Biochemical and Biophysical Research Communications 459 (2015) 340–345

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

miR-664 negatively regulates PLP2 and promotes cell proliferation and invasion in T-cell acute lymphoblastic leukaemia

Hong Zhu, Mei-hua Miao, Xue-qiang Ji, Jun Xue, Xue-jun Shao*

Department of Clinical Laboratory Diagnosis, Children's Hospital of Soochow University, Suzhou, China

ARTICLE INFO

Article history:
Received 16 February 2015
Available online 28 February 2015

Keywords:
miR-664
PLP2
T-ALL

ABSTRACT

MicroRNAs (miRNAs) play important roles in the pathogenesis of many types of cancers by negatively regulating gene expression at posttranscriptional level. However, the role of microRNAs in leukaemia, particularly T-cell acute lymphoblastic leukaemia (T-ALL), has remained elusive. Here, we identified miR-664 and its predicted target gene PLP2 were differentially expressed in T-ALL using bioinformatics methods. In T-ALL cell lines, CCK-8 proliferation assay indicated that the cell proliferation was promoted by miR-664, while miR-664 inhibitor could significantly inhibited the proliferation. Moreover, migration and invasion assay showed that overexpression of miR-664 could significantly promoted the migration and invasion of T-ALL cells, whereas miR-664 inhibitor could reduce cell migration and invasion. luciferase assays confirmed that miR-664 directly bound to the 3'untranslated region of PLP2, and western blotting showed that miR-664 suppressed the expression of PLP2 at the protein levels. This study indicated that miR-664 negatively regulates PLP2 and promotes proliferation and invasion of T-ALL cell lines. Thus, miR-664 may represent a potential therapeutic target for T-ALL intervention.

1. Introduction

Acute lymphoblastic leukemia (ALL) is a hematologic malignancy arising from hematopoietic precursors of the lymphoid lineage. ALL is the most common leukemia in pediatrics. Nearly up to 80% of ALL cases occurs in children. T-cell acute lymphoblastic leukemia (T-ALL) is the ALL transformed from developing thymocytes and resulting from cooperative genetic lesions. Generally, these genetic aberrations affect multiple biological processes, e.g. self-renewal, proliferation and survival, as well as block differentiation of precursor T cells [1].

The discovery of MicroRNAs (miRNAs) opened a new generation of the understanding of carcinogenesis in general and leukemogenesis in particular [2]. miRNAs are small, non-coding RNAs which could negatively regulate the expression of target genes by translational repression or mRNA degradation [3]. They are involved in in the management of hematopoiesis [2]. Therefore, miRNA dysregulation causes disruption of the hematopoietic system and leukemia may arise. Many miRNAs, e.g. miR-2909 [4], miRNA-193b-3p [5], miRNA-128-3p [6] and miRNA-100/99a [7], are found to play roles in the carcinogenesis of T-ALL.

In this study, we analyzed the miRNA and mRNA expression data from T-ALL samples and normal T-cell controls and identified miR-664 as a new potential T-ALL-related miRNAs with a predicted and target gene PLP2. Further wet experiment in T-ALL cell lines confirmed that miR-664 directly bound to the 3'untranslated region of PLP2, and suppressed the expression of PLP2 at the protein levels. Moreover, modulation of miR-664 expression affected proliferation and invasion of T-ALL cell lines.

2. Materials and methods

2.1. miRNA and mRNA profile data Collection

miRNA and mRNA profiles data of T-ALL and normal control samples were collected from GEO database (www.ncbi.nlm.nih.gov/gds, GSE56489, GSE41621, GSE46170). After quality control, 43 children with T-ALL and 14 age-matched healthy controls were included in miRNA analysis, while 49 children with T-ALL and 9 age-matched healthy controls were included in mRNA analysis.

2.2. Identification of differentially expressed miRNA and mRNA

The identification of differentially expressed miRNA and mRNA in children with T-ALL tissues were performed with Limma package

* Corresponding author. No 303, Jinde Road, Suzhou, Jiangsu Province, 215003, China. Fax: +86 512 67785415.
E-mail address: xuejunshao@hotmail.com (X.-j. Shao).

http://dx.doi.org/10.1016/j.bbrc.2015.02.116
0006-291X/

on R platform using download miRNA and mRNA profiles data as mentioned above. The cutline of significantly differentially expressed miRNA and mRNA is P. value <0.01 (t test).

