Biochemical and Biophysical Research Communications 450 (2014) 1241–1246

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

MicroRNA-218 inhibits the proliferation of human choriocarcinoma JEG-3 cell line by targeting Fbxw8

Dazun Shi, Zhihui Tan, Rong Lu, Wenqing Yang, Yi Zhang *

Department of Obstetric and Gynaecology, Xiangya Hospital, Xiangya Hospital of Central South University, Changsha, China

ARTICLE INFO

Article history:
Received 8 June 2014
Available online 25 June 2014

Keywords:
miR-218
Fbxw8
JEG-3
Proliferation

ABSTRACT

MicroRNAs (miRNAs) are endogenous 19–25 nucleotide noncoding single-stranded RNAs that regulate gene expression by blocking the translation or decreasing the stability of mRNAs. In this study, we showed that miR-218 expression levels were decreased while Fbxw8 expression levels were increased in human choriocarcinoma cell lines, and identified Fbxw8 as a novel direct target of miR-218. Overexpression of miR-218 inhibited cell growth arrest at G2/M phase, suppressed the protein levels of cyclin A and up-regulated the expression levels of p27 through decreasing the levels of Fbxw8. On the other hand, forced expression of Fbxw8 partly rescued the effect of miR-218 in the cells, attenuated cell proliferation decrease the percentage of cells at G2/M phase, induced cyclin A protein expression and suppressed the protein level of p27 through up-regulating the levels of Fbxw8. Taken together, these findings will shed light the role to mechanism of miR-218 in regulating JEG-3 cells proliferation via miR-218/Fbxw8 axis, and miR-218 may serve as a novel potential therapeutic target in human choriocarcinoma in the future.

1. Introduction

Choriocarcinoma typically occurs in females at the origin of the chorionic epithelium of the placenta, and is commonly related to gestation. The tumor is rapidly growing, widely metastasizing, and highly invasive of surrounding tissues. Gonadal choriocarcinomas are usually highly sensitive to various types of anti-cancer agents [1]. Currently on the clinical treatment of choriocarcinoma rely on chemotherapy and radiation therapy [1]. Choriocarcinoma of the vast majority of patients can be obtained through chemotherapy for a longer period of time, even the clinical cure, but chemotherapy failure cases are not uncommon, mainly because of the chemotherapy drug resistance, and side effects after chemotherapy generally unable to adhere to the completion of chemotherapy treatments. Clinical when such situations arise, the patient's condition and prognosis will be inestimable. Given this, there is an urgent need to develop novel strategies for the diagnosis, treatment and prognosis of choriocarcinoma.

MicroRNAs (miRNAs) are small regulatory RNAs, which control a large number of gene expressions by translational suppression and destabilization of target mRNAs [2,3]. Currently, the number of validated mature human miRNAs approaches 2000. Individual miRNAs can repress multiple genes [4,5], which implies a broad regulatory potential. Not surprisingly, miRNAs have emerged as important regulators of virtually every biological process associated with tissue development, differentiation, cellular proliferation, cell type-specific function, and homeostasis. Consequently, dysregulation of miRNAs has been implicated in dysfunction of genetic regulatory networks. Indeed, several pathological conditions have been linked to altered expression of miRNA [6–9].

Ubiquitin–proteasome pathway plays indispensable roles in many biological processes [10]. A three-enzyme cascade is involved in the protein ubiquitination: the E1ubiquitin activating enzyme, the E2 ubiquitin conjugating enzymes, and the E3 ubiquitin ligases [11]. E3 ubiquitin ligases define the specificity of the substrate and it is likely that protein ubiquitination is mainly regulated by E3s activity or E3s substrate interaction [12]. Hundreds of E3 ligases identified in eukaryotes were classified into four groups: HECT-type, U-box-type, PHD-finger-type and RING-type E3s [13]. Cullin-based E3s, one of RING-type complexes, are the largest family among them [14,15]. The Skp1-Cul1-F-box (SCF) multi-subunit complex is a well characterized E3s, and the F-box protein determines the substrate specificity. Sixty-eight human genes encoding F-box proteins have been identified [16]. F-box protein Fbxw8 (also named Fbw6 or Fbx29), contains an N-terminal F-box motif which is required for Skp1 binding, and a C-terminal WD40 repeat interacted with CUL7 (also known as

* Corresponding author. Address: Department of Obstetric and Gynaecology, Xiangya Hospital, Xiangya Hospital of Central South University, Xiangya Road No. 87, Changsha, China.
E-mail address: zhangyi5588@hotmail.com (Y. Zhang).

http://dx.doi.org/10.1016/j.bbrc.2014.06.094
0006-291X/

p193 or p185), which assembles a SCF-like E3 complex containing Skp1, Fbxw8 as well as ROC1 [17]. The function of Fbxw8 is largely unknown. Fbxw8 has been involved in the proteasomal degradation of insulin receptor substrate 1 (IRS-1) [18], and it also plays an essential role in the proliferation of cancer cells via proteolysis of cyclin A [19].

