Biochemical and Biophysical Research Communications 445 (2014) 314–319

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

miR-30a suppresses breast cancer cell proliferation and migration by targeting Eya2

Jing Fu [a,b,1], Xiaojie Xu [b,1], Lei Kang [c,1], Liying Zhou [b], Shibin Wang [d], Juming Lu [a], Long Cheng [b], Zhongyi Fan [b], Bin Yuan [b], Peirong Tian [a], Xiaofei Zheng [e], Chengze Yu [d,*], Qinong Ye [b,*], Zhaohui Lv [a,*]

[a] Department of Endocrinology, Chinese PLA General Hospital, Chinese PLA Medical School, Beijing, People's Republic of China
[b] Department of Medical Molecular Biology, Beijing Institute of Biotechnology, Beijing, People's Republic of China
[c] Department of Nuclear Medicine, Peking University First Hospital, Beijing, People's Republic of China
[d] Department of General Surgery, 307 Hospital of PLA, Beijing, People's Republic of China
[e] Beijing Institute of Radiation Medicine, Beijing, People's Republic of China

ARTICLE INFO

Article history:
Received 18 January 2014
Available online 4 February 2014

Keywords:
miR-30a
Eya2
Breast cancer
Cell proliferation
Cell migration

ABSTRACT

Eye absent (Eya) proteins are involved in cell fate determination in a broad spectrum of cells and tissues. Aberrant expression of Eya2 has been documented in a variety of cancers and correlates with clinical outcome. However, whether microRNAs (miRNAs) can regulate Eya2 expression remains unknown. Here, we show that miR-30a represses Eya2 expression by binding to the 3′-untranslated region of Eya2. Overexpression of Eya2 in miR-30a-transfected breast cancer cells effectively rescued the inhibition of cell proliferation and migration caused by miR-30a. Knockdown of Eya2 by small-interfering RNA (siRNA) in breast cancer cells mimicked the effect induced by miR-30a and abolished the ability of miR-30a to regulate breast cancer cell proliferation and migration. The miR-30a/Eya2 axis could regulate G1/S cell cycle progression, accompanied by the modulation of expression of cell cycle-related proteins, including cyclin A, cyclin D1, cyclin E, and c-Myc. Moreover, miR-30a expression was downregulated in breast cancer patients, and negatively correlated with Eya2, which was upregulated in breast cancer patients. These data suggest that the miR-30a/Eya2 axis may play an important role in breast cancer development and progression and that miR-30a activation or Eya2 inhibition may be a useful strategy for cancer treatment.

1. Introduction

MicroRNAs (miRNAs) are small noncoding RNA molecules that inhibit gene expression by interacting preferentially with the 3′-untranslated regions (3′-UTRs) of target mRNAs [1]. miRNAs have been shown to play varieties of roles in many biological processes, including control of embryo development, cell growth, differentiation, and apoptosis [2]. In recent years, extensive investigations of miRNAs have demonstrated critical roles of miRNAs in the development and progression of cancer, and some miRNAs are proposed as novel potential targets for cancer therapy [3,4].

Breast cancer is the most frequently diagnosed cancer and the leading cause of mortality for females worldwide. Distant metastasis to lung, bone marrow, liver, or brain results in poor prognosis. Recently, a number of miRNAs, such as miR-205, miR-21, and miR-17, have been shown to be involved in metastasis, cell proliferation as well as cell viability in breast cancer [5–7].

The phosphatase and transactivator eyes absent (Eya) family, which belongs to the haloacid dehalogenase family of phosphatases proteins, encodes a group of transcription cofactors necessary for eye development, and plays important roles in embryonic development and organ differentiation through the EGFR/RAS/MAPK, TGF/DPP, Wingless, Hedghog, and Notch signal pathways [8–11]. Recently, it has been reported that this family is implicated in innate immunity, DNA damage repair, photoperiodism, angiogenesis, and cancer cell growth and metastasis [12–14]. In ovarian cancer patients, Eya2 expression was upregulated, and Eya2 could promote ovarian tumor growth. Overexpression of Eya2 is significantly associated with short overall survival in advanced ovarian cancer [15]. Silencing of Eya2 significantly reduces viability, migratory capacity, and anchorage-independent growth of human papillomavirus 16 (HPV16)-transformed cervical keratinocytes [16]. Eya2 may participate in the development

* Corresponding authors. Address: Department of Medical Molecular Biology, Beijing Institute of Biotechnology, 27 Tai-Ping Lu Rd, Beijing 100850, People's Republic of China. Fax: +86 10 8827 1216 (Q. Ye). Fax: +86 10 6816 8631 (Z. Lv).
E-mail addresses: yuchengze@sina.com (C. Yu), yeqn66@yahoo.com (Q. Ye), metabolism301@126.com (Z. Lv).
[1] These authors contributed equally to this work. Chinese PLA General Hospital and Beijing Institute of Biotechnology contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.01.174
0006-291X/

of lung adenocarcinoma as a transcriptional coactivator [17]. Although Eya2 has been shown to play an important role in cancer development and progression, whether miRNAs can regulate Eya2 expression remains unknown.

In this study, we demonstrate that Eya2 is a novel target of miR-30a in breast cancer cells. miR-30a inhibited breast cancer cell proliferation and migration by directly targeting Eya2. Moreover, expression of miR-30a was downregulated in breast cancer patients and negatively correlated with Eya2 expression, which was upregulated in patients with breast cancer.