2.3. miRNA target genes prediction

Human miRNA target genes prediction were performed with miRNA sequences that downloaded from the Rfam website (http://www.sanger.ac.uk/Software/Rfam) and satisfy the established criteria [8]. 3' UTR sequences data for human genes were retrieved using EnsMart [9]. Repetitive elements in these sequences were masked by RepeatMasker [10] with repeat libraries for vertebrates, rodents, or primates, as appropriate. The target genes of miRNAs were predicted using DIANA [11], miRanda [12] and TargetScan [13] methods. The predicted target genes supported by all the three methods were selected for further analysis.

2.4. Integrative network analysis

The integrative network analysis of differentially expressed miRNAs and mRNAs was performed with Cytoscape software. The molecular network used in this analysis was constructed with predicted miRNA-mRNA interaction data and experimental validated human Protein–Protein interaction data (Downloaded from BioGrid database and HPRD database). The in-depth analysis of this network facilitated deciphering the complex interplay of differentially expressed miRNAs and corresponding target mRNAs and suggested their possible roles in the carcinogenesis of T-ALL.

2.5. Cell culture

The human T-ALL cell lines, CCRF-CEM was obtained from American Type Culture Collection (ATCC, USA). and Jurkat was obtained from the Chinese Center for Type Cultures Collections (CCTCC, China). The CCRF-CEM and Jurkat cell line was cultured in RPMI 1640 media (Life Technologies, Shanghai, China) and supplemented with 10% fetal bovine serum (FBS) (Life Technologies, Shanghai, China). Cells were maintained in a humidified atmosphere with 5% CO2 at 37 °C.

2.6. Cell transfection

CCRF-CEM and Jurkat cell lines were seeded in 24-well plates at 3×10^5 cells/wells and incubated overnight. Transfection of the