In order to identify novel microRNAs which could specifically target Fbxw8, several candidate microRNAs (miR-218, miR-30a, miR-33a, and miR-376a) were initially predicted by software analysis programme. We therefore challenged their expression in choriocarcinoma cancer cells by introduction of exogenous miRNA expression. miR-218 expression was decreased significantly in choriocarcinoma cells normalized to human trophoblast (HT) cells. In the present study, we confirmed the regulatory relationship between miR-218, a known tumor suppressive miRNA, and an oncogene, Fbxw8. We provided evidences that miR-218 could impede choriocarcinoma JEG-3 cell line proliferation, at least partly by targeting Fbxw8.

2. Materials and methods

2.1. Cells and culture

The Human trophoblast (HT) cells were isolated by trypsin-DNase I digestion, and discontinuous Percoll gradient centrifugation, as described by the previous study [20]. This assay supplies a 95% purity of HT cells. The isolated HT cells were cultured in DMEM supplemented with 2 mM glutamine, 10% heat inactivated FCS, 25 mM HEPES, 100 UI/ml penicillin and 100 mg/ml streptomycin at 37 °C in 95% air and 5% CO_2. Human choriocarcinoma cell lines JEG-3, JAR and BeWo was obtained from American Type Culture Collection (Manassas, VA, USA). JEG-3, JAR and BeWo cells were grown in DMEM medium, cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA,USA), and cultured in 95% air and 5% CO_2 at 37 °C.

2.2. Cell transfection

Ectopic expression of miR-218 in cells was achieved by transfection with miR-218 mimics (Genepharma, Shanghai, China) using Lipofectamine 2000 (Invitrogen, CA, USA). Overexpression of Fbxw8 was performed using Fbxw8 ORF expression clone (GeneCopoecia, Guangzhou, China). Cells were plated in 6-well clusters or 96-well plates and transfected for 24 h or 48 h. Transfected cells were used in further assays or RNA/protein extraction.

2.3. RNA extraction and SYBR green quantitative PCR analysis

Total RNA was extracted from cells using Trizol reagent (Invitrogen, CA, USA). Mature miR-218 expressions in cells were detected using a Hairpin-it TM miRNAs qPCR kit (Genepharma, Shanghai, China). Expression of RNU6B was used as an endogenous control. Fbxw8 expression was measured by SYBR green qPCR assay (Takara, Dalian, China). Data were processed using $2^{-\Delta\Delta CT}$ method.

2.4. Western blot analysis

Immunoblotting was performed to detect the expression of Fbxw8 cyclin A and p27 in JEG-3 cells. Cultured or transfected cells were lysed in RIPA buffer with 1% PMSF. Protein was loaded onto a SDS–PAGE minigel and transferred onto PVDF membrane. After probed with 1:1000 diluted rabbit polyclonal Fbxw8, cyclin A and p27 antibody (Abcam, MA, USA) at 4 °C overnight, the blots were subsequently incubated with HRP-conjugated secondary antibody (1:5000). Signals were visualized using ECL Substrates (Millipore, MA, USA). β-actin was used as an endogenous protein for normalization.

2.5. Luciferase reporter assay

JEG-3 cells were seeded into a 24-well plate. After cultured overnight, cells were co-transfected with the wild-type and mutated Fbxw8 3′UTR reporter plasmid, and pRL-TK plasmids, or transfected with miR-218 and miR-scrambled control precursors (miR-SCR). Luciferase assays were performed 48 h after transfection using the Dual Luciferase Reporter Assay System (Promega, WI, USA).

