or CSLCs of the A549 cell line and we confirmed that miR-183 was up-regulated in this subpopulation in both cell line and primary tumors. In this study, we focus on the exact role of miR-183 in CD133+/CD326+ CSLCs. Bioinformatics prediction is the basis for the study of miRNA, and it can effectively avoid duplication. Based on available databases including TargetScan, PicTar, and miRanda, we selected PTPN4 as the potential target gene of miR-183. PTPN4 is a widely expressed non-receptor protein tyrosine phosphatase that is involved in signal transduction and mediates cell growth, differentiation and regulates pro-apoptotic cellular functions [14]. Studies have rarely explored the role of PTPN4 in tumors, except for one study which suggested that it negatively regulates cell proliferation and motility of Hela and Hep3B cells [15].

In the present study, we found that miR-183 could suppress PTPN4 expression through binding to its 3′UTR and leading to degradation of PTPN4 mRNA as well as translational repression. Up-regulation of miR-183 in A549 CD133+/CD326+ CSLCs could play a proinvasive role by inverse repression of PTPN4 to promote lung adenocarcinoma CSLCs metastasis.

**Materials and methods**
**Cell lines and culture conditions**

A549 and HEK293T cell lines were obtained from the American Type Culture Collection and cultured in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in 5% CO2 at 37 °C. The induced spheres from A549 were cultured in DMEM supplemented with 0.4% BSA (Sigma), insulin (5 μg/mL, Sigma), basic fibroblast growth factor (bFGF, 10 ng/mL, PeproTech), and human recombinant epidermal growth factor (EGF, 20 ng/mL, PeproTech), cells were expanded once or twice per week.

**Flow cytometry analysis**

Spheroids were dissociated into single cells, washed and incubated with monoclonal antibodies specific for human PE-conjugated CD133/1 (Miltenyi, 130-080-901) and FITC-conjugated Ep-CAM (CD326, Miltenyi, 130-098-113). The dilutions and procedures were carried out according to the manufacturer’s instructions. After incubation for 30 minutes, cells were washed again and analyzed by flow cytometry.

**Immunofluorescence**

As described previously [13], spheroids were incubated with specific antibodies against CD133(1:300, Abcam, Ab5558), EP-CAM (1:300, Santa Cruz, sc53277) and then FITC-conjugated goat anti-rabbit IgG (1:400, Beyotime, A0562) and Cy3-conjugated donkey anti-goat IgG (1:400, Beyotime, A0502).

**Quantitative RT-PCR**

Real-time PCR was performed as described previously [13]. The qRT-PCR analysis was performed using an ABI7500 Prism Sequence Detection System (Applied Biosystems) with a SYBR Green kit (TAKARA). Based on the manufacturer’s instructions, we used the miRNeasy FFPE or Mini total RNA isolation kit (Qiagen) to extract the total RNA from tumor samples or cultured cells. The PTPN4 primers used were forward 5’-TGCTGGCAGCACCTACAA TG-3’ and reverse 5’- CAACCAGACTT GGCCCCCTGAT-3’, and the β-actin primers were forward 5’-TGGAGAAGAGCTATGCTGTGTA GCTGCCTG-3’ and reverse 5’-GTGCCACCAGACAGCAGTGTGGT -3’ (Qiagen). The relative gene expression levels were calculated using the comparative Ct (ΔΔCt) method, with β-actin as a loading control.

**Plasmid and lentiviral construction**

MiR-183 mimics or miR-183 inhibitor (anti-miR-183) and their negative controls (miR-control or anti-miR-control), and relative controls Lenti-miR183 or Lenti-anti-miR183 and their negative controls based on pLenti6.3/V5-DEST were constructed from Invitrogen. The open reading frame of PTPN4 was amplified by PCR using primers containing KpnI and EcoRI restriction sites and then subcloned into vector pcDNA 3.1(+) to generate the construct pcDNA6.2-PTPN4 (Invitrogen). The empty vector served as a negative control. Transfections were carried out using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer’s instructions. The miR-183 mature sequence was 5’-UAUGGCCACUGGUAGAUAUCU-3’, and the miR-183 antisense sequence was 5’-AGTGAATTCTACGAGTCCATA-3’.