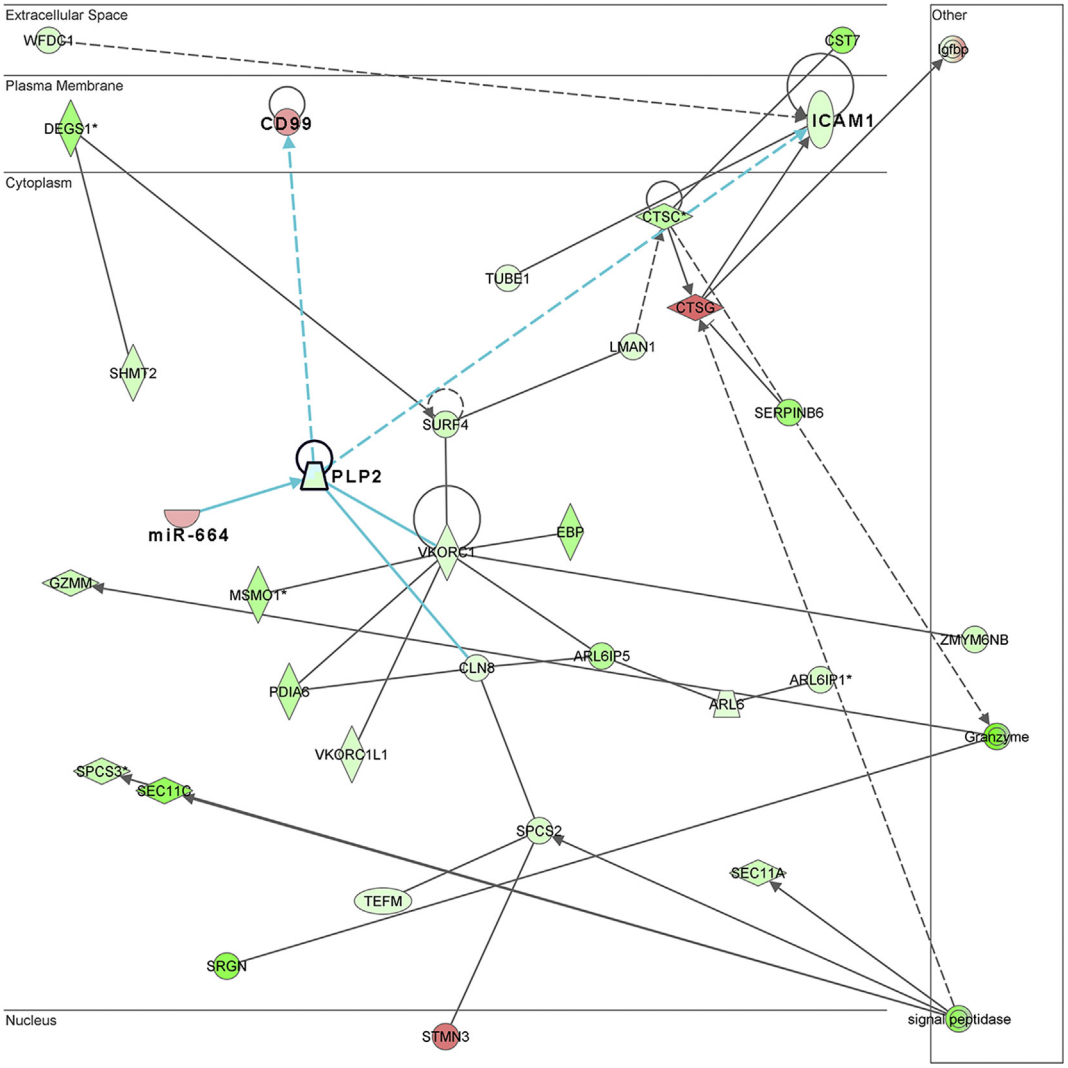

Fig. 1. Regulatory networks of differentially expressed miRNAs and corresponding target genes in T-ALL. Red means this molecule was up-regulated in ALL, while green means down-regulated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

miR-664 miRNA mimic, the anti-miR-664, inactive control cel-mir-67 (Life Technologies, Shanghai, China), or pMIR-Report vectors was taken using Lipofectamine 2000 transfection reagent (Invitrogen, Shanghai, China) with 300 nmol of miRNA or 1 μg/ml DNA plasmid, respectively. Total proteins of CCRF-CEM and Jurkat cells were isolated at 48 h after transfection.

2.7. Cell proliferation

Cell proliferations were measured using a Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). CCRF-CEM and Jurkat T-ALL cells were plated in 24-well plates at 3×10^5 cells/well. Then cells were incubated in 10% CCK-8 which was diluted in normal culture medium at 37 °C for color conversion. Proliferation rates were determined at 24, 48 and 72 h after transfection.

2.8. Cell migration and invasion

Cell invasion and migration were measured using a transwell chamber (Corning, Shanghai, China) with and without Matrigel (Invitrogen, Shanghai, China). For the determination of CCRF-CEM and Jurkat T-ALL cells invasion, transwell chambers were placed into 24-well plates, and coated with 30 μl Matrigel, then incubated at 37 °C for 40 min. In transwell assays with and without Matrigel, CCRF-CEM and Jurkat cells were trypsinized and then seeded in chambers at the density of 8×10^4 cells/well at 48 h after transfection. These cells were cultured in RPMI 1640 medium with 2% serum. Meanwhile 600 μl of 10% FBS-1640 was added to the lower chamber. After 24 h, migrated CCRF-CEM and Jurkat cells were fixed in 100% methanol for 30 min. These non-migrated CCRF-CEM and Jurkat cells were removed by cotton swabs. After that cells on the bottom surface of the membrane were stained with the 0.1% crystal violet for 20 min. Images of CCRF-CEM and Jurkat cells were taken under a phase-contrast microscope.

2.9. Luciferase assay

CCRF-CEM and Jurkat cells were seeded in 24-well plates at 1×10^5 cells/well and incubated for 24 h before transfection. In the reporter gene assay, the CCRF-CEM and Jurkat cells were co-transfected with 0.6 μg of pGL3-PLP2-3′UTR or pGL3-PLP2-3′UTR Mut plasmid, 0.06 ng of the phRL-SV40 control vector (Promega, Shanghai, China), and 100 nM miR-664 or control RNA using Lipofectamine 2000 (Invitrogen, Shanghai, China). The renilla and firefly luciferase activities were determined with a dual luciferase assay (Promega, Shanghai, China) 24 h after transfection.