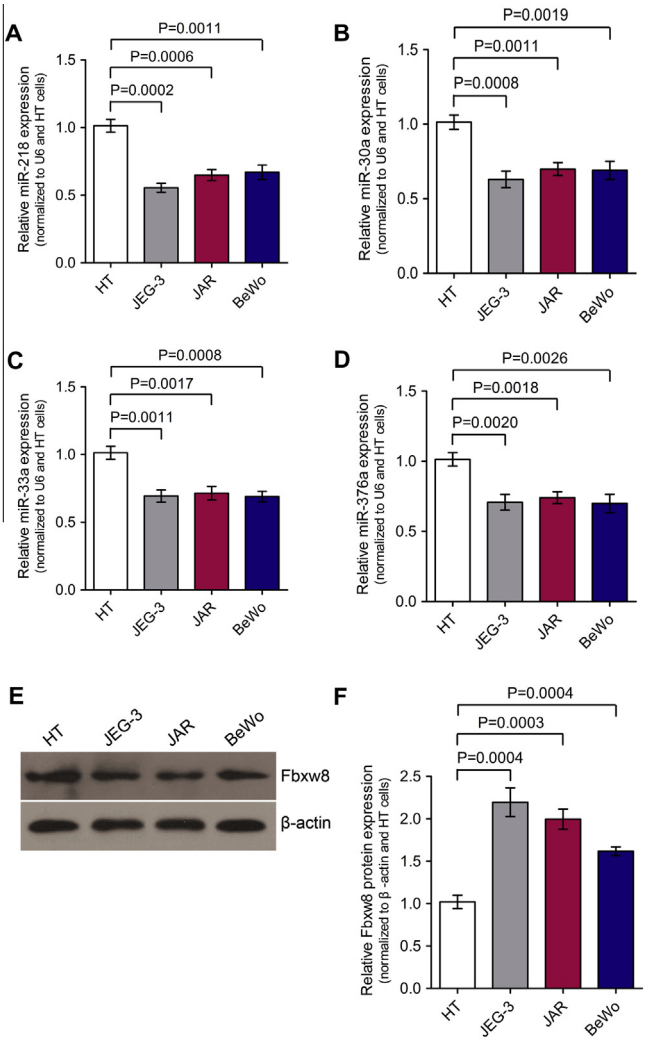

Fig. 1. The expression of miR-218 was negatively correlated with Fbxw8 protein levels in choriocarcinoma cell lines. The expression levels of miRNAs in choriocarcinoma cell lines and the human trophoblast (HT) cells were detected by qRT-PCR and normalized to that of U6. Results showed that the expression of miR-218 (A), miR-30a (B), miR-33a (C) and miR-376a (D) were significantly decreased in three choriocarcinoma cell lines compared with HT cells; (E) Increased expression of Fbxw8 was tested by Western blot in three choriocarcinoma cell lines compared with HT cells. (F) Band intensities were quantitated by Image-Pro Plus. The intensities of the bands corresponding to Fbxw8 were compared with those corresponding to β-actin. Figure is representative of three experiments with similar results. Values are mean ± SD.

2.6. MTT assay

Cell viability was evaluated using a modified MTT assay. The viability of JEG-3 cells treated with miR-218 and Fbxw8 ORF clone were assessed at five time points (on day 1, 2, 3, 4 and 5) after seeding 2×10^3 transfected cells/well into 96-well culture plates. Briefly, quantification of mitochondrial dehydrogenase activity was achieved via the enzymatic conversion of MTT [3-(4,5-dimethyldiazol-2-yl)-2,5- diphenyltetrazolium bromide; Sigma–Aldrich, MO, USA] to a colored formazan product. MTT (10 μl, 10 mg/ml) was added to the cells, incubated for 4 h, and the reaction was terminated by removal of the supernatant and addition of 100 μl DMSO to dissolve the formazan product. After 0.5 h, the optical density (OD) of each well was measured at 570 nm using a plate reader (ELx808 Bio-Tek Instruments, City, ST, USA).

2.7. Cell cycle analysis

JEG-3 cells were plated in 60-mm dishes (3.0×10^5 cells/dish). After 24 h of treatment with miR-218 and Fbxw8 ORF clone, cells were cultured for another 24 h. The cells were then fixed in 70% ethanol for 0.5 h. After washing with PBS, cells were incubated with RNase A (0.5 mg/ml) (Sigma) at 37 °C for 30 min. Finally, the cells were stained with Propidium iodide (PI) (50 μg/ml) and analyzed by fluorescence-activated cell sorter analysis (FACS) (BD, San Jose, CA, USA).

2.8. Statistical analysis

All data from 3 independent experiments were expressed as mean ± SD and processed using SPSS17.0 statistical software. The difference among the groups in these assays was estimated by Student's *t*-test. *P* value of < 0.05 was considered to be statistically significant.

3. Results

3.1. MiR-218 is frequently downregulated in choriocarcinoma cell lines

Targetscan, microRNA.org and Pictar [7,21,22] predicted that miR-30a, miR-33a, miR-376a and miR-218 could bind to and target the Fbxw8 3′ UTR. We performed SYBR green quantitative PCR analysis to quantify the expression of microRNAs in choriocarcinoma cell lines. As the results showed that miR-30a, miR-33a, miR-376a and miR-218 levels decreased in JEG-3, JAR, BeWo cells compared with HT cells. Fig. 1A showed the mean expression level of miR-218, which was significantly lower in JEG-3 cell lines than that of other choriocarcinoma cell lines. And in JEG-3 cell lines the expression of miR-218 was downregulated more than that of miR-30a, miR-33a or miR-376a after normalizing to HT cells (Fig. 1B–D). On the other hand, Western blot results showed that the Fbxw8 protein expression was up-regulated in the three choriocarcinoma cell lines compared with HT cells (Fig. 1E, F). These results indicated that higher expression levels of miR-218 were significantly associated with lower levels of Fbxw8 protein expression in the choriocarcinoma cell lines.

3.2. MiR-218 directly targets Fbxw8

To further study the relationship of miR-218 and Fbxw8 in JEG-3 cells, we transfected JEG-3 cells with miR-SCR and miR-218 mimics. Quantitative RT-PCR showed that, at 48 h after transfection, the

