MicroRNA-302a upregulation mediates chemo-resistance in prostate cancer cells

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Abstract. MicroRNAs (miRNAs) are post-transcriptional regulators that mediate the initiation and progression of human cancer. Growing evidence suggests that deregulation of miRNA expression levels underlies chemo-resistance. To investigate whether miRNA-302a (miR-302a) is involved in mediating chemo-resistance to paclitaxel in prostate cancer, a series of *in vitro* analyses were performed in paclitaxel-resistant prostate cancer PC-3PR cells and non-resistant prostate cancer PC-3 cells. It was demonstrated that the expression of miR-302a was upregulated in PC-3PR cells. Notably, ectopic expression of miR-302a also increased resistance to paclitaxel in wild-type PC-3 cells. By contrast, silencing of miR-302a in PC-3PR cells sensitized the cells to paclitaxel. Gene and protein expression analyses suggested that the miR-302a target gene breast cancer resistance protein (BCRP) may mediate chemo-resistance to paclitaxel in PC-3PR cells. In conclusion, the data suggested that elevated miR-302a levels, in part, mediate sensitivity to paclitaxel in prostate cancer through the aberrant regulation of its downstream targets, AOF2, BCRP and permeability glycoprotein 1. These data have implications for the development of novel therapeutics in prostate cancer that may improve sensitivity to chemotherapeutics.

Introduction

Prostate cancer is the most common cancer to affect men and is the second leading cause of cancer-associated mortality in men (1). Present guidelines suggest that treatment decisions should be made based on tumour stage: Surgery and radiation therapy are common methods used to treat early, localized cancer (2). In addition, paclitaxel (a taxane antimitotic agent) is used as a standard first-line chemotherapeutic agent (3,4). Despite these combinatory approaches, a number of patients relapse following primary treatment, and a substantial proportion of men with metastatic, castration-resistant, prostate cancer develop resistance towards paclitaxel and eventually succumb to the disease. The mechanisms underlying acquired paclitaxel resistance are hypothesised to involve different β-tubulin isoforms, mutations of multi-drug resistance-associated genes and/or aberrant activation of drug efflux pumps. Despite advances over previous years in the understanding of the molecular basis of resistance, treating patients with paclitaxel-resistant prostate cancer remains a clinical challenge.

MicroRNAs (miRNAs) are short, highly conserved, small non-coding RNA molecules that downregulate gene expression at the post-transcriptional level by binding to the 3'-untranslated regions (UTR) of mRNAs (5,6). miRNAs are involved in various biological processes, including tumour proliferation, promotion, invasion, angiogenesis and drug resistance (7,8). Numerous miRNAs have been demonstrated to mediate chemo-resistance in prostate cancer, including miR-148a, miR-200c, miR-205, miR-21, miR-31, miR-34 and miR-375 (2,8-12). The miR-302 miRNA family regulates cell proliferation and differentiation, and the upregulation of miR-302 has been suggested to lead to drug resistance (13-17); however, the involvement of miR-302 in the chemotherapeutic response in prostate cancer is unclear. A previous study of head and neck cancer indicated that the miR-302-mediated regulation of lysine demethylase 1B (AOF1), lysine demethylase 1A (AOF2) and DNA methyltransferase 1 (DNMT1) is associated with chemo-resistance (17). These data suggest that miR-302 may be involved in chemo-resistance towards paclitaxel in patients with prostate cancer.

The present study aimed to characterize in more detail the molecular mechanisms that enhance chemo-resistance in patients with prostate cancer. By performing a series of *in vitro* assays, increased expression levels of miR-302a in the human PC-3PR prostate cancer cell line were observed. Increased miR-302a levels significantly increased the chemo-resistance of prostate cancer cells exposed to paclitaxel. Additional analysis demonstrated that miR-302a may confer chemo-resistance

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by decreasing the expression levels of breast cancer resistance protein (BCRP), permeability glycoprotein 1 (P-glycoprotein) and AOF2.

Materials and methods

Cell culture and chemicals. The human prostate cancer PC-3 cell line [American Type Culture Collection CRL1435™; paclitaxel half maximal inhibitory concentration (IC_{50})=2.30 nM] and the paclitaxel-resistant PC-3 cells (PC-3PR; paclitaxel IC_{50}=97.87 nM) were purchased from the Guangxi Nanning Longevity Biological Technology Co., Ltd. (Guangxi, China). The cells were cultured in RPMI-1640 media (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% foetal bovine serum and 10% penicillin/streptomycin (Gibco; Thermo Fisher Scientific, Inc.) and maintained at 37˚C in a humidified incubator with a 5% CO_2 atmosphere. PC-3 and PC-3PR cells were seeded in 6-well plates ($3x10^5$ per well) and were incubated prior to exposure to paclitaxel (PC-3: 2.3 nM and PC-3PR: 97.8 nM, Cayman Chemical Company, Ann Arbor, MI, USA) for 48 h at 37˚C in a humidified incubator with a 5% CO_2 atmosphere. The cells were then harvested by 0.25% trypsinisation (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) for subsequent analyses.