2.10. Western blot

The Western blot followed a previously described protocol [14]. Proteins were separated by 12% SDS-PAGE gel and transferred onto nitrocellulose membranes (Bio-Rad, Shanghai, China). Membranes was blocked by 5% non-fat milk and incubated with anti-PLP2 antibody (Abcam, Shanghai, China) or anti-β-actin antibody (Abcam, Shanghai, China). After being washed extensively, the secondary antibody (Abcam, Shanghai, China) was then added to the system. Finally, Immunoreactive protein bands were detected with the Enhanced Chemiluminescence (ECL) system.

2.11. Statistical analysis

Experiments were repeated at least three times. Statistical analyses were performed using R 3.0.3. Values were expressed as means ± S.D. Differences between groups were estimated with T-

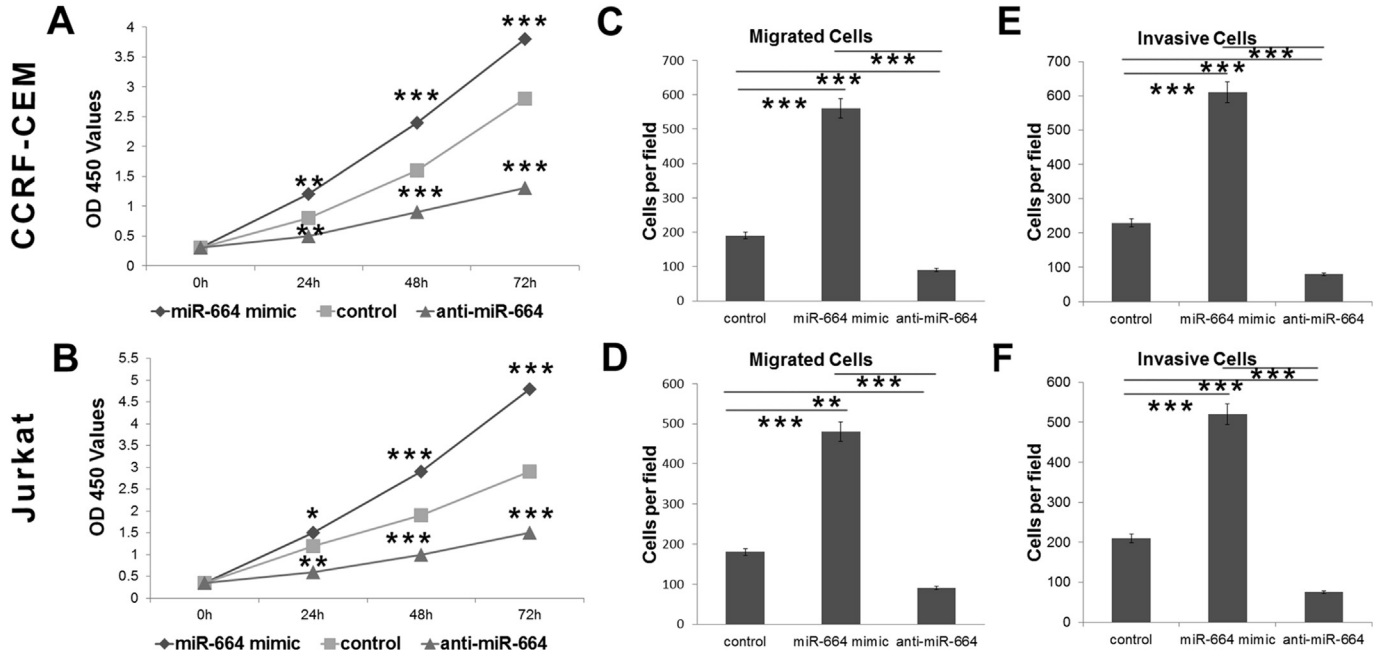

Fig. 2. miR-664 regulates T-ALL cell proliferation, migration and invasion. (A) Growth of CCRF-CEM cells was shown after transfection with miR-664 mimics or inhibitor or inactive control. The growth index as assessed at 0, 24, 48 and 72 h. (B) Growth of Jurkat cells was shown after transfection with miR-664 mimics or inhibitor or inactive control. The growth index as assessed at 0, 24, 48 and 72 h. The growth index as assessed at 0, 24, 48 and 72 h. (C) Transwell analysis of CCRF-CEM cells migration after treatment with miR-664 mimics, inhibitors or inactive control; (D) Transwell analysis of Jurkat cells migration after treatment with miR-664 mimics, inhibitors or inactive control; (E) Transwell analysis of CCRF-CEM cells invasion after treatment with miR-664 mimics, inhibitors or inactive control; (F) Transwell analysis of Jurkat cells invasion after treatment with miR-664 mimics, inhibitors or inactive control; $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

test. T-ALL analysis were considered to be significant when P. value<0.05.

