Biochemical and Biophysical Research Communications 450 (2014) 154–159

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

MicroRNA-429 induces tumorigenesis of human non-small cell lung cancer cells and targets multiple tumor suppressor genes

Yaoguo Lang [a], Shidong Xu [a], Jianqun Ma [a], Jun Wu [a], Shi Jin [b], Shoubo Cao [b], Yan Yu [b,*]

[a] Department of Thoracic Surgery, Harbin Medical University Cancer Hospital, 150 Haping Road, Harbin, Heilongjiang 150081, China
[b] Department of Medical Oncology, Harbin Medical University Cancer Hospital, 150 Haping Road, Harbin, Heilongjiang 150081, China

ARTICLE INFO

Article history:
Received 16 May 2014
Available online 24 May 2014

Keywords:
Non-small cell lung cancer
MiR-429
Metastasis
PTEN
RASSF8
TIMP2

ABSTRACT

Lung cancer is the major cause of cancer death globally. MicroRNAs are evolutionally conserved small noncoding RNAs that are critical for the regulation of gene expression. Aberrant expression of microRNA (miRNA) has been implicated in cancer initiation and progression. In this study, we demonstrated that the expression of miR-429 are often upregulated in non-small cell lung cancer (NSCLC) compared with normal lung tissues, and its expression level is also increased in NSCLC cell lines compared with normal lung cells. Overexpression of miR-429 in A549 NSCLC cells significantly promoted cell proliferation, migration and invasion, whereas inhibition of miR-429 inhibits these effects. Furthermore, we demonstrated that miR-429 down-regulates PTEN, RASSF8 and TIMP2 expression by directly targeting the 3′-untranslated region of these target genes. Taken together, our results suggest that miR-429 plays an important role in promoting the proliferation and metastasis of NSCLC cells and is a potential target for NSCLC therapy.

1. Introduction

Lung cancer is the leading cause of cancer mortality in the US [1] and it has also become a common malignancy in developing countries such as China due to increased cigarette smoking rate and air pollution [2]. There are two main types of lung cancer: small cell lung cancer and non-small cell lung cancer (NSCLC); 80% of lung cancers are NSCLCs [3]. Despite advances in surgical and chemotherapeutic interventions, the overall 5-year survival for NSCLC patient remains low (15%) and the recurrence rate is high [4,5].

MicroRNAs (miRNAs) are a class of small non-coding RNAs, which regulate gene expression post-transcriptionally by binding to a complementary sequences predominantly found in the 3′-UTR of target mRNAs and either degrade these mRNAs or inhibit translation into proteins [6,7] and are implicated in biological processes including metabolism, cell proliferation, developmental timing, apoptosis, morphogenesis and response to stress [8–10]. Emerging evidence shows that miRNA regulation correlates with human cancers and function as both oncogenes and tumor suppressors [11,12] and are potential diagnostic and prognostic biomarkers [13–16] and perhaps therapeutic targets [17,18].miRNA miR-429 belongs to a small miRNA family which includes miR-200c, miR-141, miR-200b and miR-200a [19]. It is expressed in normal biological functions [10,20] and is specifically expressed in human embryonic stem cells [21], but differentially expressed in tumor stem cells [21,22], various cancers [23–26] and other conditions [27]. The roles of miR-429 in cancers are diverse. High expression facilitates mesenchymal to epithelial transition in metastatic ovarian cancer and reduced expression predicts poor prognosis [19,28]. Increased expression of miR-429 in endometrial adenocarcinoma and infiltrating bladder urothelial carcinoma plays a crucial role in tumorigenesis [29,30]. In hepatocellular carcinoma and primary liver tumor-initiating cells (T-ICs), miR-429 positively correlates with T-ICs EPCAM expression and negatively with survival. Internalization of microvesicle-packaged miR-429 by T-ICs was reported to contribute to self-renewal, malignant proliferation, chemoresistance and tumorigenicity of hepatocytes [23].

Another study comparing NSCLC tissue and serum samples to matched healthy tissues, reported upregulation of miR-429 serum levels and its potential as an independent prognostic marker [13]. Metastatic pancreatic adenocarcinoma cells also have increased miR-429 levels [24] and upregulated miR-429 in HT-29 colorectal cancer (CRC) cells inhibits SOX2 and suppresses apoptosis [16]. Others found that miR-429 expression is dynamic during CRC progression and down-regulated in stages ii and iii [31]. miR-429 is also down-regulated in gastric carcinoma tissue and malignant

* Corresponding author. Fax: +86 45186298726.
E-mail address: yuyan@hrbmu.edu.cn (Y. Yu).

http://dx.doi.org/10.1016/j.bbrc.2014.05.084
0006-291X/

breast myoepithelioma [25,26]. In esophageal cancer, miR-429 targets Bcl-2 and SP1 and upregulation of miR-429 inhibits invasion and promotes apoptosis [17]. Similarly, in osteosarcoma, it is a down-regulated tumor-suppressor [32].

In this study, we observed that the expression of miR-429 was increased in primary NSCLS tissues and cell lines. Further analyses showed that overexpression of miR-429 promotes NSCLC cell proliferation and metastasis. Moreover, PTEN, RASSF8 and TIMP2 were identified as direct targets of miR-429.