Isolation of total RNA and reverse transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from cells harvested by 0.25% trypsinisation, according to the TRIzol (Gibco; Thermo Fisher Scientific, Inc.) manufacturer's protocol. To detect miR-302a, AOF2, BCRP and P-glycoprotein, 1 μg total RNA per sample was converted to cDNA using a cDNA Synthesis kit (Invitrogen; Thermo Fisher Scientific, Inc.). The cDNA was then amplified and detected using a SYBR-Green PCR kit (Qiagen, Duesseldorf, Germany). β-actin was used as an endogenous control. For detection of mRNAs, cDNA products were synthesized using the miScript Reverse Transcription kit (Qiagen GmbH, Hilden, Germany). Primers specific for miR-302a and the endogenous control U6 were purchased from Qiagen (Table I). RT-qPCR was performed using the miScript SYBR Green PCR kit (Qiagen GmbH). All reactions were performed in triplicate on a Bio-Rad C1000 thermal cycler (CFX-96 real-time PCR detection systems; Bio-Rad Laboratories, Inc., Hercules, CA, USA). The following thermocycling conditions were used for the qPCR: 30 sec at 95˚C; 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. Fold changes in miRNA or mRNA expression were calculated using the $2^{-\Delta\Delta Cq}$ method (18).

Transfection with miR-302a mimics, inhibitors and negative controls. The miRNA mimics and inhibitors were transiently transfected into PC-3 and PC-3PR cells using Lipofectamine® RNAiMAX Reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The miR-302a mimic (5'-ACUUAAACGUGGAUGUACUUGCU-3'), inhibitor (5'-UCGUUCAUGUAGGUGCAAAUUCA-3'), and the respective negative controls (NCs) were obtained from Ambion; Thermo Fisher Scientific, Inc. The final concentration of miR-302a mimics, inhibitors and the NC in the transfection system was 200 nM. Transfection efficiency was assessed by fluorescence microscopy (magnification, x40) and RT-qPCR, as mentioned above after 48 h. The cells were collected 48 h following transfection for flow cytometry, western blot analysis and RT-qPCR.

IC_{50} values assay. PC-3 (0, 1, 2, 4, 6 and 8 nM) and PC-3PR (0, 10, 20, 40, 80 and 120 nM) cells were treated with paclitaxel in various concentrations. Following incubation of the cells ($5x10^5$ cells/well) in a 96-well plate at 37˚C for 48 h with paclitaxel, the cell culture medium was removed. A total of 10 μl Cell-Counting Kit 8 (CCK-8) solution (Dojindo Molecular Technologies, Inc.) dissolved in 100 μl medium (as described above), added to each well and incubated at 37˚C for 1 h in the dark. Then, the optical density (OD) of each well at a wavelength of 450 nm was measured using a microplate reader. The IC_{50} was calculated using nonlinear regression modelling of the exponential data (SPSS Software v22.0; IBM Corp., Armonk, NY, USA).

Cell proliferation assay. Cell proliferation was evaluated using a CCK-8 assay, according to the manufacturer's protocol (Dojindo Molecular Technologies, Inc.). PC-3 and PC-3R cells were seeded in 96-well plates (2,000 cells/well) and 10 μl CCK-8 solution was added to each well at 48 h following transfection. The cells were then incubated at 37˚C for an additional 2 h. The absorbance values of the cultures were then measured at 450 nm using a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, Inc.).

Apoptosis assay. Apoptosis was assessed using an Annexin V-FITC Apoptosis Detection kit (556547; BD Pharmingen; BD Biosciences, San Jose, CA, USA). Cells were treated with miR-302a mimics, inhibitors or NCs, along with paclitaxel (PC-3=2.3 nM; PC-3PR=97.8 nM), then harvested in the logarithmic growth phase and washed twice with PBS. Following this, $1x10^6$ cells, which had been washed twice with PBS prior to resuspension in 1X Annexin V Binding Buffer (BD Pharmingen; BD Biosciences), were incubated with Annexin V-PE (5 μl) and 7-aminoactinomycin (5 μl) on ice for 30 min to stain the cells, followed by the addition of 400 μl 1X binding buffer to each sample. Stained cells were measured by FACSCalibur flow cytometry using Cell Quest Pro software (version 5.1) (both from BD Biosciences). Data were then analysed using FlowJo v.10 software (FlowJo LLC, Ashland, OR, USA).

Cell cycle assay. Cells were collected 48 h after transfection with the miR-302a inhibitors, mimics or NCs and stained with propidium iodide/RNase Staining Buffer (BD Pharmingen; BD Biosciences), according to the manufacturer's protocol. Stained cells were measured by flow cytometry on a FACSCalibur instrument using Cell Quest Pro Software (version 5.1) (both from BD Biosciences). The data ware analysed using FlowJo10 software (version 10.4.2; FlowJo LLC).

Western blot analysis. Cells were lysed in radioimmunoprecipitation assay lysis buffer (1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS in PBS) containing a Complete Protease Inhibitor Cocktail (Roche Diagnostics, Indianapolis, IN, USA). Protein concentration was determined using a

Table I. Primers used for reverse transcription quantitative polymerase chain reaction analysis.