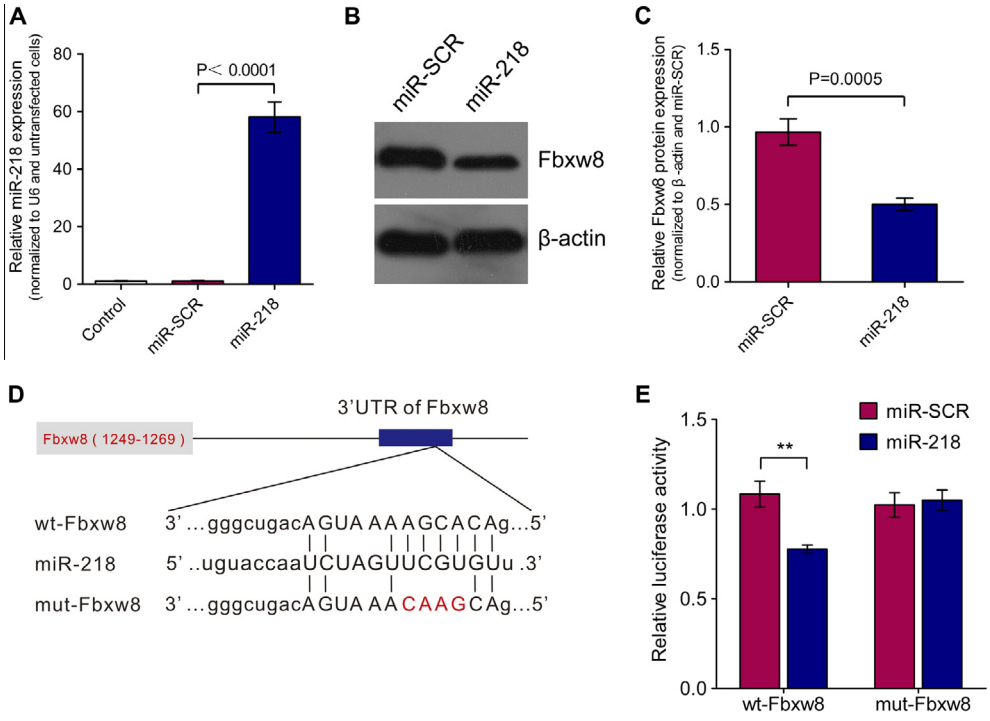

Fig. 2. miR-218 directly targets Fbxw8 by binding to its 3'UTR. (A) Up-regulated expression of miR-218 was tested by Quantitative RT-PCR in JEG-3 cell lines transfected with miR-218 mimics in comparison with the JEG-3 cell lines transfected with miR-SCR mimics. (B) Fbxw8 protein levels were detected by Western blot assay. The expression of Fbxw8 was decreased by miR-218 when compared to JEG-3 cell expressing miR-SCR. (C) Band intensities were quantitated by Image-Pro Plus. The intensities of the bands corresponding to Fbxw8 were compared to those corresponding to β-actin. (D) The predicted miR-218 binding site within Fbxw8 3′UTR and its mutated version by site mutagenesis are as shown. (E) Relative luciferase activities of Fbxw8 wild type (WT) and mutant (Mut) 3′-prime UTR regions were obtained by cotransfection of scrambled control miRNA or miR-218 precursor, and psiCHECK-2 plasmid; and calculated as the ratio of firefly/renilla activities in the cells and normalized to those of the control. The results were presented as mean ± SD from three independent experiments with each experiment in triplicate. Double asterisks indicate significant difference when compared to control. (**$P < 0.01$). Figure is representative of 3 experiments with similar results. Values are mean ± SD.

expression of miR-218 was significantly up-regulated as compared with miR-SCR (Fig. 2A). Moreover, we observed the enhanced miR-218 in JEG-3 cells significantly repressed Fbxw8 protein expression compared to cells transfected with scramble control by Western blot (Fig. 2B, C).

Additionally, To determine whether the 3′-UTR of Fbxw8 mRNA is a functional target of miR-218 in JEG-3 cells, we cloned the 3′UTR of Fbxw8 downstream to a luciferase reporter gene (wt-Fbxw8), its mutant version (mut-Fbxw8) by the binding site mutagenesis was also constructed (Fig. 2D). We co-transfected wt-Fbxw8 vector and miR-218 mimics or scramble control into JEG-3 cells. The luciferase activity of miR-218 transfected cells was significantly reduced compared to scramble control cells (Fig. 2E). Moreover, miR-218-mediated repression of luciferase activity was abolished by the mutant putative binding site (Fig. 2E).

3.3. Forced expression of Fbxw8 restored the effects of miR-218 inhibited JEG-3 cells proliferation

To validate if miR-218 regulates JEG-3 cells proliferation and cell cycle by targeting Fbxw8, we performed MTT and cell cycle assays by co-transfecting miR-218 mimics and Fbxw8 ORF clone into JEG-3 cells. MTT results showed that the forced expression of Fbxw8 restored significant inhibition on cell proliferation by miR-218 (Fig. 3A). FACS results showed that the percentage of cells at G2/M phase was increased by the treatment of miR-218, and reversed by the transfection of Fbxw8 ORF clone (Fig. 3B, C). Taken together, these results indicated that miR-218 could inhibit the proliferation of JEG-3 cells by targeting Fbxw8.