**Transwell assay**
Migration and invasion assays were performed as follows, CD133+/CD326+ CSLCs were transfected with miR-183antagomir, miR-183 mimics and their relative controls for 48 h. Transwell inserts had 6.5-mm polycarbonate membranes with 8.0 μm pores (Corning, New York, NY, and USA). Matrigel invasion assays were performed using membranes coated with Matrigel matrix (BD Science, Sparks, MD, USA). CD133+/CD326+ CSLCs at an approximate density of $1 \times 10^5$ were suspended and then seeded in the upper chambers of 24-well transwell plates with FBS-free medium while stem cell culture medium containing 10% fetal bovine serum was deposited in the lower chambers. After 18 hours, cells that migrated were stained by 0.5% crystal violet solution for 15 min and counted. For invasion assays, transwell membranes were prepared with matrigel for plating infected cells. After 24 hours cells that migrated were stained by 0.5% crystal violet solution for 15 min and counted. Each experiment was performed in duplicate.

**CKK8 assay**

Cell proliferation was analyzed using the Cell Counting Kit-8 (CCK-8, Beyotime). The cells were used for the transfection and the measurement. After transfection, cells were plated in 96-well plates at 5000 cells per well. After 24 hours, 10 μl CCK-8 solution was added to each well and incubated at 37 °C for 1 h and OD readings were determined at 450 nm.

**Experimental lung metastasis models and lentiviral infection**

The care and use of mice was performed in accordance with local ethical guidelines. The pro-metastatic activity of miR-183 was tested in a nude mouse (n=6, purchased from the Chinese Academy of Medical Sciences, Beijing, People’s Republic of China) lung metastasis model as described previously [16]. The CD133+/CD326+ CSLCs were stably infected with Lenti-miR-183 or Lenti-control containing GFP label. Treated cells ($1 \times 10^5$) were suspended in 100 μL of PBS and injected via the lateral tail vein. Mice were sacrificed and lungs were resected 28 days later after inoculation, and the metastases volumes were estimated by bioluminescence imaging using the Living Image software (Xenogen, Baltimore, MD).

**Dual-luciferase activity assay**

For luciferase reporter experiments, the wild-type and mutated 3'UTRs of PTPN4 containing the binding cites of miR-183 were subcloned into the the pmiR-RB-REPORT™ Vectors (RIBOBIO, Guangzhou, China) to obtain pmiR-RB-REPORT-PTPN4-WT and pmiR-RB-REPORT-PTPN4-MUT, respectively. The PTPN4-WT primers used to amplify specific fragments were forward 5'-GGCGGCTCGAGACCTTAACAACTCAACA-3' and reverse 5'-AATGCGGCCGCAACAGA AATTTTACCCCT-3' and the PTPN4-MUT primers were forward 5'-TGGTCACTTGAGAC TATGCTGCTCGAGAAAT-3' and reverse 5'-AGCAGCATAGGTCACAAGTGAACATAGG GAAA-3'. Synthesis and sequencing of primers were performed by GENEWIZ Inc (Suzhou, China). HEK293T cells (1 × 10^6 cells/well) were cultured in a 24-well plate and co-transfected with 40 nm miR-183 mimics or inhibitor, 50 ng of pmiR-RB-REPORT-PTPN4-WT or pmiR-RB-REPORT-PTPN4-MUT, and 2 ng of pRL-TK (Promega) using Lipofectamine 2000. After transfection for 48 h, cells were harvested and assayed using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's protocols.

**Tumor tissue samples and RNA isolation**
The 30 cases of adenocarcinoma confirmed by pathology including 15 specimens with (n=15) or without (n=15) distant metastasis based on follow-up data were selected from the Department of Pathology of the First Affiliated Hospital of Sichuan Medical University. Total RNA was isolated from formalin-fixed paraffin-embedded tissue using the miRNeasy FFPE isolation kit according to the manufacturer’s protocols (Qiagen).

**Total protein extraction and Western blotting**

Standard protein extraction and Western blotting techniques were used for analysis. Spheres were resuspended and lysed in ice-cold RIPA Lysis Buffer (Beyotime) for total protein extraction. Protein samples were separated by SDS-PAGE and then transferred to PVDF membranes (Millipore). Membranes were blocked with 5% non-fat milk, incubated with primary antibodies at 4ºC overnight, and then incubated with secondary antibodies for 1 h at 25 ºC. Specific antibodies against PTPN4 (1:300, Sigma, M8159) and β-actin (1:400, Boster, BA2305) were used.