Name	Forward	Reverse
β-actin	5'-AGCGAGCATCCCCCAAAGTT-3'	5'-GGGCACGAAGGCTCATCATT-3'
BCRP	5'-CAGGTGGAGGCAAATCTTCG-3'	5'-AGTTGTTGCAAGCCGAAGAG-3'
AOF2	5'-TTTGATCGGGTGTTCTGGGA-3'	5'-ATCGGCCAACAATCACATCG-3'
P-glycoprotein	5'-GAGCCTACTTGGTGGCACAT-3'	5'-TCCTTCCAATGTGTTCGGCA-3'
U6	5'-CGCTTCGGCAGCACATATAC-3'	5'-AAATATGGAACGCTTCACGA-3'
miR-302	5'-TGCGCTAAGTGCTTCCATGTTTT-3'	
miR-302 loop primer	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATT CGCACTGGATACGACTCACCAAA-3'	
miRNA universal primer	5'-CCAGTGCAGGGTCCGAGGTATT-3'	

BCRP, breast cancer resistance protein; AOF2, lysine demethylase 1A; P-glycoprotein, permeability glycoprotein 1; miR, microRNA.

Bio-Rad DC protein assay (Bio-Rad Laboratories, Inc.). Total proteins (30 μg) from the cell lysate were separated in 12% gel by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was then blocked in 5% non-fat milk in PBS overnight at 4˚C and incubated with primary antibodies at 37˚C for 2 h. Following washing three times with PBS + 0.05% Tween-20 for 30 min, the membrane was incubated with a 1:3,000 dilution of the secondary goat anti-mouse antibody (Biotinylated; BA-9200-1.5; Vector Laboratories, Inc., Burlingame, CA, USA) in TBS-0.05% Tween-20 for 1 h at room temperature. Following additional washes (three times), the proteins of interest were detected using a Chemiluminescent Horseradish peroxidase Antibody Detection kit (Denville Scientific, South Plainfield, NJ, USA) and the signals were captured using an electrochemiluminescent system (PerkinElmer, Inc., Waltham, MA, USA). The anti-cyclin-dependent kinase inhibitor 1 (p21) polyclonal antibody was used at a 1:1,000 dilution (cat. no. SAB4500065; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), the anti-β-actin antibody was used at a 1:5,000 dilution (cat. no. 3700; Cell Signaling Technology, Inc., Danvers, MA, USA), the anti-B-cell lymphoma-2 (Bcl-2)-associated X protein (Bax) protein antibody was used at a 1:1,000 dilution (cat. no. 2772; Cell Signaling Technology, Inc.), the anti-BCRP protein antibody was used at a 1:1,000 dilution (cat. no. 4477; Cell Signaling Technology, Inc.), the anti-Bcl-2 protein antibody was used at a 1:1,000 dilution (#15071, Cell Signaling Technology) and the anti-mouse IgG (H+L) antibody was used at a 1:1,000 dilution (cat. no. 14709; Cell Signaling Technology, Inc.).

TargetScan analysis. The 3'-UTR segments of AOF2, BCRP and P-glycoprotein were predicted to interact with miR-302a using TargetScan software (https://www. targetscan.org; release 7.2; accessed March 2018).

Statistical analysis. Data are presented as the means ± standard deviation. Student's t-tests were performed in Microsoft Excel (version 15.00; Microsoft Corporation, Redmond, WA, USA) to perform comparisons between the two groups. P<0.05 was considered to indicate a statistically significant difference.

Results

miR-302a is upregulated in prostate cancer cells and underlies paclitaxel resistance. The expression of miR-302a was first analysed in non-resistant prostate cancer PC-3 cells (Fig. 1A) and paclitaxel-resistant prostate cancer PC3-PR cells (Fig. 1B), and it was identified that miR-302a was significantly upregulated (114%) in the PC3-PR cells compared with the PC-3 cells (Fig. 1C). A CCK-8 assay of miR-302a mimic- or miR-302a inhibitor-transfected PC-3 and PC3-PR cells was then performed to determine whether miR-302a was associated with proliferation in prostate cancer cells. As indicated in Fig. 2A, in PC-3 cells transfected with miRNA NC, the IC_{30} and IC_{50} values for paclitaxel (treated with 0, 1, 2, 4, 6 or 8 nM for 48 h at 37˚C) were 0.70 and 3.20 nM, respectively, while in PC-3 cells transfected with the miR-302a mimic the IC_{30} and IC_{50} values for paclitaxel (treated with 0, 1, 2, 4, 6 or 8 nM for 48 h at 37˚C) were 1.58 and 6.12 nM, respectively. Conversely, in PC3-PR cells treated with miRNA NC, the IC_{30} and IC_{50} values for paclitaxel (treated with 0, 10, 20, 40, 80 or 120 nM for 48 h at 37˚C) were 64.80 and 91.44 nM, respectively, whereas in the PC3-PR cells treated with the miR-302a inhibitor, the IC_{30} and IC_{50} values for paclitaxel (treated with 0, 10, 20, 40, 80 or 120 nM for 48 h at 37˚C) were 55.50 and 79.64 nM, respectively. These data indicate that high levels of expression of miR-302a may enhance drug resistance in prostate cancer cells.

High miR-302a levels inhibit apoptosis in paclitaxel-resistant cells. Flow cytometry was used to quantify apoptosis in the prostate cancer cell lines treated with paclitaxel. It was identified that the apoptosis fraction in PC-3 cells transfected with the miR-302a mimic was significantly decreased compared with that of PC-3PR cells, whereas the apoptosis fraction in PC-3PR cells transfected with the miR-302a inhibitor was significantly increased compared with PC-3PR cells transfected with the NC (P<0.001) (Fig. 2B and C). These data indicate that high levels of miR-302a inhibit apoptosis.