2. Materials and methods

2.1. Cell line

The human lung cancer cell lines A549, H1299, H23, H522 and H2126 and normal lung cell line MRC-5 were obtained from the American Type Culture Collection. A549 and MRC-5 cells were grown in Dulbecco's Modified Eagle medium, and H1299, H23 and H522 were grown in RPMI-1640. In each case, medium was supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 U/ml streptomycin. H2126 cells were grown on HITES medium supplemented with 5% fetal bovine, 0.005 mg/mL insulin, 0.01 mg/mL transferrin, 30 nM sodium selenite, 10 nM hydrocortisone, 10 nM beta-estradiol, 10 mM HEPES, and 2 mM L-glutamine. Cells were incubated at 37 °C in a humidified incubator containing 5% CO_2.

2.2. MiRNA mimics, plasmids and transfection

MiR-429 mimic, miR-429 mutant (miR-429-mut) and miR-429-inhibitor (miR-429-in) were purchased from Shanghai GenePharma Co. (Shanghai, China), along with the negative control (miR-control). The transfection of microRNAs (50 nM) was performed using X-tremeGENE (Roche) per the manufacturer's instruction. The 3′UTR of PTEN, RASSF8 and TIMP2 was PCR-amplified from A549 genomic DNA and cloned downstream of luciferase gene in pGL vector (Promega). For the reporter assay, cells were cultured in 96 well plates and transfected with luciferase reporters (50 ng), and 50 nM of miR-control, miR-429 mimics or miR-429-mut. After 48 h, luciferase activity was measured using dual-luciferase reporter system (Promega). The renilla activity was used as an internal control. Each transfection was performed in triplicate.

2.3. Protein isolation and western blot

Total proteins were extracted with RIPA lysis buffer with proteinase/phosphatase inhibitors (Thermo Scientific). Lysate was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and the gel was blotted onto PVDF membrane (Millipore). The membrane was blocked in 1% BSA, and then incubated with one of the following antibodies: anti-PTEN (Abcam, ab1678), anti-RASSF8 (Abcam, ab139326), anti-TIMP2 (Abcam, ab1828), or anti-GAPDH (Santa Cruz Biotechnology, sc-25778). Horse radish peroxidase-conjugated anti-mouse and anti-rabbit IgG (Sigma–Aldrich) were incubated as the secondary antibodies. Subsequent visualization was detected with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Scientific).

2.4. RNA extraction and real time PCR (RT-PCR)

Total mRNA was extracted with mirVANA miRNA Isolation Kit (Ambion) according to the manufacturer's instructions. The expression level of miR-429 was quantified using miRNA-specific TaqMan MiRNA Assay Kit (Applied Biosystems) and normalized by U6 small nuclear RNA.

2.5. Cell growth assay

A549 cells transfected with miR-control, miR-429 or miR-429-in were seeded into 96-well culture plates. At different time points, a volume of 20 μl MTS (Sigma–Aldrich) was added into each well and the cells were incubated for additional 4 h. Cell growth was measured at wavelength of 490 nm.

2.6. Colony formation assay

The cells were seeded into six-well plates at a density of 200 cells/well after transfection and maintained in DMEM containing 10% FBS. The medium was replaced after 24 h and changed every three days. After 14 days, cells were fixed with methanol and stained with 0.1% crystal violet. Visible colonies were counted using NIH Image J software.

2.7. Cell cycle analysis

The cells were seeded into six-well plate with a density of 1×10^5 cells/well after transfection and maintained in DMEM containing 10% FBS. Cultured cells were trypsinized after 48 h and fixed with 70% ethanol at 4 °C overnight before being stained with propidium iodide (PI). DNA contents were detected by LSRII flow cytometer (BD Biosciences, San Jose, CA). Data were analyzed by Flow Jo (Tree Star, Ashland, OR).

2.8. Wound-healing assay

1×10^5 cells transfected with miR-control, miR-429 and miR-429-in were seeded into six-well plate after transfection and maintained in DMEM containing 10% FBS. A linear wound was carefully made by a 20 μl sterile pipette tip across the confluent cell monolayer, and the cell debris was removed by washing with phosphate. The wounded monolayers were then photographed at 0 and 24 h after being wounded.

2.9. Cell invasion assay

Cell invasion assay was performed in a 24-well plate with 8-μm pore size chamber inserts (BD Biosciences). 1×10^5 cells transfected with miR-control, miR-429 and miR-429-in were placed into the upper chamber per well with the Matrigel-coated membrane, which was diluted with serum-free culture medium. The lower compartment was filled with 500 μl of DMEM containing 10% fetal bovine serum as a chemoattractant. The cells were incubated at 37 °C in a 5% CO_2 humidified incubator for 24 h. Then cells were exposed to 20 μM 5-ethynyl-2′-deoxyuridine (EdU) for an additional 4 h at 37 °C. Membrane inserts were removed from the plate and stained using the ENU kit (Invitrogen). The cells were counted under six random microscopic fields for each well, using NIH Image J software.

2.10. Statistics

All values are expressed as mean ± SEM. Differences between groups were analyzed by one way ANOVA followed by Bonferroni post hoc analyses as appropriate. A p value less than 0.05 was considered significant.

3. Results

3.1. MiR-429 was overexpressed in human NSCLC tissues and cell lines

To investigate the role of miR-429 in human NSCLC, we first searched the public database for miR-429 expression in NSCLC

patient samples and healthy controls. Analysis using database NCBI/GEO/GSE16025 [33] showed that miR-429 was significantly overexpressed in NSCLC samples (Fig. 1A, $p < 0.01$). To further confirm the association of miR-429 and NSCLC, we examined miR-429 expression in several NSCLC cancer cell lines using RT-PCR. The data showed that miR-429 expression was substantially increased in all five cell lines (A549, H23, H522, H1299, H2126) derived from NSCLC, compared with the normal MRC-5 lung cells (Fig. 1B). Thus, these data suggest that miR-429 is upregulated in NSCLC.