**Statistical analysis**

Data from all experiments were presented as the means ± SEM from at least three independent experiments and analyzed by Student’s t-test unless otherwise specified (Pearson’s correlation). The correlation between miR-183 levels and PTPN4 was also calculated by Spearman’s correlation. P-values < 0.05 were considered to be statistically significant.

**Results**

miR-183 expression was up-regulated in CD133+/CD326+ TICs

According to our previous study mentioned above, we successfully induced CD133+/CD326+ CSLCs from A549 cells (Fig. 1a-c) and again affirmed that miR-183 was up-regulated in CD133+/CD326+ CSLCs compared to normal A549 cells (Fig. 1c). These data are in agreement with our previous report that demonstrated up-regulation of miR-183 by miRNA microarray and validation in the A549 cell line and primary samples [13].

MiR-183 promotes the motility of CD133+/CD326+ CSLCs in vivo and in vitro

To confirm the function of miR-183 in CD133+/CD326+ CSLCs, we established stable miR-183 overexpressed or knockdown CD133+/CD326+ CSLCs using a lentiviral system as described above. The relevant variation of miR-183 expression in cells infected with miR-183 and anti-miR183 was confirmed by qRT-PCR (Fig 2a). To assess changes in cell migration, CD133+/CD326+ CSLCs that overexpressed miR-183 or the negative control were allowed to migrate through a transwell membrane into complete media. Compared to the negative control, overexpression of miR-183 led to the promotion of the motility of CD133+/CD326+ CSLCs (Fig. 2d). Then, to evaluate cell invasion capability, miR-183 was overexpressed in CD133+/CD326+ CSLCs that were plated on membranes precoated with matrigel. As shown in Figure 2e, miR-183 overexpression significantly promoted the invasion of CD133+/CD326+ CSLCs. In intravenous injection assays, bioluminescence imaging revealed that fluorescence signals in the miR-183 overexpressed group were significantly higher than the control group, which indicated that more metastasis is formed in the lungs after miR-183 overexpression (Fig. 2b). In order to rule out the possibility that the promotion of metastasis by miR-183 may in part be caused by increasing cell proliferation, we analyzed cell proliferation using the CCK8 assay. As shown in Figure 2c, there were no differences in cell proliferation. To further
investigate the proinvasive role of miR-183, we examined the effects of inhibiting miR-183 on the phenotype of metastasis in CD133+/CD326+ CSLCs. As expected, inhibition of miR-183 markedly reduced the invasive capabilities of CD133+/CD326+ CSLCs (Fig. 2d, 2e). Collectively, our data suggest that miR-183 greatly contributes to the metastasis and invasion of CD133+/CD326+ CSLCs.

**PTPN4 was a direct target of miR-183**

To explore the molecular mechanism of miR-183 on the motility of CD133+/CD326+ CSLCs, we searched for potential target genes of miR-183 using three publicly available databases including TargetScan, PicTar, and miRanda. The above algorithms indicated that PTPN4 was a theoretical target of miR-183 (Fig. 3a), and it was then selected for further analysis. We conducted a luciferase reporter assay to confirm whether PTPN4 was a direct target of miR-183. PTPN4 wild-type (WT) or mutant (MUT) 3'-UTRs were cloned into pGL3 luciferase reporter vectors, and co-transfected with miR-183 or control mimics into HEK 293T cells. The results revealed that miR-183 overexpression caused a clear decrease in relative luciferase activity (Fig. 3b). Furthermore, qRT-PCR and western blotting analyses showed that miR-183 knockdown significantly promoted the PTPN4 mRNA and protein levels in CD133+/CD326+ CSLCs (Fig. 3c). Together, these results strongly support that miR-183 directly suppresses PTPN4 by means of mRNA degradation as well as translational repression.

**PTPN4 mediates miR-183-induced migration and invasion in CD133+/CD326+ CSLCs**

To further examine whether miR-183 promotes invasiveness of CD133+/CD326+ CSLCs through PTPN4, we performed a rescue experiment by overexpressing PTPN4 in the presence or absence of miR-183 expression in CSLCs. After co-transfection, the expression of PTPN4 was analyzed by western blot (Fig. 4a). Consistent with the expression of target proteins, miR-183 could enhance the invasiveness of CD133+/CD326+ CSLCs, and the decreased metastatic potential was also confirmed in PTPN4-overexpressing cells compared to control cells. Furthermore, concomitant overexpression of miR-183 and PTPN4 could partially abrogate miR-183-induced invasiveness of CD133+/CD326+ CSLCs (Fig. 4 b,c). Thus, these findings demonstrate that PTPN4 is a functional target of miR-183.