3.4. Forced expression of Fbxw8 restored the effects of miR-218 inhibited Fbxw8, cyclin A expression and induced p27 expression

As we showed above, miR-218 targeted Fbxw8 in JEG-3 cells. Therefore, we wondered whether over-expression of Fbxw8 was enough to reverse the expression of miR-218 inhibited or induced downstream molecules. Fbxw8 ORF clone was transfected into miR-218 mimics transfected cells. As shown in Fig. 4A–C, forced expression of Fbxw8 could restore the Fbxw8 and cyclin A protein expression inhibited by miR-218. Similarly, over-expression of Fbxw8 also restored miR-218-induced p27 expression in JEG-3 cells (Fig. 4A, D). These results indicated that miR-218 inhibited cyclin A and induced p27 signaling pathway by targeting Fbxw8.

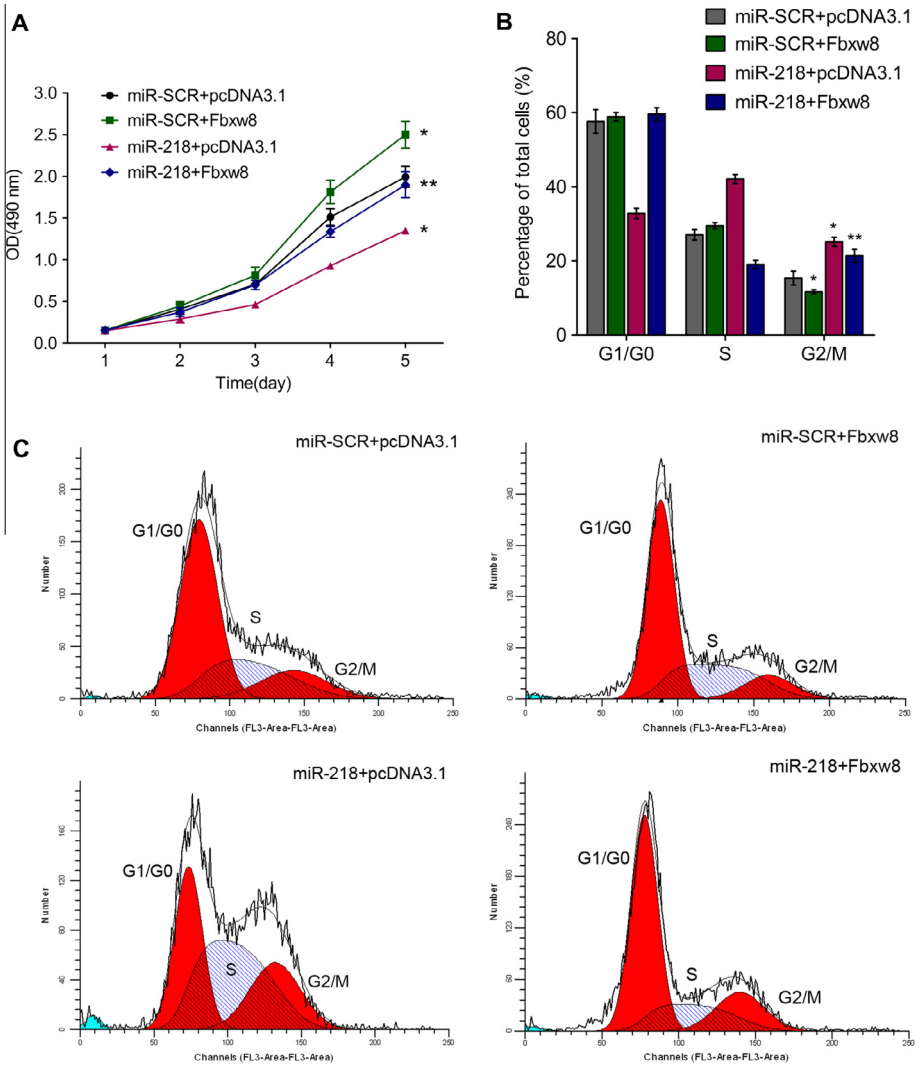

Fig. 3. Force expression of Fbxw8 restored the inhibitory effects of miR-218 mimics in proliferation and cell cycle of JEG-3 cell lines. (A) Cell growth of JEG-3 was measured by MTT assay. (B) Cell cycle measured using FCM analysis. (C) Percentage of cell cycle distribution. The asterisk indicates significant difference when miR-218 mimics or Fbxw8 compared with miR-SCR; while two asterisks indicate significant difference when miR-218 mimics + Fbxw8 compared with miR-218 mimics or Fbxw8. ($^{**}P < 0.01$) Figure is representative of 3 experiments with similar results. Values are mean ± SD.