3.2. MiR-429 promotes NSCLC cell proliferation

To explore the role of miR-429 in NSCLC tumorigenesis, we examined the effect of miR-429 overexpression and inhibition on the proliferation of A549 NSCLC cell lines. The cells were transfected with miR-429 mimic (miR-429), miR-429 inhibitor (miR-429-in) or the miR scramble control oligonucleotides. RT-PCR showed that miR-429 was significantly increased in cells transfected with miR-429 mimics and decreased in the miR-429 inhibitor group compared with the miR-control (Fig. 2A). The MTS assay showed that the overexpression of miR-429 significantly promoted the proliferation of A549 cells, whereas the inhibition of miR-429 suppressed cell proliferation (Fig. 2B). Furthermore, we assessed cell cycle by flow cytometry. As shown in Fig. 2C, transfection of the miR-429 decreased the percentage of cells in G1 peak but increased that in the S peak. Consistently, miR-429-in led to cell cycle arrests in A549 cells. The colony formation assay was performed to further confirm the effect of miR-429 on NSCLC cell proliferation, and data indicated the overexpression of miR-429 significantly increased colony numbers in A549 cell cultures, whereas knockdown of miR-429 expression obviously decreased colony formation (Fig. 2D). Taken together, these results demonstrate that miR-429 promotes NSCLC cell growth.

3.3. MiR-429 induces NSCLC cell migration and invasion

Next, we examined the effect of miR-429 on cell migration and invasion. As shown in Fig. 3A, compared with controls, the migratory capabilities of A549 cells transfected with the miR-429-mimic were dramatically increased, as the wound in miR-429-mimic group was completely healed within 24 h, while only 70% closure was reached at the same time in miR-control group. In contrast, cells in the miR-429 inhibitor group had lower migration ability than controls. Matrigel invasion assays were also performed, and exogenously increase of miR-429 expression enhanced cell invasion by >100%. The number of invasive cells demonstrated a 60% reduced ability to invade through Matrigel membranes (Fig. 3B). Collectively, these results suggest that miR-429 stimulates NSCLC cell migration and invasion.

3.4. MiR-429 directly targets PTEN, RASSF8 and TIMP2

To gain insights into the biological implications of miR-429 on lung cancer tumorigenesis, we used TargetScan, DIANA, miRanda for putative human protein-coding gene targets of miR-429. The tumor suppressor genes PTEN, RASSF8 and TIMP2 were predicted to have miR-429-binding elements in their 3′-UTRs with high-fidelity scores (Fig. 4A and B). To test whether the predicted miR-429-binding sites in the 3′-UTR of the three target genes were responsible for miR-429 regulation, we cloned the 3′-UTR regions downstream of a luciferase reporter gene and co-transfected these vectors together with miR-control, miR-429 and miR-429-in into A549 cells respectively. The luciferase activity of cells transfected with miR-429 was significantly decreased compared with the miR-control. However, the mutation of miR-429 clearly abrogated the repression of the luciferase activity (Fig. 4C). To further investigate whether miR-429 regulated these targets, the protein expression levels of PTEN, RASSF8 and TIMP2 were tested by western blot. As shown in Fig. 4D, overexpression of miR-429 greatly changed the protein levels of PTEN, RASSF8 and TIMP2, whereas the protein expression of these target protein was not affected by mutant miR-429. These results suggest that PTEN, RASSF8 and TIMP2 are direct targets of miR-429 and these three targets might mediate the promotive effect of miR-429 on tumorigenesis.

4. Discussion

In this study we first performed a database search for miR-429 expression in human NSCLC and healthy controls, and found that miR-429 is significantly overexpressed in NSCLC samples. We further confirmed this in five NSCLC derived cell lines.

To explore the significance of miR-429 upregulation in NSCLC, several assays were performed. First we conducted a cell growth assay on A549 cells to assess whether differential expression of miR-429 could affect proliferation. We found that upregulation of miR-429 expression significantly promoted proliferation and that inhibition of miR-429 suppressed cell proliferation. A549 cells over-expressing miR-429 were also found to be significantly more migratory and invasive than non-transfected cells. Cells transfected with miR-429 inhibitor had significant reduction in invasion activity.

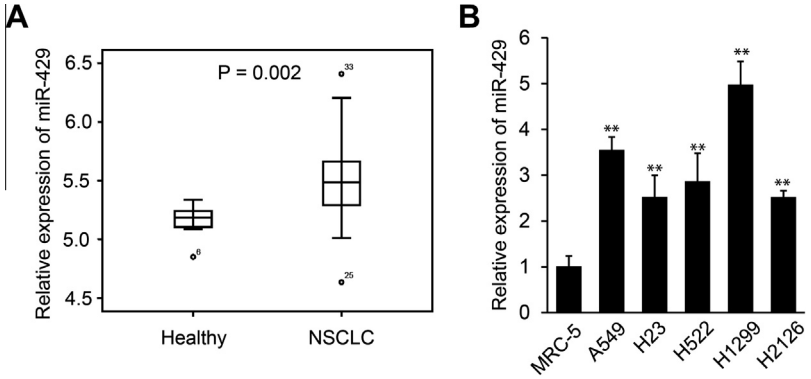

Fig. 1. MiR-429 expression is upregulated in NSCLC tumor tissues and cell lines. (A) Box-plot analysis of miR-429 levels in ten normal lung tissue samples and 61 NSCLC patient samples from a public database (NCBI/GEO/GSE16025; $n = 71$, $p = 0.002$). (B) RT-PCR analysis of miR-429 levels in one normal human lung cell line (MRC-5) and five human NSCLC cell lines (A549, H23, H522, H1299, H2126). U6 snRNA served as the loading control. Data are shown as mean ± SEM of three independent experiments; $^{**}p \leqslant 0.01$ versus MRC-5 cells.