3. Results

3.1. miR-664 and its predicted target genes were differentially expressed in T-ALL

The comparison of the transcriptome data from T-ALL samples and normal healthy control identified that 33 miRNAs and 345 genes were differentially expressed in ALL. Further miRNA targets prediction analysis and integrative network analysis indicated that there are potential regulatory interaction between differentially expressed miR-664 and PLP2 in T-ALL (Fig. 1). In this study miR-664 was found to be up-regulated in the 43 T-ALL children samples and its predicted target PLP2 was down-regulated in the 49 children with T-ALL. It imply that miR-664 might have more possibility to take part in the carcinogenesis of T-ALL. Therefore, we selected miR-664 for further functional investigation in the human T-ALL cell line CCRF-CEM and Jurkat.

3.2. Overexpression of miR-664 promoted T-ALL cell proliferation, migration and invasion

We explored the potential impact of miR-664 in T-ALL cell proliferation, migration and invasion in CCRF-CEM and Jurkat cell lines. CCRF-CEM and Jurkat cells were transfected with miR-664 mimics or inhibitor or inactive control cel-mir-67. CCK-8 proliferation assay indicated that the cell proliferation was promoted in both of the miR-664-mimics-transfected T-ALL cell lines compared with inactive control cel-mir-67-transfected cell lines (Fig. 2A and B). Conversely, miR-664 inhibitor could significantly inhibit the proliferation of the CCRF-CEM and Jurkat cells (Fig. 2A and B). Interestingly, migration and invasion assay showed that overexpression of miR-664 could significantly promoted the migration and invasion of CCRF-CEM and Jurkat cells compared with the