Effect of miR-302a on paclitaxel-induced G2/M arrest. Flow cytometry was used to assess the cell cycle profile in the 2 prostate cancer cell lines. Compared with the PC-3 cells transfected with the miRNA NC, the proportion of cells in the G1/G0 phase

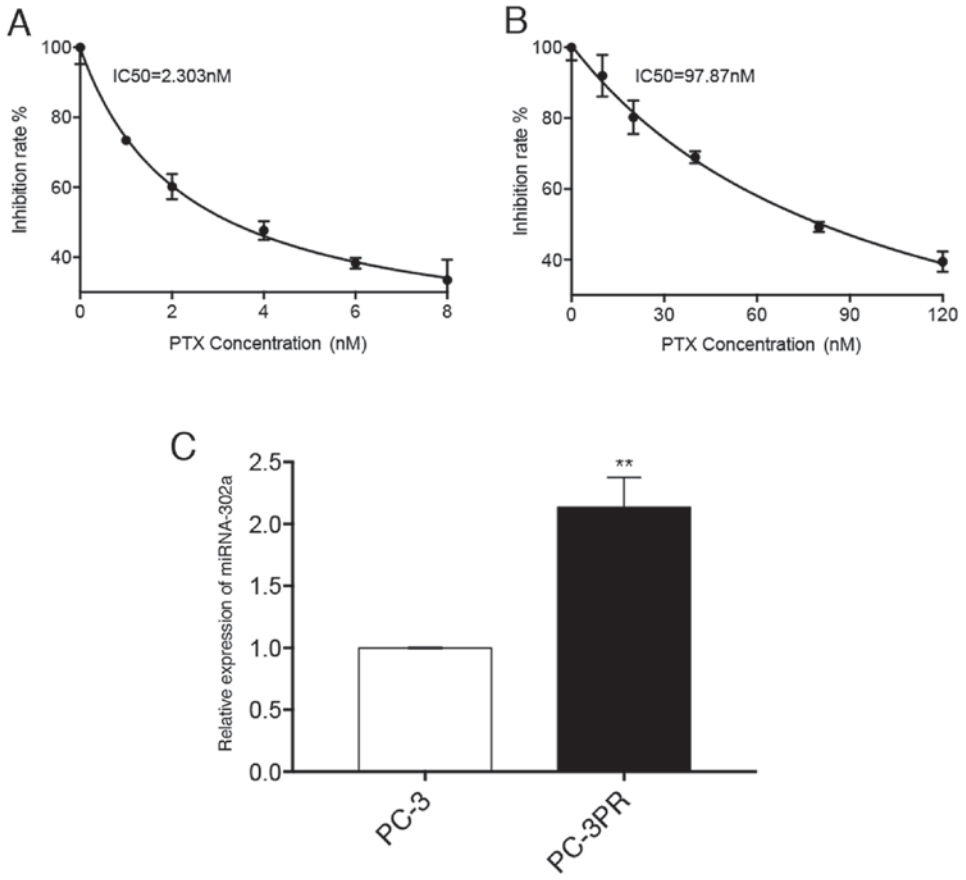

Figure 1. Difference in miR-302a expression levels between PC-3 and PC-3PR cells. (A) PC-3 cells were treated with different concentrations (0, 1, 2, 4, 6 and 8 nM) of PTX for 48 h. (B) PC-3PR cells were treated with different concentrations (0, 10, 20, 40, 80 and 120 nM) of PTX for 48 h. Inhibition of cell proliferation was assessed using the CCK-8 assay. (C) The expression levels of miR-302a were assessed by reverse transcription quantitative polymerase chain reaction. **P<0.01 vs. PC-3. miR, microRNA; PTX, paclitaxel.

in PC-3 cells transfected with the miR-302a mimics was significantly increased, the proportion of G2/M cells was significantly decreased and the proportion of cells in S phase did not change (Fig. 2D and E). In PC-3PR cells transfected with the miR-302a inhibitor, the proportion of cells in the G1/G0 phase was significantly decreased, and the proportion of cells in the G2/M phase and S phase cells were significantly increased compared with the PC-3PR cells transfected with the miRNA NC. These data indicate that high levels of miR-302a expression attenuate cell cycle arrest at G2/M stage.

High levels of miR-302a promote AOF2, BCRP and P-glycoprotein expression in paclitaxel-resistant cells. To determine the effect of miR-302a expression levels on paclitaxel-induced downstream gene expression, miRNA target prediction algorithms (http://targetscan.org) were used to screen miR-302a target genes, and AOF2, BCRP and P-glycoprotein were identified as tentative targets of miR-302a (Fig. 3A-C). RT-qPCR was then used to quantify the relative expression of miR-302a, AOF2, BCRP and P-glycoprotein. As indicated in Fig. 3D-G, compared with non-resistant prostate cancer cells (PC-3 cells transfected with the miRNA NC), the levels of miR-302a, AOF2, BCRP and P-glycoprotein were significantly upregulated in PC-3 cells transfected with miR-302a, and in PC-3PR cells transfected with the miRNA NC. The opposite effect was observed in PC-3PR cells transfected with the miR-302a inhibitor. These results suggest that miR-302a increases the expression of downstream targets AOF2, BCRP and P-glycoprotein.

miR-302a regulates apoptosis and cycle-associated proteins in paclitaxel-resistant cells. The effects of miR-302a on the paclitaxel-induced expression of BCRP, the apoptosis-associated proteins Bax and Bcl-2 and the cell cycle regulator p21 were then assessed in prostate cancer cells. As indicated in Fig. 4, compared with non-resistant prostate cancer cells (PC-3 cells transfected with the miRNA NC), the expression of BCRP and Bcl-2 were significantly upregulated at the protein level in PC-3 cells transfected with miR-302a and in resistant PC-3PR cells transfected with the miRNA NC, whereas the expression levels of Bax and p21 were significantly downregulated. The opposite effect was observed in the PC-3PR cells transfected with the miR-302a inhibitor. These data support the hypothesis that high levels of miR-302a increase the expression of BCRP and Bcl-2 and inhibit the expression of the pro-apoptotic protein Bax and the cell cycle regulator p21.