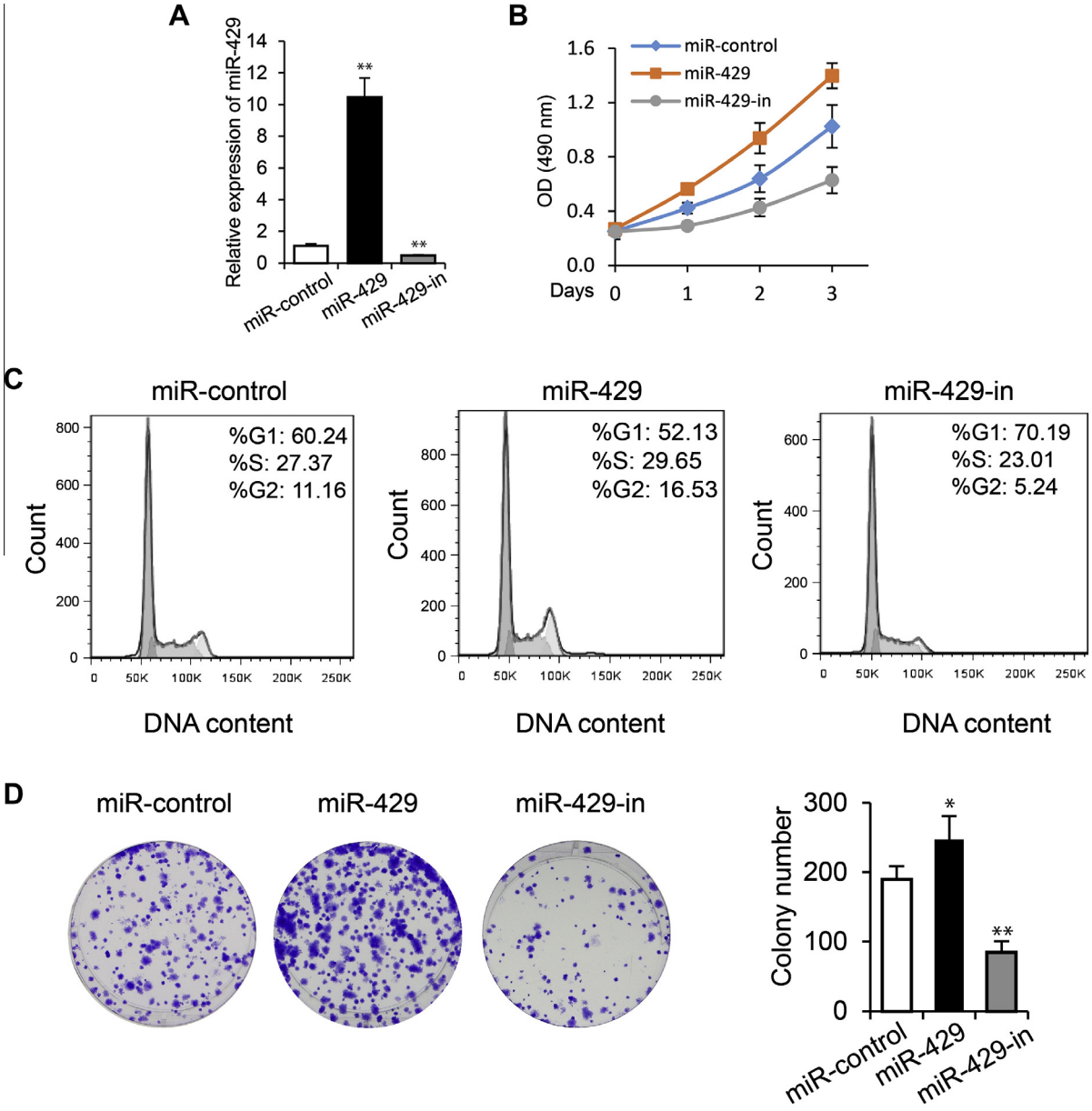

Fig. 2. MiR-429 boosts cell growth in A549 cells. (A) RT-PCR analyses of miR-429 levels in miR-control, miR-429 and miR-429-in transfected A549 cells. (B) Effects of miR-429 and miR-429-in on tumor cell proliferation at 0, 1, 2 and 3 days using MTS assay. (C) Cell cycle profiles of A549 cells transfected with miR-control, miR-429 and miR-429-in. (D) Representative micrographs (left) and quantification (right) of colony formation in A549 cells transfected with miR-control, miR-429 and miR-429-in. Data are shown as mean ± SEM of three independent experiments; $^{*}p \leqslant 0.05$; $^{**}p \leqslant 0.01$ versus cells transfected with miR-control.