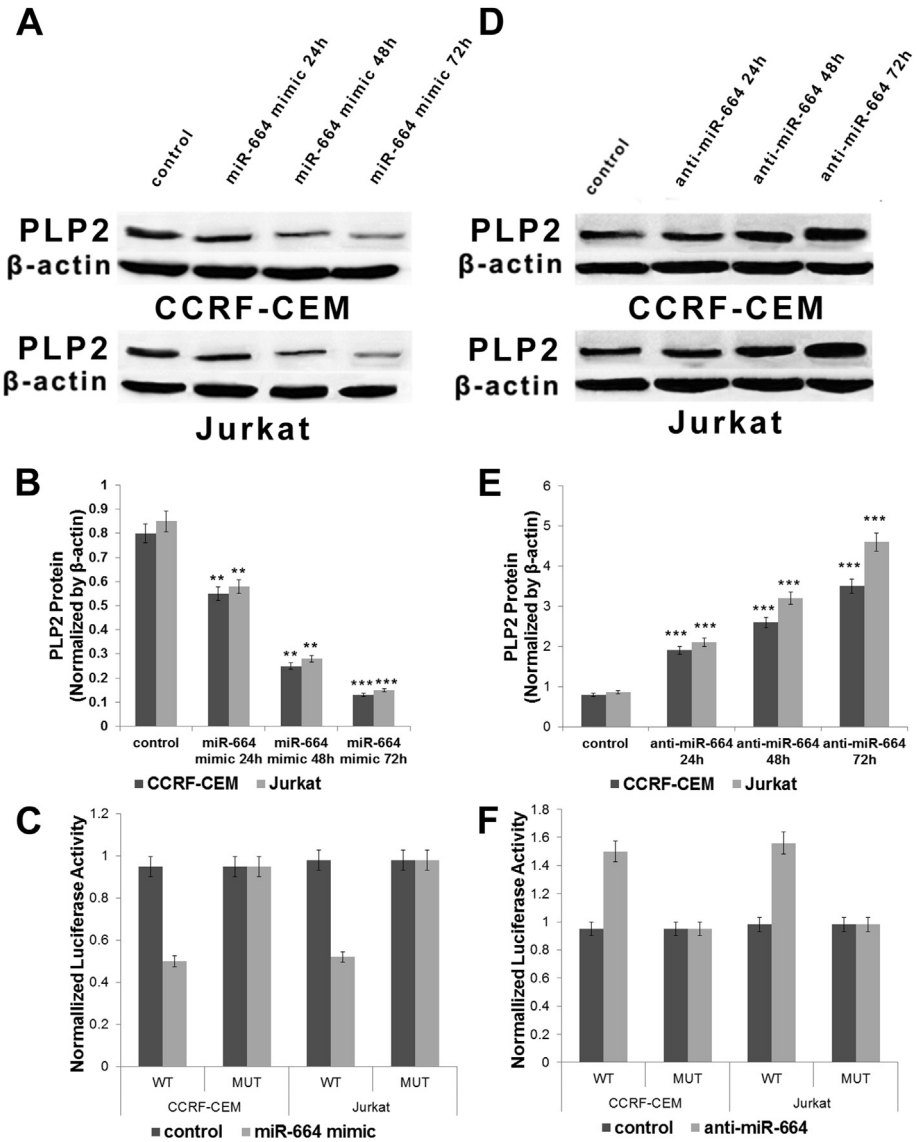

Fig. 3. miR-664 targets PLP2 in T-ALL cells. (A.B) Protein of PLP2 significantly decreased with time after transfection with miR-664 mimics in CCRF-CEM and Jurkat cells; (C) The analysis of the relative luciferase activities of PLP2-WT, PLP2-MUT in CCRF-CEM and Jurkat cells after transfection with miR-664 mimics; (D,E) Protein of PLP2 increased with time after transfection with anti-miR-664 in CCRF-CEM and Jurkat cells; (F) The analysis of the relative luciferase activities of PLP2-WT, PLP2-MUT in CCRF-CEM and Jurkat cells after transfection with anti-miR-664; *p < 0.05, **p < 0.01, and ***p < 0.001.

inactive cel-mir-67 control, whereas anti-miR-664 could inhibited cell migration and invasion in both of the CCRF-CEM and Jurkat cells (Fig. 2C–2F).

3.3. MiR-664 targets and negatively regulates PLP2 in T-ALL cells

As predicted by DIANA [11], miRanda [12] and TargetScan [13], there was complementarity between has-miR-664 and the 3′ UTR of PLP2. Overexpression of miR-664 significantly reduced the protein levels of PLP2 in T-ALL cells (Fig. 3A and B). Conversely, miR-664 inhibitor significantly increased the protein levels of PLP2 in T-ALL cells (Fig. 3D and E). The effect of miR-664 on the translation of PLP2 mRNA into protein was then assessed by using a luciferase reporter assay (Fig. 3C and F). Enforced expression of miR-664 remarkably reduced the luciferase activity of the reporter gene with the wild type construct but not with the mutant PLP2 3′UTR construct (Fig. 3C), while the miR-664 inhibitor remarkably enhanced the luciferase activity of the reporter gene with the wild type construct but not with the mutant PLP2 3′UTR construct (Fig. 3F). These evidences indicate that miR-664 directly targeted the 3′UTR region of PLP2.

4. Discussion

In the last decades, miRNAs have be identified to be major transcriptional regulators involved in many biological processes such as cell differentiation and carcinogenesis [15]. Globally miRNA expression profiles of tumors have provided valuable understandings of the molecular mechanisms underlying carcinogenesis [16]. Thus we compared miRNA and mRNA expression data between T-ALL samples and healthy normal controls, and made further miRNA –target-gene prediction and integrative molecular network analysis to identify potential miRNAs and corresponding target genes involved in carcinogenesis of ALL. The results showed that miR-664 was up-regulated in the 43 T-ALL children samples and its predicted target PLP2 was down-regulated in the 49 children with T-ALL.