2. Materials and methods

2.1. Plasmids, cell lines and reagents

The sequence of miRNA precursor of hsa-miR-30a was cloned into pcDNA3.0 (Invitrogen). Wild-type and mutated miR-30a putative targets on Eya2 3′-UTR were cloned into pmir-GLO dual-luciferase miRNA target expression vector (Promega). Briefly, the 3′-UTR of human Eya2 gene was obtained by PCR using the following primers: 5′-CGGAATTCCAGGATCAGCAGCATCTCCACC-3′ (forward) and 5′-CCGCTCGAGTTCAAAATGTAAACGTGGTTTTA-3′ (reverse). To introduce mutations into the seed sequences of the predicted miR-30a target sites within the Eya2 3′-UTR, recombinant PCR was performed using the above-mentioned primers and the following primers: 5′-GAAGAGGGGGGCATCAAGAAGGAATG-3′ (forward) and 5′-CATTCCTTCTTGATGCCCCCCTCTTC-3′ (reverse). Stable cell lines overexpressing miR-30a were generated by lentiviral transduction using pCDH plasmid carrying miR-30a (System Biosciences). Anti-cyclin D1, anti-cyclin A, and anti-cyclin E antibodies were purchased from Santa Cruz Biotechnology Inc. and anti-Eya2 from Sigma.

2.2. Luciferase reporter assay

Cells were harvested and analyzed for luciferase and β-galactosidase activities as previously described [18]. The experiments were performed in triplicate and reproduced at least 3 times.

2.3. miRNA extraction and quantitative RT-PCR

Total RNA from tissues or cell lines containing miRNA was extracted using the miRcute miRNA isolation kit (Tiangen). Target miRNA was reverse transcribed to cDNA using the miRcute miRNA First-Strand cDNA synthesis Kit (Tiangen). miRNA expression was determined with miScript SYBR Green PCR Kit (Qiagen). Eya2 mRNA expression was assessed by quantitative RT-PCR with the following primers: 5′-CA CTCCCTGAAGGCACTAAACCTCATC-3′ (forward), and 5′-CTGCATTATC CTCTCGAAGCAGCTCTC-3′ (reverse). The relative quantification value of the target, normalized to a control, was calculated by the comparative Ct methods.

2.4. Cell growth and colony formation assays

Anchorage-dependent cell growth was assessed by the CCK-8 Kit (Dojindo Laboratories) according to the manufacturer's instructions. For colony formation assay, transfected cells were seeded in 6-well plates at 2000 cells per well. Two weeks later, colonies were fixed with 4% paraformaldehyde and stained with crystal violet for 30 min. The number of colonies with diameters of more than 1.5 mm was counted.

2.5. Cell migration assays

Wound healing assays were used to determine cell migration. Briefly, transfected cells grown in 6-well plates as confluent monolayers were mechanically scratched using a 1-ml pipette tip to create the wound. Cells were washed with PBS to remove the debris and were cultured for 48 h to allow wound healing.

2.6. Clinical samples

Thirty pairs of human breast cancer tumor samples and adjacent noncancerous tissues were obtained from the Chinese PLA General Hospital and the Chinese PLA No. 307 Hospital, with the informed consent of patients and with approval for experiments from these two hospitals and Beijing Institute of Biotechnology. None of the breast cancer patients had received any chemotherapy prior to surgery. Tissue samples were used for miRNA extraction. Clinical information was collected from patient records.

2.7. Statistics

Statistical significance in the luciferase activity, cell growth and migration were determined by two-tailed Student's *t* test. Differences between breast tumors and normal tissue adjacent to breast tumors were assessed by the Mann–Whitney *U* test. Correlation between expression of miR-30a and Eya2 was examined by Spearman correlation analysis. Statistical calculations were performed using SPSS 17.0. *p* values of less than 0.05 were considered statistically significant.

3. Results

3.1. miR-30a inhibits Eya2 expression by targeting its 3′-UTR

To investigate the role of Eya2 in breast cancer, we used two target prediction programs, TargetScan and miRanda, to screen for miRNAs that target Eya2. Our analysis predicted several potential Eya2-targeting miRNAs, among which, only miR-30a could inhibit Eya2 expression in ZR75-1 and MCF-7 cells by Western blot analysis (Fig. 1A). In contrast, miR-30a inhibition increased Eya2 expression in the above-mentioned cell lines (Fig. 1B). It should be noted that miR-30a did not modulate the mRNA level of Eya2, suggesting that this regulation is post-transcriptional (Supplementary Fig. S1A).

To confirm whether Eya2 is a direct and specific target of miR-30a, we transfected ZR75-1 and MCF-7 cells with Eya2 3′-UTR or 3′-UTR mutated luciferase reporter and the expression plasmid for miR-30a. miR-30a decreased the Eya2 3′-UTR reporter activity in MCF-7 cells. However, miR-30a did not affect the luciferase activity of the mutant reporter in which the binding sites for miR-30a were mutated. Similar results were obtained in ZR75-1 cells (Fig. 1C). Taken together, these results suggest that miR-30a inhibits Eya2 expression by directly targeting its 3′-UTR in breast cancer cells.

3.2. miR-30a suppresses breast cancer cell proliferation and migration through inhibition of Eya2 expression

To determine the effect of miR-30a on breast cancer cells, we performed cell proliferation and colony formation assays in ZR75-1 and MCF-7 cell lines. The results revealed that overexpression of miR-30a reduced the proliferation and colony formation of these cell lines (Fig. 2A and C and Supplementary Fig. S1B and C). Overexpression of Eya2 reversed the effect of miR-30a on proliferation in these two cell lines (Fig. 2A and Supplementary Fig. S1B). In contrast, miR-30a inhibition increased proliferation and colony formation of the above-mentioned cell lines (Fig. 1B and D). These results suggest that miR-30a inhibits breast cancer cell growth by targeting Eya2.