When assessing the cell cycle of A549 cells by flow cytometry, it was found that miR-429-mimic transfected cells yielded a population with more cells in the S-phase, less cells in G1-phase and that inhibition of miR-429 led to cell cycle arrest. The S-phase is the period in which a cell performs DNA replication and the G1/S transition is a major checkpoint in the regulation of the cell cycle. Our data suggests that miR-429 affects mechanisms that control the G1/S transition in NSCLC cells.

To explore downstream effects of miR-429 overexpression in NSCLC cells, we developed a luciferase-reporter system to assess whether miR-429 can regulate known tumor suppressors and found that miR-429 targets PTEN, RASSF8 and TIMP2.

PTEN is a dual specificity phosphatase that has been heavily implicated in many cancers [34–36], and shown previously to play essential roles in apoptosis and cell cycle arrest by regulating the phosphatidylinositol 3,4,5,-trisphosphate and Akt signaling pathways [37]. Deletion of PTEN from both alleles has also been associated with advanced stage tumors and metastasis [38]. Thus it seems likely that our observation of increased cell numbers in the S-phase and decreased numbers in the G1-phase was caused by the modulation of PTEN by miR-429.

RASSF8 is critical for maintaining adherent junction function, is involved in regulating migration of epithelial cells and inhibits cell proliferation [39]. This protein has been previously reported to be down-regulated in A549 and H520 cell lines, and restoring RASSF8 expression inhibits anchorage-independent growth in A549 cells and reduces clonogenic activity in H520 cells [40]. This is consistent with our observations that A549 cells have down-regulated RASSF8 expression caused by miR-429, and increased proliferation and migration ability.

TIMP2 is a matrix-metalloproteinase inhibitor, thought to be a suppressor of metastasis. Metalloproteinases are involved in extracellular matrix (ECM) degradation and regulation of such activity is crucial to development, remodeling, morphogenesis, and tissue

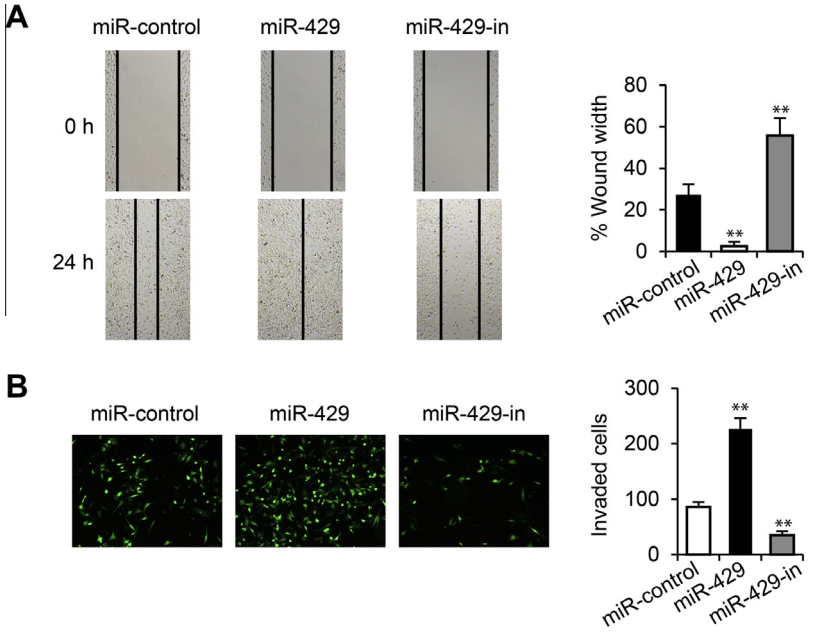

Fig. 3. MiR-429 induces cell migration and invasion in A549 cells. (A) Wound-healing assay for A549 cells transfected with miR-control, miR-429 and miR-429-in at 0 and 24 h. The relative wound width at 24 h was quantified in the right panel. (B) Transwell invasion assay in A549 cells transfected with miR-control, miR-429 and miR-429-in at 24 h after cells were seeded. The invaded cell numbers are quantified in the right panel; $^{**}p \leqslant 0.01$, versus A549 cells transfected with miR-control. Data are shown as mean ± SEM of three independent experiments.