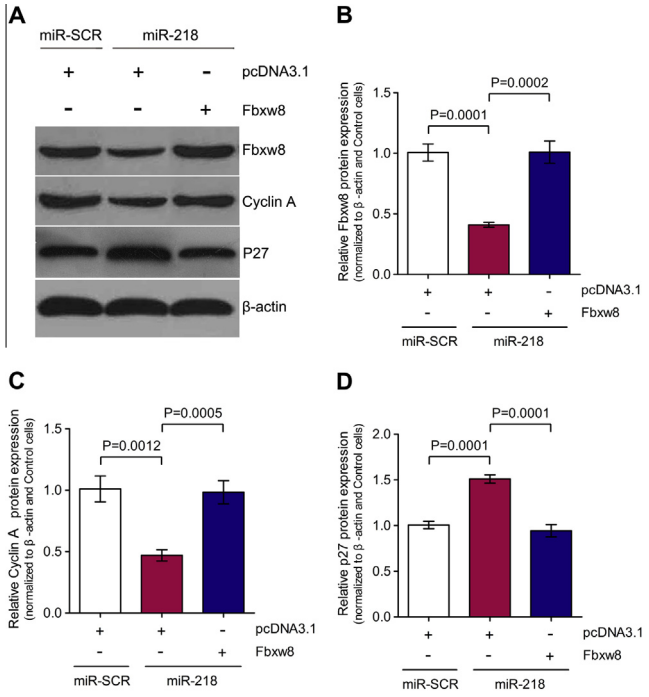

Fig. 4. Force expression of Fbxw8 restored the effects of miR-218 mimics in Fbxw8 pathway. JEG-3 cells treated by miR-218 mimics (50 nmol/L) were transfected with Fbxw8 plasmid at 2 μg per well. (A) After treatment for 48 h, the levels of specific proteins were analyzed by immunoblotting. Band intensities were quantitated by Image-Pro Plus. The intensities of the bands corresponding to Fbxw8, Cyclin D1, p27 were compared with those corresponding to β-actin. (B, C) Up-regulation of Fbxw8 reversed the inhibitory effect of miR-218 mimics in levels of Fbxw8 and Cyclin D1 protein expression. (D) Over-expression of Fbxw8 restored p27 protein expression induced by miR-218 mimics. Figure is representative of 3 experiments with similar results. Values are mean ± SD.

4. Discussion

In the present study, we tried to identify a novel miRNA which regulates the expression of Fbxw8, and evaluate its effects on the proliferation of JEG-3 cells. After the screening of miRNAs targeting the 3′-UTR of Fbxw8 mRNA (Fig. 1), miR-218 was predicted to be located in the 3′-UTR. To demonstrate that miR-218 directly targets the 3′-UTR, we analyzed the luciferase activity using the vector containing the two alleles. The luciferase vector with the mutant-type allele showed significantly less activity than that with the wild-type vector, as shown in Fig. 2. Overexpression of miR-218 decreased the luciferase activity, suggesting that miR-218 directly repressed Fbxw8 expression. Transfection with the miR-218 mimics significantly decreased Fbxw8 mRNA and protein levels in a precursor miRNA concentration dependent manner (Fig. 2). It was suggested that miR-218 suppresses the expression of the endogenous Fbxw8 protein by controlling the stability of Fbxw8 mRNA transcripts. Taking these observations into consideration, miR-218 acts as a repressive regulator as to Fbxw8.

MicroRNAs are newly identified modulators of many complicated signaling pathways involved in various human diseases [2,3]. It has been reported that antitumor drugs can change miRNA expression profiles in tumor cells, and that chemotherapy drug-resistant tumors are also correlated with miRNA expression level. Therefore, the variation in miRNA expression profiles may be one mecha nism by which drug treatment inhibits tumors [23–25]. Our results obtained from quantitative RT-PCR validated that miR-218, one of the potential microRNAs target the Fbxw8 3′UTR, was commonly down-regulated in JEG-3 cell lines (Fig. 1). These findings suggested that miR-218 was involved in the processes of the proliferation of JEG-3 cells. Other reports showed that miR-218 acts as tumor-suppressive miRNA in several kinds of tumors, including gastric cancer [26], lung cancer [27], cervical cancer [28], head and neck cancer [29] and bladder cancer [30]. Our findings highlighted the importance of miRNA as a potential tumor suppressor in a solid cancer.

Fbxw8 substrates include many proteins involved in regulating cell growth and differentiation, including cyclin A, p27, et al. [10,31]. Therefore, Fbxw8 regulated the cell cycle, cell growth and differentiation. There were evidences that Fbxw8 had a tumor inducer function; deletion or mutation of the Fbxw8 gene has been reported in many different types of tumors [10,31,32]. In this study, we raised the hypothesis that miR-218 might be involved in inhibiting Fbxw8 expression regulated JEG-3 cells proliferation process. Interestingly, the levels of miR-218 were negatively correlated with Fbxw8 protein levels in choriocarcinomas cell lines (Fig. 1). We up-regulated Fbxw8 expression in JEG-3 cells and found that Fbxw8 restored miR-218 inhibited cell proliferation and increased the percentage of cells at G2/M phase of the cell cycle (Fig. 3). Finally, we confirmed the link between miR-218 and Fbxw8, and found that miR-218 inhibited JEG-3 cell proliferation via targeting Fbxw8.