Discussion

Paclitaxel is a common chemotherapeutic agent that is efficacious in a number of types of cancer, but in certain patients, its efficacy is limited due to the development

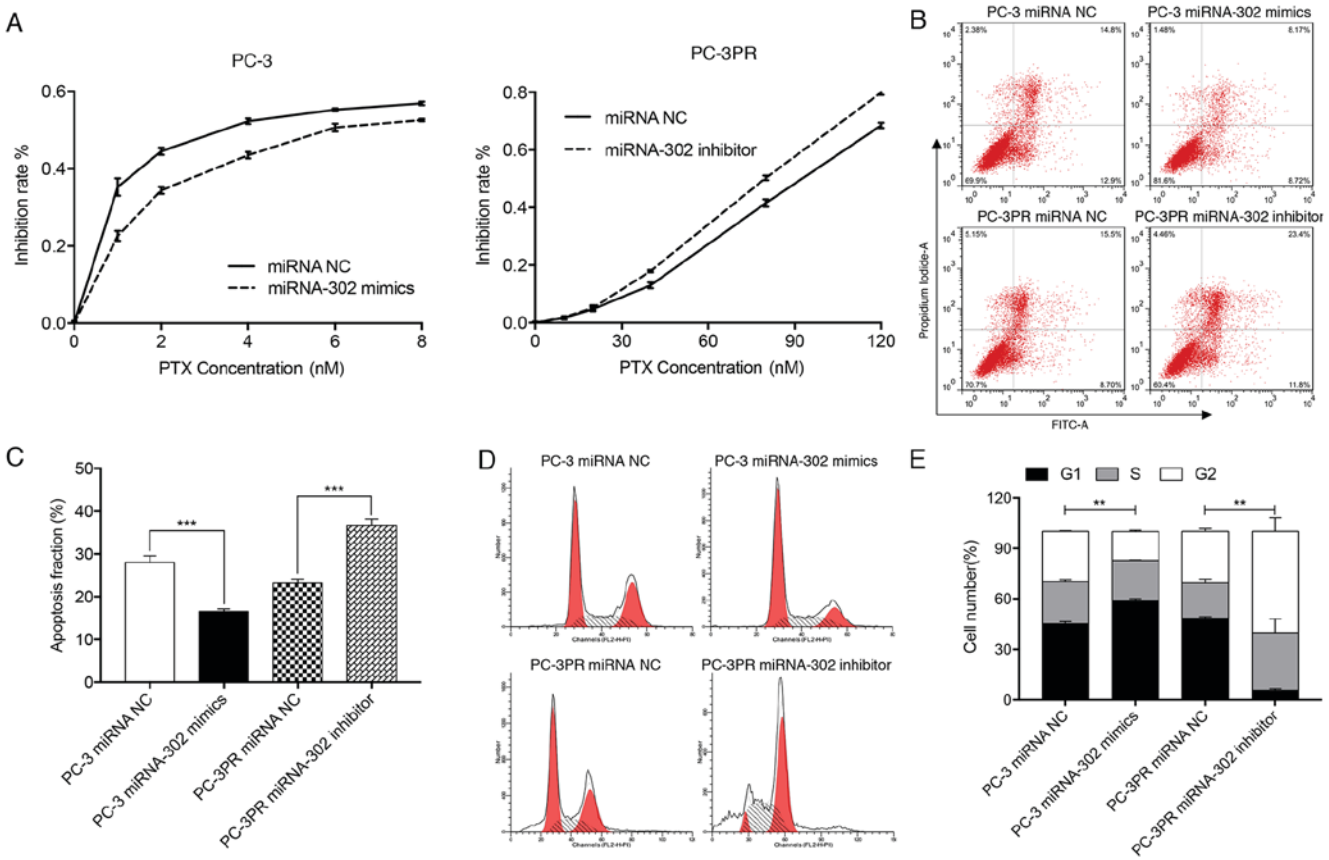

Figure 2. Effect of miR-302a on apoptosis, cell cycle and chemotherapy sensitivity in PC-3 and PC-3PR cells. (A) Inhibition of cell proliferation was assessed using a Cell Counting Kit-8 assay. PC-3 cells were transfected with a miR-302a or NC mimics in the presence of PTX (2.3 nM) for 48 h; PC-3PR cells were transfected with the miR-302a or NC inhibitors in the presence of PTX (97.8 nM) for 48 h. (B) Cells were stained with Annexin V-FITC and propidium iodide, and then assessed by flow cytometry. (C) Quantification of flow cytometry data. (D) Cells were stained with PI/RNase staining buffer. (E) Quantification of cell cycle analysis data. **P<0.01 and ***P<0.001. miRNA, microRNA; NC, negative control; FITC, fluorescein isothiocyanate; PTX, paclitaxel.