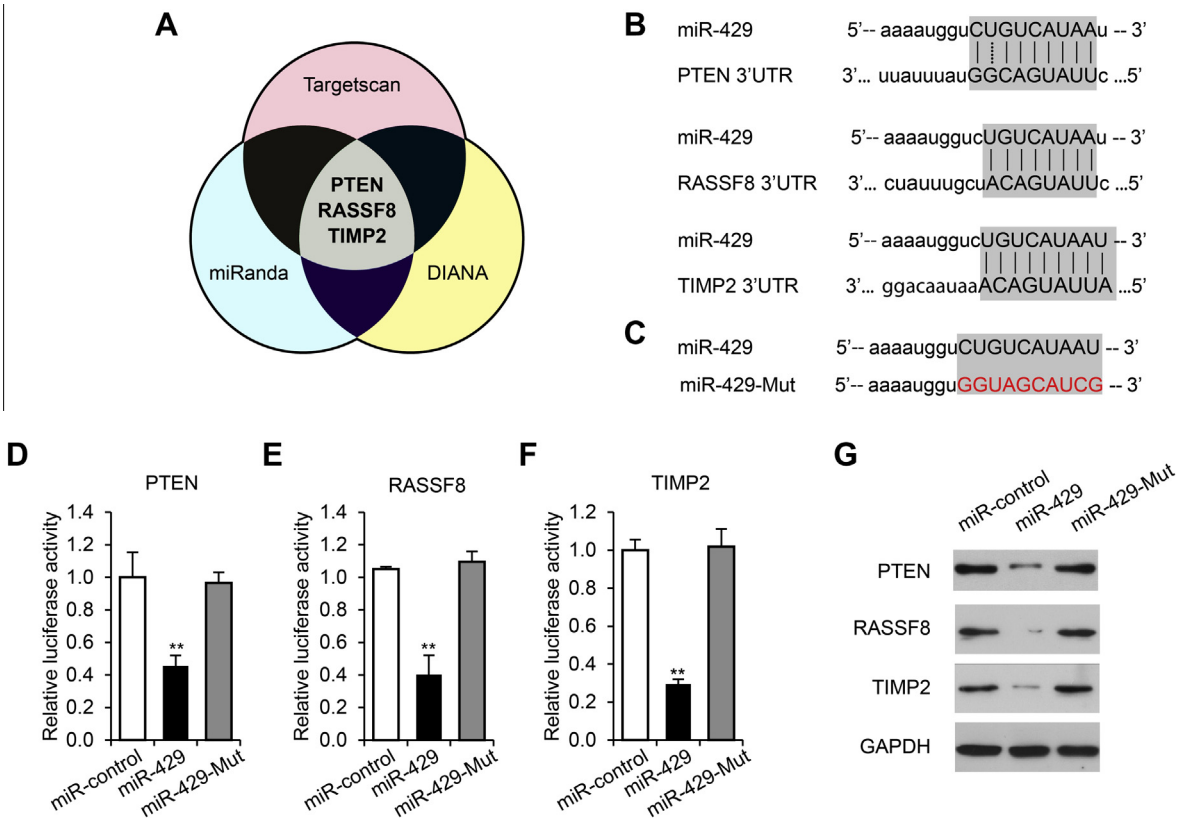

Fig. 4. PTEN, RASSF8 and TIMP2 are direct targets of miR-429. (A) Venn diagram showing PTEN, RASSF8 and TIMP8 genes are identified as predicted targets of miR-429 using three algorithms; Targetscan, miRanda and DIANA. (B) Sequences of the putative miR-429 binding sites in the 3′-UTR of PTEN, RASSF8 and TIMP2. (C) Ten nucleotides (red) of miR-429-mut were mutated to prevent the broad binding of miR-429 and its targets. (D) Luciferase assay of A549 cells transfected with hRluc-PTEN-3′UTR, and 50 nM of miR-control, miR-429, or miR-429-mut. (E) Luciferase assay of A549 cells transfected with hRluc-RASSF8-3′UTR, and 50 nM of miR-control, miR-429, or miR-429-mut. (F) Luciferase assay of A549 cells transfected with hRluc-TIMP2-3′UTR, and 50 nM of miR-control, miR-429, or miR-429-mut. (G) Western blot analysis of PTEN, RASSF8 and TIMP2 in A549 cells transfected with 50 nM miR-control, miR-429 or the miR-429-mut. $^{**}p \leqslant 0.01$, versus A549 cells transfected with miR-control. Data are shown as mean ± SEM of three independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

repair. Dysregulation of TIMP2 has been found in several cancers [41–44]. In our experiment, TIMP2 was found to be down-regulated by miR-429, and when miR-429 overexpressing cells grown in a mono-layer were wounded; they exhibited increased repair activity. This suggests that the dysregulation of TIMP2 by miR-429 is a mechanism relating to this process.

We here provide confounding evidence that upregulated expression of miR-429 in NSCLC modulates core mechanisms in the control of cell cycle progression, proliferation, migration and invasion, by inhibiting expression of tumor suppressors PTEN, RASSF8 and TIMP2. The differential expression of miR-429 in NSCLC can potentially be utilized in diagnostic applications and therapeutic interventions.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

None.