**Inverse correlation between miR-183 and PTPN4 in human lung adenocarcinoma tissues**

To further investigate whether the miR-183-induced modulation of PTPN4 is of clinical relevance, we first assessed the expression levels of PTPN4 in clinical lung adenocarcinoma tissues. PTPN4 levels were lower in lung adenocarcinoma tissues with metastasis compared to the non-metastatic lung adenocarcinoma tissues (P < 0.05, Fig. 5a). We next correlated PTPN4 with miR-183 expression in the same lung adenocarcinoma specimens. A statistically significant inverse correlation was observed between PTPN4 mRNA levels and miR-183 (P < 0.05, Fig. 5b).

**Discussion**

In a previous study, we successfully separated a cellular subpopulation marked by CD133+/CD326+ from the A549 cell line and identified that this subpopulation represented CSLCs because they possessed capacities of multi-differentiation, stronger tumorigenesis, and higher expression of stem cell-associated genes. Following further investigation using miRNA microarray and quantitative RT-PCR on both cell line and primary samples, we found that miR-183 was upregulated in the CD133+/CD326+ subpopulation compared to normal A549 cells [13].

Different roles of miR-183 in tumorigenesis and metastasis were highlighted in various tumors.
MiR-183 overexpression represented a potential oncogenic role in tumorigenesis and invasion in several cancer types including synovial sarcoma, rhabdomyosarcoma and colon cancer, by targeting EGR1 and PTEN [17], gastric and oesophageal cancers by targeting PDCD4 to promote proliferation and invasion [18, 19], and renal cancer cells by targeting phosphatase 2A to increase cell proliferation and invasion [20]. Contrarily, a tumor suppressor role was found in 801D cells, where up-regulation of miR-183 repressed invasion and migration by targeting Ezrin [21]. A tumor suppressor role was also demonstrated in pancreatic cancer stem cells by negatively regulating ZEB1 to eliminate some traits of CSCs [22]. In our present study, we aimed to discover the function of miR-183 in regulating the biological behavior of CD133+/CD326+ CSLCs in NSCLC. Following transwell assays, we first found that overexpression of miR-183 could enhance migration and invasion activities of CD133+/CD326+ CSLCs, while inhibition of miR-183 reduced invasiveness of the cells (Fig. 2d, 2e). The same proinvasive role of miR-183 was further validated in nude mice in vivo (Fig. 2b). In addition, we performed the CCK8 assay to explore the influence on cell proliferation and found no obvious differences between the control group and the miR-183 overexpression group (Fig. 2c). These data indicate that miR-183 plays a proinvasive role in CD133+/CD326+ CSLCs.

Following affirmation of the proinvasive ability in CD133+/CD326+ CSLCs, we next explored the potential targets of miR-183 on the motility of CD133+/CD326+ CSLCs. Based on three publicly available databases, PTPN4 was identified as a theoretical target of miR-183 (Fig. 3a). PTPN4 is a widely expressed non-receptor protein tyrosine phosphatase involved in signal transduction that mainly plays pro-apoptotic [14] and proinvasive roles [15]. Because few studies have explored its role in tumors, we selected PTPN4 as a target gene of miR-183. We conducted luciferase reporter assays to confirm that PTPN4 was a direct target of miR-183. As expected, our results revealed that miR-183 overexpression caused a clear decrease in relative luciferase activity (Fig. 3b). Furthermore, qRT-PCR and western blotting analyses showed that miR-183 knockdown significantly promoted PTPN4 mRNA and protein levels in CD133+/CD326+ CSLCs (Fig. 3c). Therefore, these results strongly support a direct suppression of PTPN4 by miR-183 through mRNA degradation as well as translational repression. The above results were again verified by a rescue experiment through overexpression of PTPN4 with or without the presence of miR-183 expression in CSLCs. Concomitant overexpression of miR-183 and PTPN4 could partially abrogate miR-183-induced invasiveness of CD133+/CD326+ CSLCs (Fig. 4 b,c), indicating that PTPN4 was a functional target of miR-183.