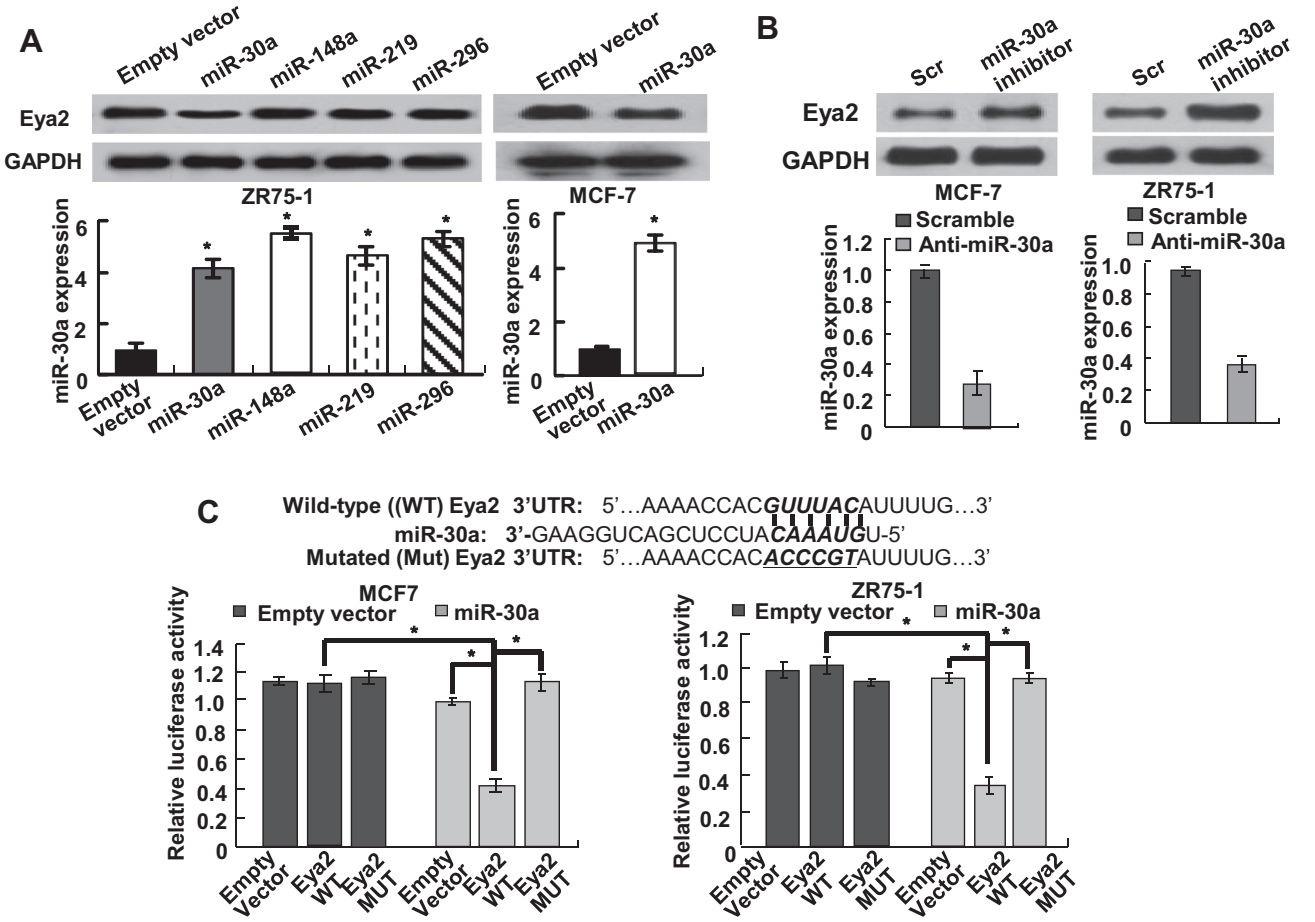

Fig. 1. miR-30a inhibits Eya2 expression by targeting its 3′-UTR. (A and B) Immunoblot analysis of the indicated breast cancer cell lines transfected with empty vector, miR-30a, miR-148a, miR-219, or miR-296 (A), or scramble vector (Scr) or miR-30a inhibitor (B). Histograms under the immunoblot graphs show corresponding expression levels of miRNAs by real-time RT-PCR. (C) miRNA luciferase reporter assays in MCF-7 and ZR75-1 cells transfected with wild-type or mutated Eya2 reporter and miR-30a. The top panel indicates wild-type and mutant forms of putative miR-30a target sequences of Eya2 3′-UTR. Bold and italicized font indicates the putative miR-30a binding sites within human Eya2 3′-UTR. Underlining indicates the mutations introduced into the Eya2 3′-UTR. Eya2 WT, wild-type Eya2 3′-UTR; Eya2 Mut, mutated Eya2 3′-UTR. All values shown are mean ± SD of triplicate measurements and have been repeated 3 times with similar results ($^*p < 0.01$).

Next, we examined the effects of miR-30a on migration capacity of breast cancer cells. miR-30a overexpression suppressed cell migration in MCF-7 and ZR75-1 cells using a wound-healing assay (Fig. 2E and Supplementary Fig. S1D). Eya2 reexpression in miR-30a-transfected ZR75-1 or MCF-7 cells reversed the effects of miR-30a on cell migration (Fig. 2E and Supplementary Fig. S1D). In contrast, miR-30a inhibition increased ZR75-1 cell migration (Fig. 1F).