References

[1] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, CA Cancer J. Clin. 63 (2013) (2013) 11–30.
[2] J. She, P. Yang, Q. Hong, C. Bai, Lung cancer in China: challenges and interventions, Chest 143 (2013) 1117–1126.
[3] A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, M.J. Thun, Cancer statistics, CA Cancer J. Clin. 59 (2009) (2009) 225–249.
[4] M. Reck, What future opportunities may immuno-oncology provide for improving the treatment of patients with lung cancer?, Ann Oncol. 23 (2012) viii28–viii34.
[5] Y.E. Miller, Pathogenesis of lung cancer: 100 year report, Am. J. Respir. Cell Mol. Biol. 33 (2005) 216–223.
[6] E.V. Makeyev, T. Maniatis, Multilevel regulation of gene expression by microRNAs, Science 319 (2008) 1789–1790.
[7] N. Bushati, S.M. Cohen, MicroRNA functions, Annu. Rev. Cell Dev. Biol. 23 (2007) 175–205.
[8] I. Alvarez-Garcia, E.A. Miska, MicroRNA functions in animal development and human disease, Development 132 (2005) 4653–4662.
[9] A.K. Leung, P.A. Sharp, MicroRNA functions in stress responses, Mol. Cell 40 (2010) 205–215.
[10] B. Yan, L.H. Zhao, J.T. Guo, J.L. Zhao, MiR-429 regulation of osmotic stress transcription factor 1 (OSTF1) in tilapia during osmotic stress, Biochem. Biophys. Res. Commun. 426 (2012) 294–298.
[11] J.T. DeSano, L. Xu, MicroRNA regulation of cancer stem cells and therapeutic implications, AAPS J. 11 (2009) 682–692.
[12] A. Esquela-Kerscher, F.J. Slack, Oncomirs – microRNAs with a role in cancer, Nat. Rev. Cancer 6 (2006) 259–269.
[13] W. Zhu, J. He, D. Chen, B. Zhang, L. Xu, H. Ma, X. Liu, Y. Zhang, H. Le, Expression of miR-29c, miR-93, and miR-429 as potential biomarkers for detection of early stage non-small lung cancer, PLoS ONE 9 (2014) e87780.
[14] N.A. Schultz, C. Dehlendorff, B.V. Jensen, J.K. Bjerregaard, K.R. Nielsen, S.E. Bojesen, D. Calatayud, S.E. Nielsen, M. Yilmaz, N.H. Hollander, K.K. Andersen, J.S. Johansen, MicroRNA biomarkers in whole blood for detection of pancreatic cancer, JAMA 311 (2014) 392–404.
[15] F.E. Ahmed, N.C. Ahmed, P.W. Vos, C. Bonnerup, J.N. Atkins, M. Casey, G.J. Nuovo, W. Naziri, J.E. Wiley, H. Mota, R.R. Allison, Diagnostic microRNA markers to screen for sporadic human colon cancer in stool: I. Proof of principle, Cancer Genomics Proteomics 10 (2013) 93–113.
[16] J. Li, L. Du, Y. Yang, C. Wang, H. Liu, L. Wang, X. Zhang, W. Li, G. Zheng, Z. Dong, MiR-429 is an independent prognostic factor in colorectal cancer and exerts its anti-apoptotic function by targeting SOX2, Cancer Lett. 329 (2013) 84–90.
[17] Y. Wang, M. Li, W. Zang, Y. Ma, N. Wang, P. Li, T. Wang, G. Zhao, MiR-429 up-regulation induces apoptosis and suppresses invasion by targeting Bcl-2 and SP-1 in esophageal carcinoma, Cell. Oncol. (Dordr.) 36 (2013) 385–394.
[18] J. Krutzfeldt, N. Rajewsky, R. Braich, K.G. Rajeev, T. Tuschl, M. Manoharan, M. Stoffel, Silencing of microRNAs in vivo with 'antagomirs', Nature 438 (2005) 685–689.
[19] X. Hu, D.M. Macdonald, P.C. Huettner, Z. Feng, I.M. El Naqa, J.K. Schwarz, D.G. Mutch, P.W. Grigsby, S.N. Powell, X. Wang, A miR-200 microRNA cluster as prognostic marker in advanced ovarian cancer, Gynecol. Oncol. 114 (2009) 457–464.
[20] H. Hasuwa, J. Ueda, M. Ikawa, M. Okabe, MiR-200b and miR-429 function in mouse ovulation and are essential for female fertility, Science 341 (2013) 71–73.
[21] X. Nissan, J.A. Denis, M. Saidani, G. Lemaitre, M. Peschanski, C. Baldeschi, MiR-203 modulates epithelial differentiation of human embryonic stem cells towards epidermal stratification, Dev. Biol. 356 (2011) 506–515.
[22] D.E. Jung, J. Wen, T. Oh, S.Y. Song, Differentially expressed microRNAs in pancreatic cancer stem cells, Pancreas 40 (2011) 1180–1187.
[23] L. Li, J. Tang, B. Zhang, W. Yang, M. Liugao, R. Wang, Y. Tan, J. Fan, Y. Chang, J. Fu, F. Jiang, C. Chen, Y. Yang, J. Gu, D. Wu, L. Guo, D. Cao, H. Li, G. Cao, M. Wu, M.Q. Zhang, L. Chen, H. Wang, Epigenetic modification of MiR-429 promotes liver tumour-initiating cell properties by targeting Rb binding protein 4, Gut (2014) (Epub ahead of print).
[24] S.T. Mees, W.A. Mardin, C. Wendel, N. Baeumer, E. Willscher, N. Senninger, C. Schleicher, M. Colombo-Benkmann, J. Haier, EP300 – a miRNA-regulated metastasis suppressor gene in ductal adenocarcinomas of the pancreas, Int. J. Cancer 126 (2010) 114–124.
[25] T. Sun, C. Wang, J. Xing, D. Wu, MiR-429 modulates the expression of c-myc in human gastric carcinoma cells, Eur. J. Cancer 47 (2011) 2552–2559.
[26] C.L. Bockmeyer, M. Christgen, M. Muller, S. Fischer, P. Ahrens, F. Langer, H. Kreipe, U. Lehmann, MicroRNA profiles of healthy basal and luminal mammary epithelial cells are distinct and reflected in different breast cancer subtypes, Breast Cancer Res. Treat. 130 (2011) 735–745.