Increased expression of miR-664 has been found in hepatocellular carcinoma [17]. The relationship between miR-664 and T-ALL has never been reported before. Thus, our further study intended to clarify the biological function of miR-664 in T-ALL. Enforced expression of miR-664 enhanced proliferation, migration and invasion of CCRF-CEM and Jurkat cells, which suggest that miR-664 plays a critical role in the carcinogenesis of T-ALL and may be a potential diagnostic and predictive biomarker. Next, we addressed the molecular mechanism of miR-664 in promoting proliferation, migration and invasion in T-ALL cells. In this study, Western blots and luciferase assays showed that PLP2 is a target gene of miR-664. PLP2 encodes an integral membrane protein that localizes to the endoplasmic reticulum, which can multimerize and may function as an ion channel. PLP2 has been reported to be associated with melanoma metastasis [18]. PLP2 protein is necessary for down-regulation of human CD99 protein in plasma membrane [19]. This implied that reduced PLP2 protein may lead to increased CD99 protein level. Congruously, the up-regulation of human CD99 was observed in the 49 T-ALL children, in which PLP2 is down-regulated (Fig. 1). The up-regulation of CD99 was also observed previously in pediatric B and T leukemias [20,21]. CD99 encodes a cell surface glycoprotein involved in leukocyte migration, T-cell adhesion, and T-cell death [22,23]. The up-regulation of CD99 seems to be part of the downstream mechanisms of miR-664 in the carcinogenesis of T-ALL.

In conclusion, our results have shown that enforced expression of miR-664 promoted T-ALL cell proliferation, migration and invasion through directly targeting and down-regulated PLP2, and further potentially contributing to the up-regulation of CD99. This novel miR-664/PLP2 axis may provide new insights into the mechanisms underlying tumor metastasis, and inhibition of miR-664 may be a potential therapeutic strategy for the treatment of T-ALL in the future.

Conflict of interest

Authors have declared that no competing interest exists.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.116.