To further determine whether miR-30a exerts its function through inhibition of Eya2, we investigated the effect of miR-30a on cell proliferation and migration in Eya2 knockdown cells. As expected, Eya2 knockdown had similar effects to miR-30a overexpression on the regulation of breast cancer cell proliferation and migration. Importantly, Eya2 knockdown abolished the ability of miR-30a to regulate these effects (Fig. 2G and H), indicating that Eya2 is a key mediator of miR-30a function.

3.3. miR-30a induces G1 cell cycle arrest and regulates the expression of cell cycle-related proteins in breast cancer cells

Since miR-30a can suppress the proliferation of breast cancer cells, we examined the effect of miR-30a on the cell cycle of breast cancer cells by flow cytometry analysis. ZR75-1 and MCF-7 cells overexpressing miR-30a had a significant decrease in the S-phase population and an increase in the G0/G1 population compared with cells transfected with empty vector (Fig. 3A and Supplementary Fig. 2A). Moreover, Eya2 overexpression reversed these effects (Fig. 3A). In contrast, miR-30a inhibition had opposite effects in ZR75-1 cells (Fig. 3B).

Next, we examined the effects of miR-30a on expression of cell cycle-related genes, some of which are also Eya2 target genes, such as cyclin D1 and c-Myc. miR-30a reduced the expression of cyclin D1, cyclin A, cyclin E, and c-Myc in ZR75-1 and MCF-7 cells (Fig. 3C and Supplementary Fig. S2B), while inhibition of miR-30a had opposite effects (Fig. 3D).

To further investigate whether miR-30a regulates the expression of the above-mentioned genes through Eya2, we determined the effect of miR-30a on modulation of these genes in Eya2 knockdown cells. As expected, Eya2 knockdown had similar effects to miR-30a overexpression on the regulation of these genes (Fig. 3E). Importantly, Eya2 knockdown abolished the ability of miR-30a to regulate these effects (Fig. 3E), suggesting that miR-30a regulates the expression of the above-mentioned genes via Eya2.

3.4. Expression of miR-30a and Eya2 and correlation between miR-30a and Eya2 in breast cancer patients

We assessed the miR-30a expression levels in 30 pairs of primary breast cancer and their corresponding noncancerous tissues