[27] W. Wu, Y. Qin, Z. Li, J. Dong, J. Dai, C. Lu, X. Guo, Y. Zhao, Y. Zhu, W. Zhang, B. Hang, J. Sha, H. Shen, Y. Xia, Z. Hu, X. Wang, Genome-wide microRNA expression profiling in idiopathic non-obstructive azoospermia: significant up-regulation of miR-141, miR-429 and miR-7-1-3p, Hum. Reprod. 28 (2013) 1827–1836.
[28] J. Chen, L. Wang, L.V. Matyunina, C.G. Hill, J.F. McDonald, Overexpression of miR-429 induces mesenchymal-to-epithelial transition (MET) in metastatic ovarian cancer cells, Gynecol. Oncol. 121 (2011) 200–205.
[29] P. Xie, F. Xu, W. Cheng, J. Gao, Z. Zhang, J. Ge, Z. Wei, X. Xu, Y. Liu, Infiltration related miRNAs in bladder urothelial carcinoma, J. Huazhong Univ. Sci. Technol. Med. Sci. 32 (2012) 576–580.
[30] J. Snowdon, X. Zhang, T. Childs, V.A. Tron, H. Feilotter, The microRNA-200 family is upregulated in endometrial carcinoma, PLoS ONE 6 (2011) e22828.
[31] Y. Sun, S. Shen, H. Tang, J. Xiang, Y. Peng, A. Tang, N. Li, W. Zhou, Z. Wang, D. Zhang, B. Xiang, J. Ge, G. Li, M. Wu, X. Li, MiR-429 identified by dynamic transcriptome analysis is a new candidate biomarker for colorectal cancer prognosis, OMICS 18 (2014) 54–64.
[32] X. Liu, Y. Liu, S. Wu, X. Shi, L. Li, J. Zhao, H. Xu, Tumor-suppressing effects of miR-429 on human osteosarcoma, Cell Biochem. Biophys. (2014) (Epub ahead of print).
[33] M. Raponi, L. Dossey, T. Jatkoe, X. Wu, G. Chen, H. Fan, D.G. Beer, MicroRNA classifiers for predicting prognosis of squamous cell lung cancer, Cancer Res. 69 (2009) 5776–5783.
[34] B. Ling, L. Chen, Q. Liu, J. Yang, Gene expression correlation for cancer diagnosis: a pilot study, Biomed. Res. Int. 2014 (2014) 253804.
[35] M.K. McConechy, J. Ding, J. Senz, W. Yang, N. Melnyk, A.A. Tone, L.M. Prentice, K.C. Wiegand, J.N. McAlpine, S.P. Shah, C.H. Lee, P.J. Goodfellow, C.B. Gilks, D.G. Huntsman, Ovarian and endometrial endometrioid carcinomas have distinct CTNNB1 and PTEN mutation profiles, Mod. Pathol. 27 (2014) 128–134.
[36] F.L. Day, R.N. Jorissen, L. Lipton, D. Mouradov, A. Sakthianandeswaren, M. Christie, S. Li, C. Tsui, J. Tie, J. Desai, Z.Z. Xu, P. Molloy, V. Whitehall, B.A. Leggett, I.T. Jones, S. McLaughlin, R.L. Ward, N.J. Hawkins, A.R. Ruszkiewicz, J. Moore, D. Busam, Q. Zhao, R.L. Strausberg, P. Gibbs, O.M. Sieber, PIK3CA and PTEN gene and exon mutation-specific clinicopathologic and molecular associations in colorectal cancer, Clin. Cancer Res. 19 (2013) 3285–3296.
[37] H. Sun, R. Lesche, D.M. Li, J. Liliental, H. Zhang, J. Gao, N. Gavrilova, B. Mueller, X. Liu, H. Wu, PTEN modulates cell cycle progression and cell survival by regulating phosphatidylinositol 3,4,5,-trisphosphate and Akt/protein kinase B signaling pathway, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 6199–6204.
[38] B. Kwabi-Addo, D. Giri, K. Schmidt, K. Podsypanina, R. Parsons, N. Greenberg, M. Ittmann, Haploinsufficiency of the PTEN tumor suppressor gene promotes prostate cancer progression, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 11563–11568.
[39] F.E. Lock, N. Underhill-Day, T. Dunwell, D. Matallanas, W. Cooper, L. Hesson, A. Recino, A. Ward, T. Pavlova, E. Zabarovsky, M.M. Grant, E.R. Maher, A.D. Chalmers, W. Kolch, F. Latif, The RASSF8 candidate tumor suppressor inhibits cell growth and regulates the Wnt and NF-kappaB signaling pathways, Oncogene 29 (2010) 4307–4316.
[40] F.S. Falvella, G. Manenti, M. Spinola, C. Pignatiello, B. Conti, U. Pastorino, T.A. Dragani, Identification of RASSF8 as a candidate lung tumor suppressor gene, Oncogene 25 (2006) 3934–3938.
[41] H. Nagase, R. Visse, G. Murphy, Structure and function of matrix metalloproteinases and TIMPs, Cardiovasc. Res. 69 (2006) 562–573.
[42] D.C. Gurgel, J.T. Valenca-Junior, C.A. Dornelas, R.B. Vieira, J.T. Maia-Filho, R.C. Lima-Junior, R.A. Ribeiro, P.R. Almeida, Immunoexpression of metalloproteinases 2 and 14 and TIMP-2 inhibitor in main types of primary gastric carcinomas and lymph node metastasis, Pathol. Oncol. Res. (2014) (Epub ahead of print).
[43] M.I. Ellina, P. Bouris, A.J. Aletras, A.D. Theocharis, D. Kletsas, N.K. Karamanos, EGFR and HER2 exert distinct roles on colon cancer cell functional properties and expression of matrix macromolecules, Biochim. Biophys. Acta (2014) (Epub ahead of print).
[44] D. Bajracharya, B. Shrestha, A. Kamath, A. Menon, R. Radhakrishnan, Immunohistochemical correlation of matrix metalloproteinase-2 and tissue inhibitors of metalloproteinase-2 in tobacco associated epithelial dysplasia, Dis. Markers 2014 (2014) 197813.