of drug resistance. Therefore, it is critical to determine optimal chemotherapeutic protocols and identify predictive markers of resistance to assist in stratifying patients at risk. Increasing evidence suggests that various miRNAs are involved in the development of chemo-resistance. miRNA screening may be useful to identify the subgroups of patients who are paclitaxel-resistant and determine the molecular mechanisms of chemo-resistance. By directly targeting protein-coding genes, miRNAs may inhibit genes that are required for paclitaxel-induced apoptosis, cell cycle arrest or the signalling pathways that render cells resistant to therapy. Numerous miRNAs have been implicated in controlling chemo-resistance in prostate cancer. The present study addressed the effects of miR-302a on paclitaxel resistance and its downstream targets using *in vitro* assays.

miRNAs are the most commonly studied class of non-coding RNAs (~22 nucleotides); these RNAs cause post-transcriptional gene silencing by regulating the translation of mRNAs into proteins. The miR-302/367 cluster is formed from 4 highly homologous miRNAs, miR-302b, miR-302c, miR-302a, miR-302d, and miR-367, in a 5'-to-3' direction (15). The present study demonstrated that the upregulation of miR-302a leads to chemo-resistance and tumour progression; however, this result contradicts other studies indicating that in other tumour types it targets key oncogenes mediating chemo-sensitivity (19,20). Previous studies have revealed that miR-302/367 is involved in maintaining stemness and reprogramming somatic cells into induced pluripotent stem cells (13,14,21,22). Other studies have suggested that miR-302 maintains the tumorigenesis of human pluripotent stem cells by inhibiting cyclin dependent kinase 4/6 (CDK4/6) and CDK2 (15,23).

miR-302b mediates cell proliferation by inhibiting the epidermal growth factor receptor/RAC-beta serine/threonine protein kinase/G1/S-specific cyclin-D1 signalling pathway in hepatocellular carcinoma cells (24,25). A previous study in head and neck cancer demonstrated that miR-302 was associated with chemo-resistance by regulating AOF1/AOF2/DNMT1 (17). These data suggest that chemo-resistance in prostate cancer may also be associated with changes in the expression of these candidate genes. Therefore, RT-qPCR and western blot analysis of a sub-set of these genes in paclitaxel resistant and non-resistant prostate cancer cell lines was performed in the present study, and it was confirmed that there was an association between elevated miR-302a expression levels and increased expression of AOF2, BCRP, P-glycoprotein and Bcl-2, along with decreased levels of Bax and p21 expression.

Overexpression of AOF2, also known as lysine-specific histone demethylase 1A, correlates with poor survival and castration-resistant prostate cancer (26). Conversely, AOF2 inhibition decreases v-myc avian myelocytomatosis viral oncogene homolog expression in poorly differentiated prostate cancer cell lines and exhibits a therapeutic benefit in paclitaxel-resistant