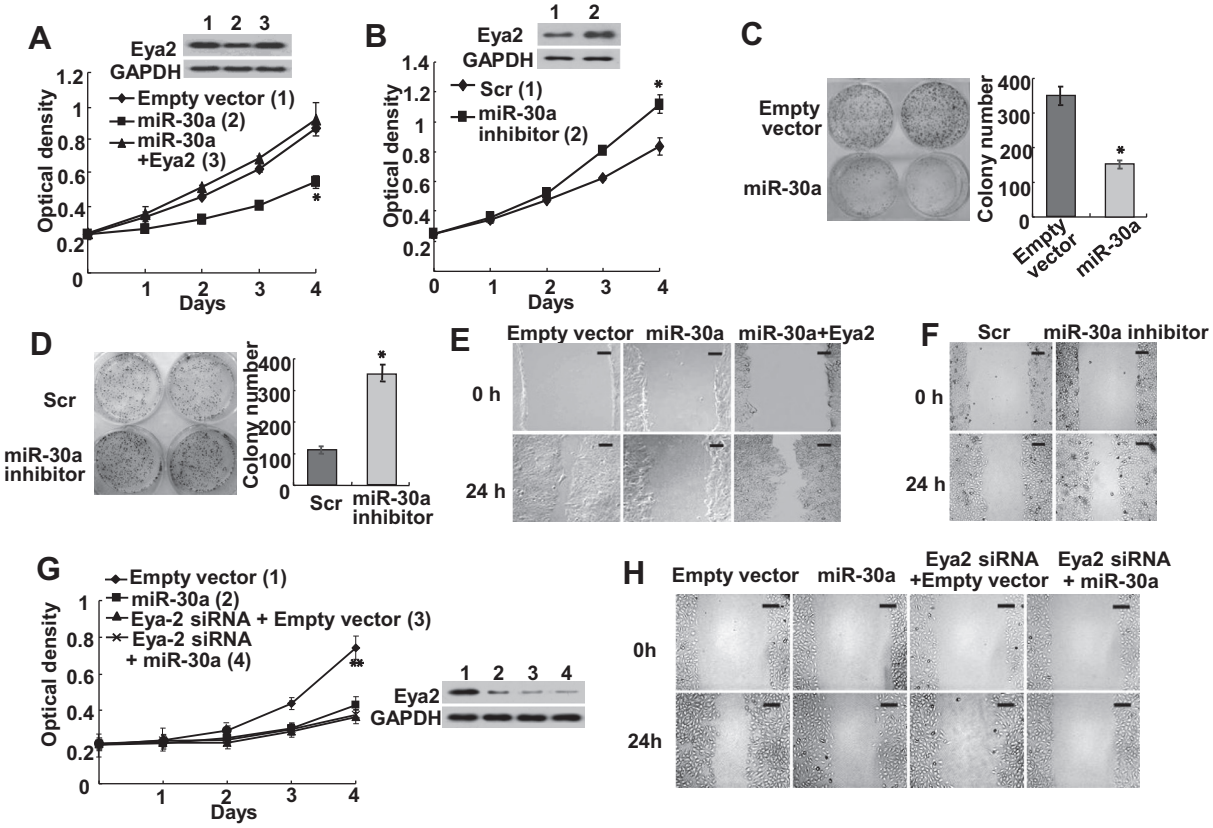

Fig. 2. miR-30a suppresses cell proliferation and migration through inhibition of Eya2 expression. (A and B) ZR75-1 cells expressing miR-30a or miR-30a plus Eya2 (A) and ZR75-1 cells transfected with miR-30a inhibitor (B) were cultured in regular medium. At specified times, cell numbers were determined by CCK-8 assay. The representative immunoblot shows Eya2 expression. (C and D) ZR75-1 cells transfected with miR-30a (C) or miR-30a inhibitor (D) were plated and assayed for colony number after 3 weeks. Representative images show colonies in plates (left panels). (E and F) Wound-healing assays were conducted in ZR75-1 cells transfected with miR-30a or miR-30a plus Eya2 (E) or miR-30a inhibitor (F). Cell migration was measured 24 h after cells were scratched. Scale bar, 100 μm. (G and H) ZR75-1 cells transfected with miR-30a, Eya2 siRNA, miR-30a plus Eya2 siRNA, or miR-30a together with Eya2 siRNA as indicated were cultured in regular medium. At specified times, cell number (G) and migration (H) were determined as in (A) and (E), respectively. The representative immunoblot indicates Eya2 expression (G). All values shown are mean ± SD of triplicate measurements and have been repeated 3 times with similar results ($^{*}p < 0.05$, $^{**}p < 0.01$ versus corresponding control).

by qRT-PCR. Compared with their corresponding noncancerous counterparts, miR-30a expression was decreased in breast cancer tissues (Fig. 4A and Supplementary Fig. S3A, $p = 2.64 \times 10^{-5}$). Next, we used immunohistochemistry to detect Eya2 protein expression in 30 pairs of breast cancer tissues and matched nontumor tissues. The results showed that Eya2 expression was up-regulated in breast cancer tissues ($p = 4.86 \times 10^{-5}$) (Fig. 4B). We confirmed the specificity of the Eya2 antibody by immunohistochemical staining of breast cancer samples incubated with normal IgG or anti-Eya2 pre-incubated with its antigen (Supplementary Fig. S2B and C). In agreement with miR-30a inhibition of Eya2 in cultured cells, expression of miR-30a negatively correlated with Eya2 expression in breast cancer samples ($p = 3.27 \times 10^{-5}$, $r = -0.56$) (Fig. 4C). Taken together, these data strongly suggest important pathological roles of miR-30a and Eya2 in breast cancer.

4. Discussion

Accumulating studies have reported that miRNAs are aberrantly expressed in many types of human cancers. Therefore, better understanding of the gene networks orchestrated by these miRNAs may help exploit the full potential of miRNAs in regards to cancer diagnosis and treatment. miR-30a has been found to be one of the miRNAs that is downregulated in various types of solid tumor, including colon cancer, prostate cancer, lung cancer, and liver cancer [19–22]. However, the tumor suppressive role of miR-30a is still poorly understood. In the present study, we report for the first time that miR-30a plays an important role in the suppression of breast cancer cell proliferation and migration via targeting Eya2.

The Eya proteins (Eya1–4) are the mammalian counterparts of the eyes absent gene product in Drosophila. Eyes absent is one member of the retinal determination network which was shown to function in gonadogenesis, myogenesis, limb formation, neurogenesis, thymus and kidney development, and cell cycle control [23–25]. Eya proteins function as both tyrosine and threonine phosphatases and form bipartite transcriptional activators by interacting with a member of the sine oculis (Drosophila) family of proteins, termed Six proteins in vertebrates [26,27]. Eya proteins activate genes essential for the development of multiple organs and the phosphatase function of Eya has been shown to be required for normal development [25,28]. Recently, Eya proteins have been reported to be overexpressed in several types of cancers, including breast, lung, cervix, and kidney cancers [16,17,14]. Eya2 overexpression in breast cancer cells increased proliferation, transformation, and migration, and correlated with poor prognosis/outcomes in breast cancer [29,14]. Eya2 expression has been shown to be upregulated in epithelial ovarian cancer, where it was associated with shortened overall survival [15]. More recently, Eya overexpression has been observed in glioblastoma, and brain, cervical, colon, and hematopoietic cancers [30,31,16,32,33]. Conceivably, downregulation of Eya2 expression may be a promising way to treat cancer. In this study, we showed that the suppressive effect of miR-30a on Eya2 expression could lead to the decreased