References

[1] P. Van Vlierberghe, R. Pieters, H.B. Beverloo, J.P. Meijerink, Molecular-genetic insights in paediatric T-cell acute lymphoblastic leukaemia, Br. J. Haematol. 143 (2008) 153–168.
[2] D. Schotte, R. Pieters, M.L. Den Boer, MicroRNAs in acute leukemia: from biological players to clinical contributors, Leukemia 26 (2012) 1–12.
[3] D.P. Bartel, MicroRNAs: target recognition and regulatory functions, Cell 136 (2009) 215–233.
[4] D. Malik, D. Kaul, N. Chauhan, R.K. Marwaha, miR-2909-mediated regulation of KLF4: a novel molecular mechanism for differentiating between B-cell and T-cell pediatric acute lymphoblastic leukemias, Mol. Cancer 13 (2014) 175.
[5] E. Mets, J. Van der Meulen, G. Van Peer, M. Boice, P. Mestdagh, I. Van de Walle, T. Lammens, S. Goossens, B. De Moerloose, Y. Benoit, N. Van Roy, E. Clappier, B. Poppe, J. Vandesompele, H.G. Wendel, T. Taghon, P. Rondou, J. Soulier, P. Van Vlierberghe, F. Speleman, MicroRNA-193b-3p acts as a tumor suppressor by targeting the MYB oncogene in T-cell acute lymphoblastic leukemia, Leukemia (Sep 18 2014), http://dx.doi.org/10.1038/leu.2014.276 [Epub ahead of print].
[6] E. Mets, G. Van Peer, J. Van der Meulen, M. Boice, T. Taghon, S. Goossens, P. Mestdagh, Y. Benoit, B. De Moerloose, N. Van Roy, B. Poppe, J. Vandesompele, H.G. Wendel, P. Van Vlierberghe, F. Speleman, P. Rondou, MicroRNA-128-3p is a novel oncomiR targeting PHF6 in T-cell acute lymphoblastic leukemia, Haematologica 99 (2014) 1326–1333.
[7] X.J. Li, X.Q. Luo, B.W. Han, F.T. Duan, P.P. Wei, Y.Q. Chen, MicroRNA-100/99a, deregulated in acute lymphoblastic leukaemia, suppress proliferation and promote apoptosis by regulating the FKBP51 and IGF1R/mTOR signalling pathways, Br. J. Cancer 109 (2013) 2189–2198.
[8] V. Ambros, B. Bartel, D.P. Bartel, C.B. Burge, J.C. Carrington, X. Chen, G. Dreyfuss, S.R. Eddy, S. Griffiths-Jones, M. Marshall, M. Matzke, G. Ruvkun, T. Tuschl, A uniform system for microRNA annotation, RNA 9 (2003) 277–279.
[9] A. Kasprzyk, D. Keefe, D. Smedley, D. London, W. Spooner, C. Melsopp, M. Hammond, P. Rocca-Serra, T. Cox, E. Birney, EnsMart: a generic system for fast and flexible access to biological data, Genome Res. 14 (2004) 160–169.
[10] M. Tarailo-Graovac, N. Chen, Using RepeatMasker to identify repetitive elements in genomic sequences, Curr. Protoc. Bioinforma. Chapter 4 (2009). Unit 4 10.
[11] M.D. Paraskevopoulou, G. Georgakilas, N. Kostoulas, M. Reczko, M. Maragkakis, T.M. Dalamagas, A.G. Hatzigeorgiou, DIANA-LncBase: experimentally verified and computationally predicted microRNA targets on long non-coding RNAs, Nucleic Acids Res. 41 (2013) D239–D245.
[12] B. John, A.J. Enright, A. Aravin, T. Tuschl, C. Sander, D.S. Marks, Human MicroRNA targets, PLoS Biol. 2 (2004) e363.
[13] B.P. Lewis, C.B. Burge, D.P. Bartel, Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets, Cell 120 (2005) 15–20.
[14] J. Li, X. Kong, J. Zhang, Q. Luo, X. Li, L. Fang, MiRNA-26b inhibits proliferation by targeting PTGS2 in breast cancer, Cancer Cell. Int. 13 (2013) 7.
[15] W.K. Wu, C.W. Lee, C.H. Cho, D. Fan, K. Wu, J. Yu, J.J. Sung, MicroRNA dysregulation in gastric cancer: a new player enters the game, Oncogene 29 (2010) 5761–5771.
[16] J. Miao, S. Wu, Z. Peng, M. Tania, C. Zhang, MicroRNAs in osteosarcoma: diagnostic and therapeutic aspects, Tumour Biol. 34 (2013) 2093–2098.
[17] H. Yang, M.E. Cho, T.W. Li, H. Peng, K.S. Ko, J.M. Mato, S.C. Lu, MicroRNAs regulate methionine adenosyltransferase 1A expression in hepatocellular carcinoma, J. Clin. Invest 123 (2013) 285–298.
[18] Y. Sonoda, M. Warita, T. Suzuki, H. Ozawa, Y. Fukuda, M. Funakoshi-Tago, T. Kasahara, Proteolipid protein 2 is associated with melanoma metastasis, Oncol. Rep. 23 (2010) 371–376.
[19] R.T. Timms, L.M. Duncan, I.A. Tchasovnikarova, R. Antrobus, D.L. Smith, G. Dougan, M.P. Weekes, P.J. Lehner, Haploid genetic screens identify an essential role for PLP2 in the downregulation of novel plasma membrane targets by viral E3 ubiquitin ligases, PLoS Pathog. 9 (2013) e1003772.

[20] Z. Husak, M.N. Dworzak, CD99 ligation upregulates HSP70 on acute lymphoblastic leukemia cells and concomitantly increases NK cytotoxicity, Cell. Death Dis. 3 (2012) e425.
[21] D.M. Dorfman, M. Kraus, A.R. Perez-Atayde, R.L. Barnhill, G.S. Pinkus, S.R. Granter, CD99 (p30/32MIC2) immunoreactivity in the diagnosis of leukemia cutis, Mod. Pathol. 10 (1997) 283–288.
[22] T.H. Lin, H.C. Wu, Y.C. Hsieh, C.E. Tseng, R. Ichinohasama, S.S. Chuang, CD4 and CD8 double-negative adult T-cell leukemia/lymphoma with monomorphic cells expressing CD99: a diagnostic challenge in a country non-endemic for human T-cell leukemia virus, Pathol. Int. 63 (2013) 132–137.
[23] T. Terada, TDT (−), KIT (+), CD34 (+), CD99 (+) precursor T lymphoblastic leukemia/lymphoma, Int. J. Clin. Exp. Pathol. 5 (2012) 167–170.