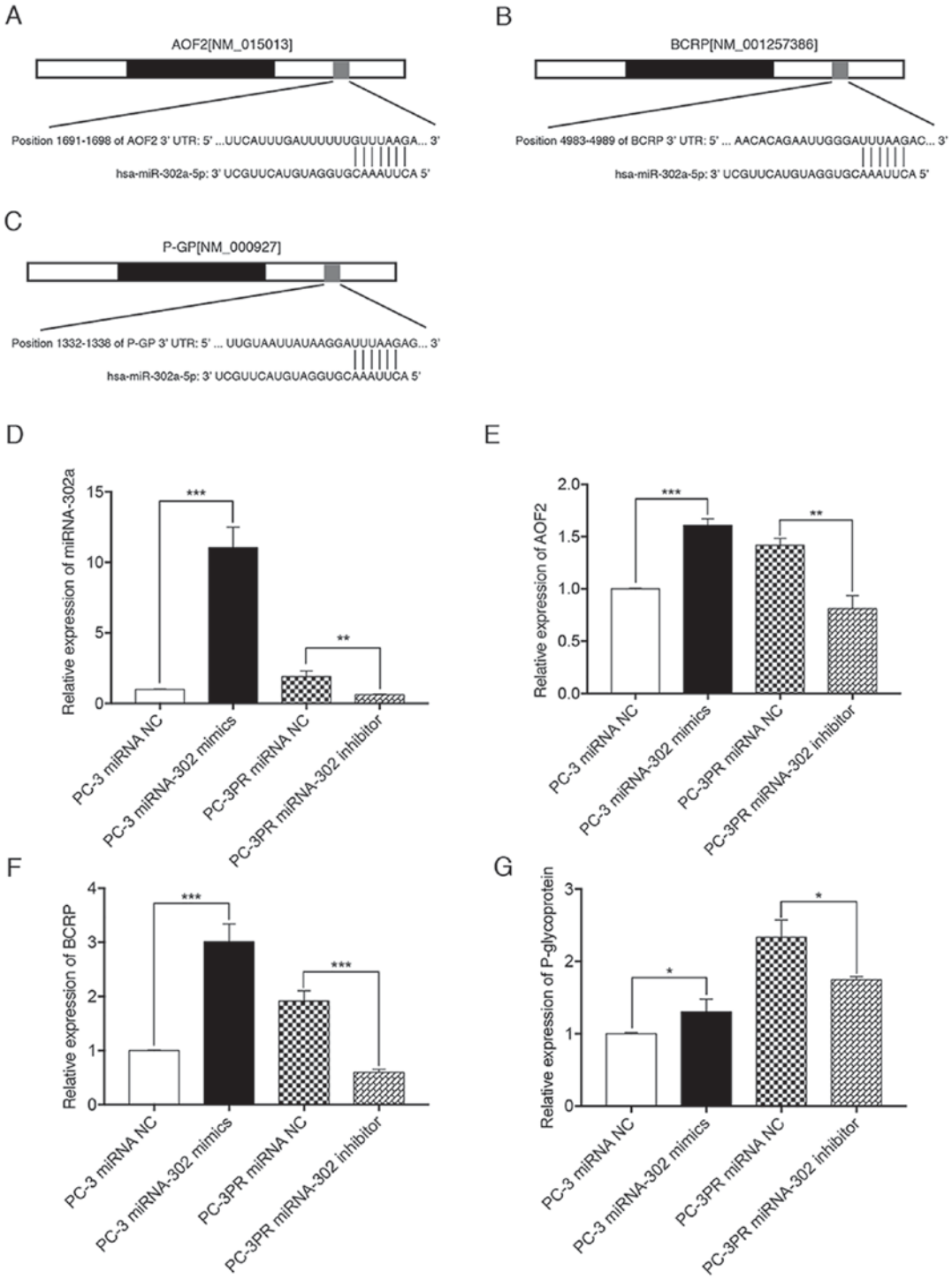

Figure 3. miR-302a increases the expression of downstream genes, AOF2, BCRP and P-glycoprotein. PC-3 cells were transfected with the miR-302a or NC mimics in the presence of paclitaxel (2.3 nM) for 48 h. PC-3PR cells were transfected with the miR-302a inhibitor or NC inhibitors in the presence of paclitaxel (97.8 nM) for 48 h. Binding sites of miR-302a in 3'-UTR of human (A) AOF2, (B) BCRP and (C) P-glycoprotein mRNA were predicted with miRNA target prediction algorithms. The expression levels of (D) miR-302a, (E) AOF2, (F) BCRP and (G) P-glycoprotein were assessed by qPCR. *P<0.05, **P<0.01 and ***P<0.01. miRNA, microRNA; NC, negative control; AOF2, lysine demethylase 1A; BCRP, breast cancer resistance protein; P-glycoprotein, permeability glycoprotein 1; UTR, untranslated region; hsa, *Homo sapiens*.

prostate cancer (27). BCRP and P-glycoprotein are drug efflux transporters that belong to the adenosine 5'-triphosphate binding cassette family of proteins. BCRP and P-glycoprotein are located in the apical membrane of epithelial cells, where they transport drug substrates including paclitaxel out of cancer cells (28-30). Proteins of the Bcl-2 family, particularly Bcl-2 and Bax, are also involved in mitochondria-mediated apoptotic pathways (31). In terms of cell-cycle kinetics, the kinase inhibitor protein p21 binds to the CDK4 or 6 complex to inhibit progression through the G1 phase of the cell cycle (32).

In summary, the data from the present study suggested that increased miR-302a expression serves an important role in mediating resistance to paclitaxel in prostate cancer cells. AOF2, BCRP and P-glycoprotein are all miR-302a targets