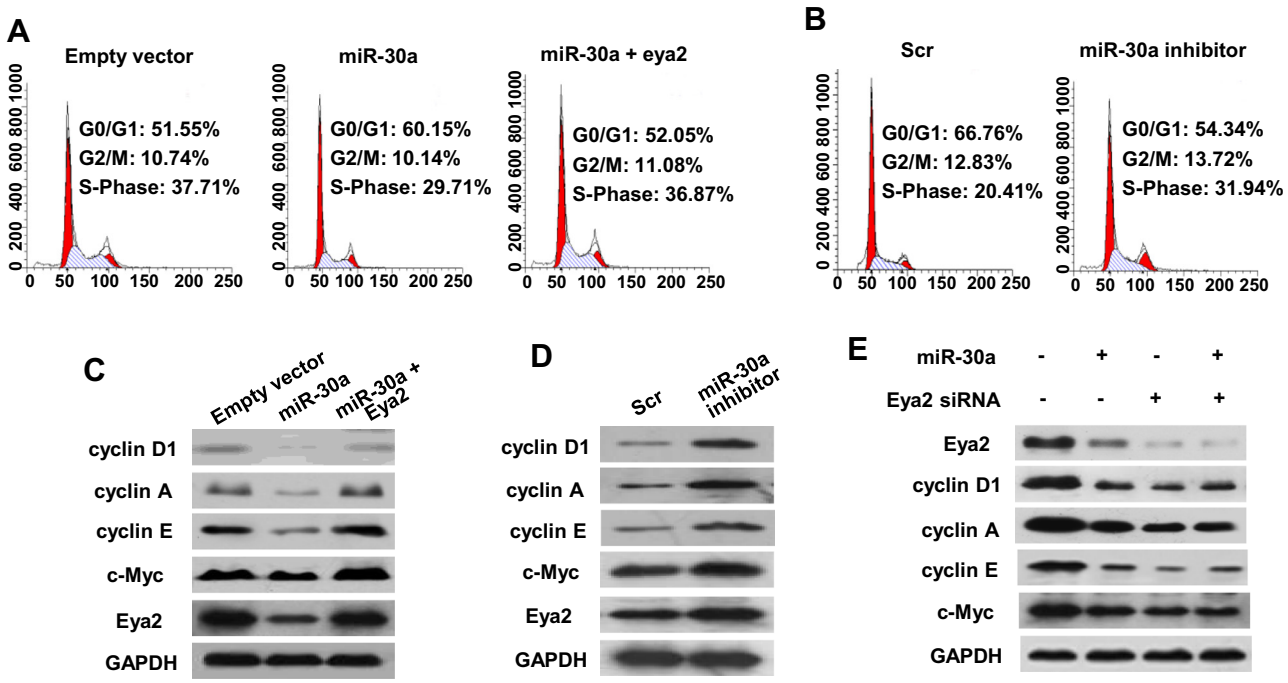

Fig. 3. miR-30a induces G1/S cell cycle arrest in breast cancer cells. (A and B) ZR75-1 cells transfected with miR-30a or miR-30a plus Eya2 (A) or miR-30a inhibitor (B) were analyzed by flow cytometry. The experiments have been repeated 3 times with similar trend. (C and D) Immunoblot analysis of ZR75-1 cells transfected with miR-30a or miR-30a plus Eya2 (C) or miR-30a inhibitor (D). (E) Immunoblot analysis of ZR75-1 cells transfected with miR-30a, Eya2 siRNA, or miR-30a plus Eya2 siRNA.

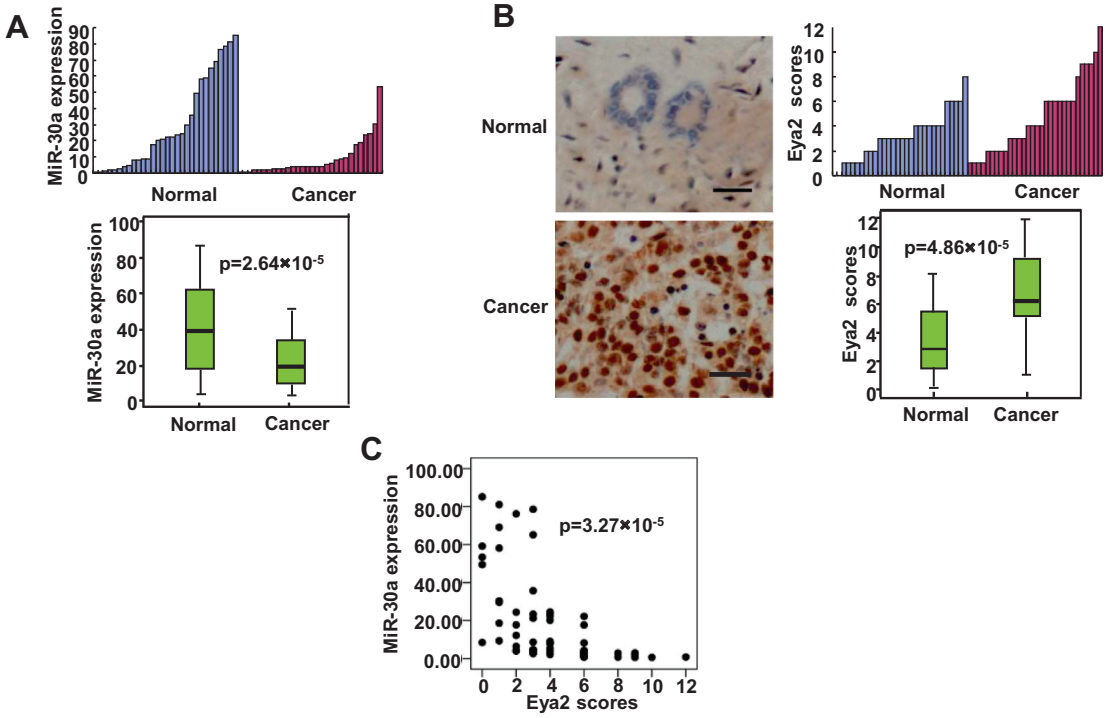

Fig. 4. Expression of miR-30a and Eya2 in patients with breast cancer. (A) miR-30a expression in human cancerous breast tissues and adjacent normal breast tissues was plotted using real-time RT-PCR ($n = 30$). (B) Representative immunohistochemical staining of Eya2 in cancerous breast tissues and adjacent normal breast tissues. Scale bar, 50 μm. Eya2 expression scores were plotted and compared (right panels). (C) The relationship between miR-30a and Eya2 expression was detected by Spearman rank correlation analysis in breast cancer samples.

proliferation and migration of breast cancer cells. Furthermore, introduction of miR-30a in Eya2 knockdown cells lost its ability to inhibit the proliferation and migration of breast cancer cells. These data suggest that Eya2 and miR-30a act in the same pathway and that activation of miR-30a may be useful for treatment of cancer with Eya2 overexpression.