It was reported that Fbxw8 expression contributed to cancer cells proliferation through cyclin A and p27 pathway [32]. Cyclin A regulates G2/M progression in cancer cells and is overexpressed in various malignant neoplasms [33]. As a result, it is a potential target for cancer therapeutics [34]. Transcriptional regulation of cyclin A has been extensively studied and is well understood [35–37]. It is stimulated when, for example, various mitogenic signals activate the Ras/Raf/MEK/ERK (MAPK) cascade. After synthesis following MAPK cascade activation, cyclin A associates with CDK4/6, p21 Cip1 or p27 Kip1 [38,39]. The cyclin-dependent kinase inhibitor p27 is an important regulator of the cell cycle in mammalian cells, which negatively controls cell cycle progression by directly repressing cyclin/CDK2 complexes [29,40]. P27 plays a crucial role in the regulation of JEG-3 proliferation [41]. Our results confirmed a vital molecular relationship among miR-218, Fbxw8, cyclin A and p27. We showed that, at protein level, up-regulation of miR-218 expression in JEG-3 cells effectively suppressed Fbxw8 and cyclin A and induced p27 expression, and forced expression of Fbxw8 could reverse the expression of cyclin A and p27. It suggested a potential inverse relevance of miR-218 and Fbxw8 in JEG-3 and the main effect of Fbxw8 of the cells is autocrine affect, because the miR-218 down-regulated the level of the cellular Fbxw8 mRNA and protein. Additionally, these results suggested miR-218 might inhibit cyclin A and induce p27 expression via Fbxw8 signaling. These results verified that miR-218 inhibited the expression of cyclin A and p27 via Fbxw8 signaling. Taken together, these findings sufficiently consolidated that miR-218 played a suppressive role in cellular proliferation, at least, in part due to directly inhibiting Fbxw8 expression.

In conclusion, we newly described miR-218/Fbxw8 link and provided a potential mechanism for contribution to JEG-3 cells proliferation. As a result, restoration of miR-218 expression could have an important implication for the clinical management of JEG-3 cells. However, further studies are needed to determine the extent to which miR-218 regulate the growth of choriocarcinoma by targeting Fbxw8 in vitro.

References

[1] T. May, D.P. Goldstein, R.S. Berkowitz, Current chemotherapeutic management of patients with gestational trophoblastic neoplasia, Chemother. Res. Pract. 2011 (2011) 806256.
[2] V. Ambros, The functions of animal microRNAs, Nature 431 (2004) 350–355.
[3] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.