The clinical relevance analysis in 30 cases with or without metastases showed that there was a greater expression of PTPN4 in specimens with distant metastasis compared to those without distant metastasis. The expression of PTPN4 was inversely correlated with the expression of miR-183. These clinical data indicate the relevance of the involvement of the miR-183/PTPN4 axis in the distant metastasis of lung adenocarcinoma.

Taken together, our findings demonstrated that miR-183 plays a proinvasive role in CD133+/CD326+ CSLCs through repression of PTPN4. The miR-183/PTPN4 axis might be a novel therapeutic target for regulating CD133+/CD326+ CSLCs in NSCLC. In the future, experiments should be designed to examine other targets of miR-183 in this subpopulation. The coordination of these target genes may lead to the interpretation of the exact function of miR-183 in NSCLC carcinogenesis.

References
1. Long HX, Xiang T, Qi W, Huang J, Chen J, He L, et al. CD133+ ovarian cancer stem-like cells promote non-stem cancer cell metastasis via CCL5 induced epithelial-mesenchymal transition.
Gastric cancer stem cell. Phosphatase er cells by downregulating theeses breast cancer metastasis. enchymal transition.


Saver AL, Li LH, Subramanian S. MicroRNA miR-183 functions as an oncogene by targeting the

Legends of figures

Fig. 1 MiR-183 expression was up-regulated in CD133+/CD326+ CSLCs. a. Cell morphology: A549 cells grew into tight spheres after induction. b. Cytometric CD133/CD326 expression in spheroids. c. Immunofluorescence of CD133 (green) and CD326 (red) expression on spheroids. d. Relative miR-183 expression between A549 cells and CD133+/CD326+ CSLCs.

Fig. 2 MiR-183 promotes the invasion and metastasis of CD133+/CD326+ CSLCs. a. The expression levels of miR-183 were tested by qRT-PCR in CD133+/CD326+ CSLCs transfected with miR-183, anti-183, and their respective controls. b. Representative bioluminescent images of lungs in nude mice at 28 days after inoculation via the tail vein. c. Cell proliferation of CD133+/CD326+ CSLCs transfected with miR-183 or control by CCK8 assay. d. The effect of miR-183 overexpression or knockdown on motility of CD133+/CD326+ CSLCs using transwell migration assay and e. invasion assay.

Fig. 3 PTPN4 was a direct target of miR-183. a. The wild-type and mutant of putative miR-183 target sequences in PTPN4 3’UTRs. b. Analysis of the luciferase activity of PTPN4 3’UTR WT and MUT vectors in HEK293T cells by miR-183. c. PTPN4 mRNA levels in the indicated cells were examined by qRT-PCT. d. PTPN4 protein in the indicated cells were examined by western blot.

Fig. 4 PTPN4 mediates miR-183-induced migration and invasion in CD133+/CD326+ CSLCs. a. PTPN4 protein levels were analyzed by western blot in CD133+/CD326+ CSLCs transfected with miR-183 in the presence or absence of PTPN4 up-regulation. b-c. The invasive ability of transfected CSLCs was detected by the transwell assay.

Fig. 5 Inverse correlation between miR-183 and PTPN4 in human lung adenocarcinoma tissues. a. The expression of PTPN4 in primary human lung adenocarcinoma tissues with (n=15) or without metastasis (n=15) by qRT-PCR.
b. The inverse correlation between miR-183 and PTPN4 in human lung adenocarcinoma tissues determined by Spearman’s correlation analysis.
Figure

A 5’-UCCCUUAUGUUCACUGUGCCCAUA-3’ PTPN4-3’UTR-WT

3’-UCACUUAAGAUGGUCAACGGAUAU-5’ hsa-miR-183

5’-UCCCUUAUGUUCACUGCAAAACUA-3’ PTPN4-3’UTR-MUT

B

Relative luciferase activity

Control

miR-183

WT

MUT

* P-value

C

Relative PTPN4 mRNA expression

Control

anti-miR-control

anti-miR-183

* P-value

D

PTPN4

β-actin

110 KDa

42 KDa

Control

anti-miR-control

anti-miR-183

* P-value
Figure

(A) Comparison of Relative PTPN4 expression in tissues without metastasis and tissues with metastasis.

(B) Scatter plot showing the correlation between Relative PTN4 expression and Relative PTPN4 expression. The correlation coefficient is $r = -0.7018$, and the p-value is $P < 0.0001$. 

Tissues without metastasis vs. Tissues with metastasis.