Eya proteins are predominately expressed in progenitor cells where they regulate both cell proliferation and cell survival. It

has been reported that overexpression of Tristetraprolin proteins decrease Eya2 mRNA stability in MCF7 cells, leading to reduced cell viability in Drosophila and human cells [14]. In this study, we present a novel mechanism for the regulation of Eya2 expression involving miR-30a inhibition of Eya2 at the translational level. Transcriptional targets of mammalian Eya proteins include the protooncogene c-Myc and the important cell cycle regulators cyclin D1 and cyclin A. c-Myc and cyclin D1 play critical roles both in cell proliferation and migration [34,35]. We showed that Eya2 can increase the expression of c-Myc, cyclin A, cyclin D1, and cyclin E, all of which are involved in G1/S cell cycle progression. Although the miR-30a/Eya2 axis was shown to regulate the expression of these important genes, the exact underlying molecular mechanisms remain to be elucidated.

In conclusion, our study demonstrated that miR-30a can inhibit breast cancer cell proliferation and migration through inhibiting Eya2 expression. Expression of miR-30a, which is downregulated in breast cancer patients, negatively correlates with Eya2 expression, which is upregulated in breast cancer patients. These findings outline the importance of the miR-30a/Eya2 axis in breast cancer development and progression.

Conflict of interest

The authors have declared that no conflict of interest exists.

Acknowledgments

This work was supported by Major State Basic Research Development Program (2011CB504202 and 2012CB945100), National Natural Science Foundation (81372161, 31100604 and 81101065).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.174.