[4] D. Baek, J. Villen, C. Shin, F.D. Camargo, S.P. Gygi, D.P. Bartel, The impact of microRNAs on protein output, Nature 455 (2008) 64–71.
[5] Y. Tay, J. Rinn, P.P. Pandolfi, The multilayered complexity of ceRNA crosstalk and competition, Nature 505 (2014) 344–352.
[6] C.M. Croce, G.A. Calin, miRNAs, cancer, and stem cell division, Cell 122 (2005) 6–7.
[7] A. Esquela-Kerscher, F.J. Slack, Oncomirs-microRNAs with a role in cancer, Nat. Rev. Cancer 6 (2006) 259–269.
[8] M.V. Latronico, G. Condorelli, RNA silencing: small RNA-mediated posttranscriptional regulation of mRNA and the implications for heart electropathophysiology, J. Cardiovasc. Electrophysiol. 20 (2009) 230–237.
[9] A.K. Pandey, P. Agarwal, K. Kaur, M. Datta, MicroRNAs in diabetes: tiny players in big disease, Cell. Physiol. Biochem. 23 (2009) 221–232.
[10] T. Cardozo, M. Pagano, The SCF ubiquitin ligase: insights into a molecular machine, Nat. Rev. Mol. Cell. Biol. 5 (2004) 739–751.
[11] A. Hershko, A. Ciechanover, The ubiquitin system, Annu. Rev. Biochem. 67 (1998) 425–479.
[12] R.J. Deshaies, SCF and Cullin/Ring H2-based ubiquitin ligases, Annu. Rev. Cell. Dev. Biol. 15 (1999) 435–467.
[13] K.I. Nakayama, K. Nakayama, Ubiquitin ligases: cell-cycle control and cancer, Nat. Rev. Cancer 6 (2006) 369–381.
[14] D. Guardavaccaro, M. Pagano, Oncogenic aberrations of cullin-dependent ubiquitin ligases, Oncogene 23 (2004) 2037–2049.
[15] M.D. Petroski, R.J. Deshaies, Function and regulation of cullin-RING ubiquitin ligases, Nat. Rev. Mol. Cell. Biol. 6 (2005) 9–20.
[16] J. Jin, T. Cardozo, R.C. Lovering, S.J. Elledge, M. Pagano, J.W. Harper, Systematic analysis and nomenclature of mammalian F-box proteins, Genes Dev. 18 (2004) 2573–2580.
[17] T. Arai, J.S. Kasper, J.R. Skaar, S.H. Ali, C. Takahashi, J.A. DeCaprio, Targeted disruption of p185/Cul7 gene results in abnormal vascular morphogenesis, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 9855–9860.
[18] X. Xu, A. Sarikas, D.C. Dias-Santagata, G. Dolios, P.J. Lafontant, S.C. Tsai, W. Zhu, H. Nakajima, H.O. Nakajima, L.J. Field, R. Wang, Z.Q. Pan, The CUL7 E3 ubiquitin ligase targets insulin receptor substrate 1 for ubiquitin-dependent degradation, Mol. Cell 30 (2008) 403–414.
[19] H. Okabe, S.H. Lee, J. Phuchareon, D.G. Albertson, F. McCormick, O. Tetsu, A critical role for FBXW8 and MAPK in cyclin A degradation and cancer cell proliferation, PLoS One 1 (2006) e128.
[20] X. Wu, D.J. Li, M.M. Yuan, Y. Zhu, M.Y. Wang, The expression of CXCR4/CXCL12 in first-trimester human trophoblast cells, Biol. Reprod. 70 (2004) 1877–1885.
[21] H.W. Hwang, J.T. Mendel, MicroRNAs in cell proliferation, cell death, and tumorigenesis, Br. J. Cancer 94 (2006) 776–780.
[22] 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.
[23] M. Fabbri, MicroRNAs and cancer: towards a personalized medicine, Curr. Mol. Med. 13 (2013) 751–756.
[24] H. Li, B.B. Yang, Friend or foe: the role of microRNA in chemotherapy resistance, Acta Pharmacol. Sin. 2013 (34) (2013) 870–879.
[25] Q. Wu, Z. Yang, Y. Shi, D. Fan, MiRNAs in human cancers: the diagnostic and therapeutic implications, Curr. Pharm. Des. (2014) (Epub ahead of print).
[26] C. Gao, Z. Zhang, W. Liu, S. Xiao, W. Gu, H. Lu, Reduced microRNA-218 expression is associated with high nuclear factor kappa B activation in gastric cancer, Cancer 116 (2010) 41–49.
[27] M.R. Davidson, J.E. Larsen, I.A. Yang, N.K. Hayward, B.E. Clarke, E.E. Duhig, L.H. Passmore, R.V. Bowman, K.M. Fong, MicroRNA-218 is deleted and downregulated in lung squamous cell carcinoma, PLoS One 5 (2010) e12560.
[28] I. Martinez, A.S. Gardiner, K.F. Board, F.A. Monzon, R.P. Edwards, S.A. Khan, Human papillomavirus type 16 reduces the expression of microRNA-218 in cervical carcinoma cells, Oncogene 27 (2008) 2575–2582.
[29] A.I. Wald, E.E. Hoskins, S.I. Wells, R.L. Ferris, S.A. Khan, Alteration of microRNA profiles in squamous cell carcinoma of the head and neck cell lines by human papillomavirus, Head Neck 33 (2011) 504–512.
[30] S. Tatarano, T. Chiyomaru, K. Kawakami, H. Enokida, H. Yoshino, H. Hidaka, T. Yamasaki, K. Kawahara, K. Nishiyama, N. Seki, M. Nakagawa, MiR-218 on the genomic loss region of chromosome 4p15.31 functions as a tumor suppressor in bladder cancer, Int. J. Oncol. 39 (2011) 13–21.
[31] H. Wang, Y. Chen, P. Lin, L. Li, G. Zhou, G. Liu, C. Logsdon, J. Jin, J.L. Abbruzzese, T.H. Tan, H. Wang, The CUL7/F-box and WD repeat domain containing 8 (CUL7/Fbxw8) ubiquitin ligase promotes degradation of hematopoietic progenitor kinase 1, J. Biol. Chem. 289 (2014) 4009–4017.
[32] P. Lin, J. Fu, B. Zhao, F. Lin, H. Zou, L. Liu, C. Zhu, H. Wang, X. Yu, Fbxw8 is involved in the proliferation of human choriocarcinoma JEG-3 cells, Mol. Biol. Rep. 38 (3) (2011) 1741–1747.
[33] M. Malumbres, M. Barbacid, To cycle or not to cycle: a critical decision in cancer, Nat. Rev. Cancer 1 (2001) 222–231.
[34] Q. Yu, Y. Geng, P. Sicinski, Specific protection against breast cancers by cyclin A ablation, Nature 411 (2001) 1017–1021.
[35] C.J. Sherr, J.M. Roberts, Living with or without cyclins and cyclin dependent kinases, Genes Dev. 18 (2004) 2699–2711.
[36] O. Tetsu, F. McCormick, b-Catenin regulates expression of cyclin A in colon carcinoma cells, Nature 398 (1999) 422–426.
[37] O. Tetsu, F. McCormick, Proliferation of cancer cells despite CDK2 inhibition, Cancer Cell 3 (2003) 233–245.
[38] J. LaBaer, M.D. Garrett, L.F. Stevenson, J.M. Slingerland, C. Sandhu, et al., New functional activities for the p21 family of CDK inhibitors, Genes Dev. 11 (1997) 847–862.
[39] M. Cheng, P. Olivier, J.A. Diehl, M. Fero, M.F. Roussel, et al., The p21(Cip1) and p27(Kip1) CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts, EMBO J. 18 (1999) 1571–1583.
[40] B. Vogelstein, K.W. Kinzler, Cancer genes and the pathways they control, Nat. Med. 10 (2004) 789–799.
[41] S.T. Kim, S.K. Lee, M.C. Gye, The expression of Cdk inhibitors p27kip1 and p57kip2 in mouse placenta and human choriocarcinoma JEG-3 cells, Placenta 26 (2005) 73–80.