References

[1] H.W. Hwang, J.T. Mendell, MicroRNAs in cell proliferation, cell death, and tumorigenesis, Br. J. Cancer 94 (2006) 776–780.
[2] I. Alvarez-Garcia, E.A. Miska, MicroRNA functions in animal development and human disease, Development 132 (2005) 4653–4662.
[3] G.A. Calin, C.M. Croce, MicroRNA signatures in human cancers, Nat. Rev. Cancer 6 (2006) 857–866.
[4] A. Gaur, D.A. Jewell, Y. Liang, D. Ridzon, J.H. Moore, C. Chen, V.R. Ambros, M.A. Israel, Characterization of microRNA expression levels and their biological correlates in human cancer cell lines, Cancer Res. 67 (2007) 2456–2468.
[5] C. Piovan, D. Palmieri, L.G. Di, L. Braccioli, P. Casalini, G. Nuovo, M. Tortoreto, M. Sasso, I. Plantamura, T. Triulzi, C. Taccioli, E. Tagliabue, M.V. Iorio, C.M. Croce, Oncosuppressive role of p53-induced miR-205 in triple negative breast cancer, Mol. Oncol. 6 (2012) 458–472.
[6] B. Song, C. Wang, J. Liu, X. Wang, L. Lv, L. Wei, L. Xie, Y. Zheng, X. Song, MicroRNA-21 regulates breast cancer invasion partly by targeting tissue inhibitor of metalloproteinase 3 expression, J. Exp. Clin. Cancer Res. 29 (2010) 29.
[7] H. Li, C. Bian, L. Liao, J. Li, R.C. Zhao, MiR-17-5p promotes human breast cancer cell migration and invasion through suppression of HBP1, Breast Cancer Res. Treat. 126 (2011) 565–575.
[8] S.J. Silver, E.L. Davies, L. Doyon, I. Rebay, Functional dissection of eyes absent reveals new modes of regulation within the retinal determination gene network, Mol. Cell. Biol. 23 (2003) 5989–5999.
[9] A. Baonza, M. Freeman, Control of Drosophila eye specification by wingless signalling, Development 129 (2002) 5313–5322.
[10] J.P. Kumar, K. Moses, EGF receptor and Notch signaling act upstream of Eyeless/Pax6 to control eye specification, Cell 104 (2001) 687–697.
[11] K.L. Kenyon, S.S. Ranade, J. Curtiss, M. Mlodzik, F. Pignoni, Coordinating proliferation and tissue specification to promote regional identity in the Drosophila head, Dev. Cell 5 (2003) 403–414.
[12] N. Krishnan, D.G. Jeong, S.K. Jung, S.E. Ryu, A. Xiao, C.D. Allis, S.J. Kim, N.K. Tonks, Dephosphorylation of the C-terminal tyrosyl residue of the DNA damage-related histone H2A.X is mediated by the protein phosphatase eyes absent, J. Biol. Chem. 284 (2009) 16066–16070.
[13] J. Sun, Z. Karoulia, E.Y. Wong, M. Ahmed, K. Itoh, P.X. Xu, The phosphatase-transcription activator EYA1 is targeted by anaphase-promoting complex/Cdh1 for degradation at M-to-G1 transition, Mol. Cell. Biol. 33 (2013) 927–936.
[14] S.M. Farabaugh, D.S. Micalizzi, P. Jedlicka, R. Zhao, H.L. Ford, Eya2 is required to mediate the pro-metastatic functions of Six1 via the induction of TGF-beta signaling, epithelial-mesenchymal transition, and cancer stem cell properties, Oncogene 31 (2012) 552–562.
[15] L. Zhang, N. Yang, J. Huang, R.J. Buckanovich, S. Liang, A. Barchetti, C. Vezzani, A. O'Brien-Jenkins, J. Wang, M.R. Ward, M.C. Courreges, S. Fracchioli, A. Medina, D. Katsaros, B.L. Weber, G. Coukos, Transcriptional coactivator Drosophila eyes absent homologue 2 is up-regulated in epithelial ovarian cancer and promotes tumor growth, Cancer Res. 65 (2005) 925–932.
[16] M. Bierkens, O. Krijgsman, S.M. Wilting, L. Bosch, A. Jaspers, G.A. Meijer, C.J. Meijer, P.J. Snijders, B. Ylstra, R.D. Steenbergen, Focal aberrations indicate EYA2 and hsa-miR-375 as oncogene and tumor suppressor in cervical carcinogenesis, Genes Chromosom. Cancer 52 (2013) 56–68.
[17] J.T. Guo, L.H. Ding, C.Y. Liang, N.K. Zhou, Q.N. Ye, Expression of EYA2 in non-small cell lung cancer, Chin. J. Oncol. 31 (2009) 528–531.
[18] X.J. Xu, Z.Y. Fan, L. Kang, J.Q. Han, C.Y. Jiang, X.F. Zheng, Z.M. Zhu, H.B. Jiao, J. Lin, K. Jiang, L.H. Ding, H. Zhang, L. Cheng, H.J. Fu, Y. Song, Y. Jiang, J.H. Liu, R.F. Wang, N. Du, Q.N. Ye, Hepatitis B virus X protein represses miRNA-148a to enhance tumorigenesis, J. Clin. Invest. 123 (2013) 630–645.
[19] Y. Ma, P. Zhang, J. Yang, Z. Liu, Z. Yang, H. Qin, Candidate microRNA biomarkers in human colorectal cancer: systematic review profiling studies and experimental validation, Int. J. Cancer 130 (2012) 2077–2087.
[20] A. Watahiki, Y. Wang, J. Morris, K. Dennis, H.M. O'Dwyer, M. Gleave, P.W. Gout, Y. Wang, MicroRNAs associated with metastatic prostate cancer, PLoS ONE 6 (2011) e24950.
[21] Y. Yang, X. Li, Q. Yang, X. Wang, Y. Zhou, T. Jiang, Q. Ma, Y.J. Wang, The role of microRNA in human lung squamous cell carcinoma, Cancer Genet. Cytogenet. 200 (2010) 127–133.
[22] W. Wang, H. Lin, L. Zhou, Q. Zhu, S. Gao, H. Xie, Z. Liu, Z. Xu, J. Wei, X. Huang, S. Zheng, MicroRNA-30a-3p inhibits tumor proliferation, invasiveness and metastasis and is downregulated in hepatocellular carcinoma, Eur. J. Surg. Oncol. (2013) 0748–7983.
[23] J.J. Fabrizio, M. Boyle, S. DiNardo, A somatic role for eyes absent (eya) and sine oculis (so) in Drosophila spermatocyte development, Dev. Biol. 258 (2003) 117–128.
[24] X. Li, V. Perissi, F. Liu, D.W. Rose, M.G. Rosenfeld, Tissue-specific regulation of retinal and pituitary precursor cell proliferation, Science 297 (2002) 1180–1183.
[25] P.X. Xu, W. Zheng, C. Laclef, P. Maire, R.L. Maas, H. Peters, X. Xu, Eya1 is required for the morphogenesis of mammalian thymus, parathyroid and thyroid, Development 129 (2002) 3033–3044.
[26] W. Zheng, L. Huang, Z.B. Wei, D. Silvius, B. Tang, P.X. Xu, The role of Six1 in mammalian auditory system development, Development 130 (2003) 3989–4000.
[27] F. Fougerousse, M. Durand, S. Lopez, L. Suel, J. Demignon, C. Thornton, H. Ozaki, K. Kawakami, P. Barbet, J.S. Beckmann, P. Maire, Six and Eya expression during human somitogenesis and MyoD gene family activation, J. Muscle Res. Cell Motil. 23 (2002) 255–264.
[28] P.X. Xu, J. Adams, H. Peters, M.C. Brown, S. Heaney, R. Maas, Eya1-deficient mice lack ears and kidneys and show abnormal apoptosis of organ primordia, Nat. Genet. 23 (1999) 113–117.
[29] R.N. Pandey, R. Rani, E.J. Yeo, M. Spencer, S. Hu, R.A. Lang, R.S. Hegde, The eyes absent phosphatase-transactivator proteins promote proliferation, transformation, migration, and invasion of tumor cells, Oncogene 29 (2010) 3715–3722.
[30] P.R. Godoy, S.S. Mello, D.A. Magalhaes, F.S. Donaires, P. Nicolucci, E.A. Donadi, G.A. Passos, E.T. Sakamoto-Hojo, Ionizing radiation-induced gene expression changes in TP53 proficient and deficient glioblastoma cell lines, Mutat. Res. 756 (2013) 46–55.
[31] S.J. Miller, Z.D. Lan, A. Hardiman, J. Wu, J.J. Kordich, D.M. Patmore, R.S. Hegde, T.P. Cripe, J.A. Cancelas, M.H. Collins, N. Ratner, Inhibition of eyes absent homolog 4 expression induces malignant peripheral nerve sheath tumor necrosis, Oncogene 29 (2010) 368–379.
[32] E. Hoekstra, M.P. Peppelenbosch, G.M. Fuhler, The role of protein tyrosine phosphatases in colorectal cancer, Biochim. Biophys. Acta 2012 (1826) 179–188.
[33] M.B. Potts, S. Cameron, Cell lineage and cell death: *Caenorhabditis elegans* and cancer research, Nat. Rev. Cancer 11 (2011) 50–58.
[34] S. Kanazawa, L. Soucek, G. Evan, T. Okamoto, B.M. Peterlin, C-Myc recruits P-TEFb for transcription, cellular proliferation and apoptosis, Oncogene 22 (2003) 5707–5711.
[35] V. Baldin, J. Lukas, M.J. Marcote, M. Pagano, G. Draetta, Cyclin D1 is a nuclear protein required for cell cycle progression in G1, Genes Dev. 7 (1993) 812–821.