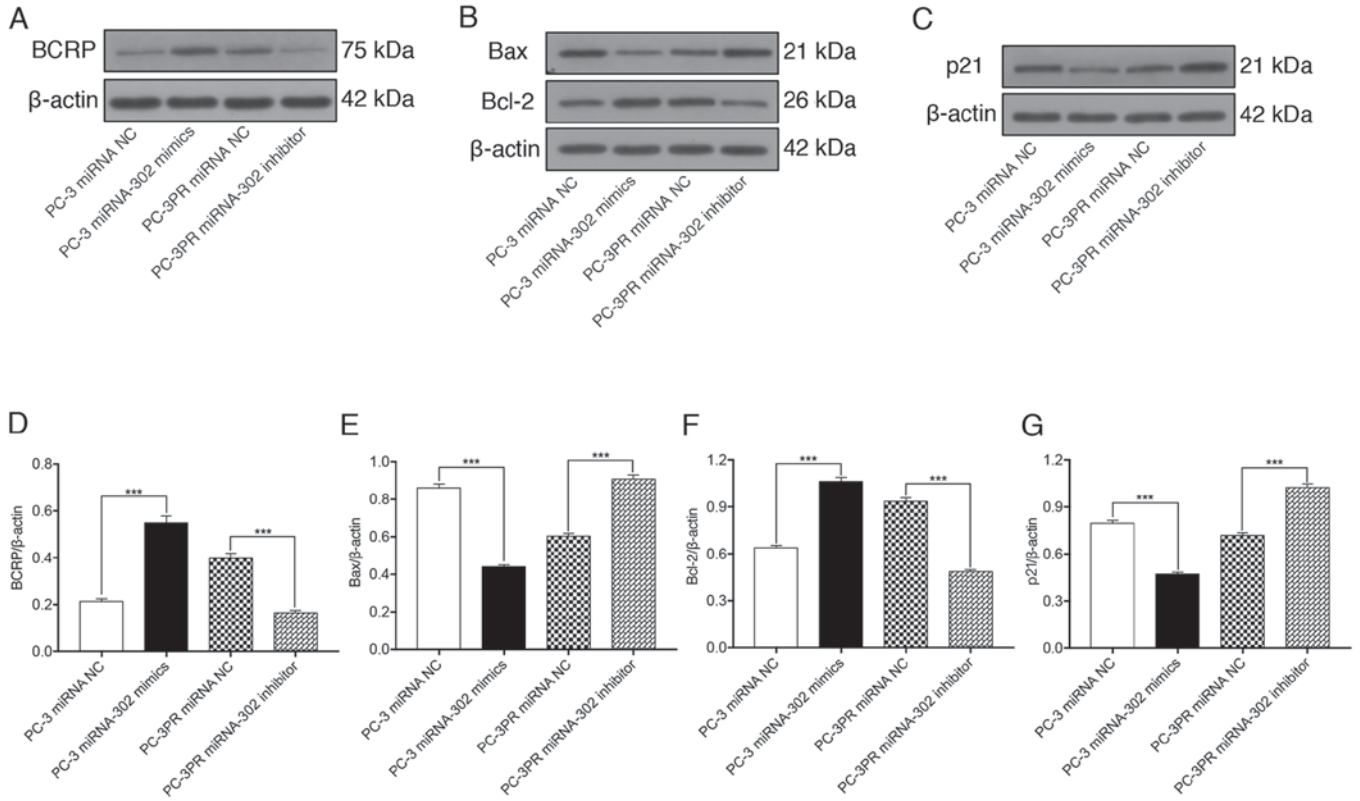

Figure 4. Effects of miR-302a on the expression of apoptosis and cell cycle-associated proteins in prostate cancer cells. PC-3 cells were transfected with the miR-302a or NC mimics in the presence of paclitaxel (2.3 nM) for 48 h. PC-3PR cells were transfected with the miR-302a or NC inhibitors in the presence of paclitaxel (97.8 nM) for 48 h. The expression levels of (A) BCRP, (B) Bax, Bcl-2 and (C) p21 were assessed. Protein lysates were collected and assayed by western blot analysis. (D) Quantification of BCRP western blot analysis data. (E) Quantification of Bax western blot analysis data. (F) Quantification of Bcl-2 western blot analysis data. (G) Quantification of p21 western blot analysis data. ***P<0.01. miRNA, microRNA; NC, negative control; AOF2, lysine demethylase 1A; BCRP, breast cancer resistance protein; Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X protein.

and may mediate miR-302a-induced chemo-resistance to paclitaxel in PC-3PR cells. In addition, miR-302a may also participate in drug resistance by regulating the expression of the apoptosis-associated proteins Bcl-2 and Bax and the cell cycle-associated protein p21. This novel avenue of study, based on miR-302a targeting AOF2, BCRP and P-glycoprotein, will assist in improving the understanding of the molecular basis of chemo-resistance, and will guide the development of novel therapeutics for prostate cancer in the future.

A limitation of the present study is that although TargetScan analysis was performed, a luciferase assay was not conducted due to funding limitations, which would have determined whether the regulation of AOF2, BCRP and P-glycoprotein luciferase expression was dependent on the binding of complementary 3'UTR sequences to the miR-302a seed sequence. The other limitation is that only one prostate cancer cell line was analysed in the present study, as cancer resistant cells are difficult to obtain. In future studies, other prostate cancer paclitaxel-resistant cell lines will be included, to explore the role of miR-302a in paclitaxel resistance in prostate cancer in more detail.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

YW and XW conceived and designed the study. YW, LH and ZQ performed the experiments. YW wrote the paper. YW, LH, ZQ and XW reviewed and edited the manuscript. All authors have read and approved the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2015. CA Cancer J Clin 65: 5-29, 2015.
2. Bhatnagar N, Li X, Padi SK, Zhang Q, Tang MS and Guo B: Downregulation of miR-205 and miR-31 confers resistance to chemotherapy-induced apoptosis in prostate cancer cells. Cell Death Dis 1: e105, 2010.
3. Petrylak DP, Tangen CM, Hussain MH, Lara PN Jr, Jones JA, Taplin ME, Burch PA, Berry D, Moinpour C, Kohli M, *et al*: Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. N Engl J Med 351: 1513-1520, 2004.
4. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Théodore C, James ND, Turesson I, *et al*: Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med 351: 1502-1512, 2004.
5. Fang L, Li H, Wang L, Hu J, Jin T, Wang J and Yang BB: MicroRNA-17-5p promotes chemotherapeutic drug resistance and tumour metastasis of colorectal cancer by repressing PTEN expression. Oncotarget 5: 2974-2987, 2014.
6. Rokavec M, Öner MG, Li H, Jackstadt R, Jiang L, Lodygin D, Kaller M, Horst D, Ziegler PK, Schwitalla S, *et al*: IL-6R/STAT3/miR-34a feedback loop promotes EMT-mediated colorectal cancer invasion and metastasis. J Clin Invest 124: 1853-1867, 2014.
7. Choi N, Park J, Lee JS, Yoe J, Park GY, Kim E, Jeon H, Cho YM, Roh TY and Lee Y: miR-93/miR-106b/miR-375-CIC-CRABP1: A novel regulatory axis in prostate cancer progression. Oncotarget 6: 23533-23547, 2015.
8. Fujita Y, Kojima K, Ohhashi R, Hamada N, Nozawa Y, Kitamoto A, Sato A, Kondo S, Kojima T, Deguchi T and Ito M: MiR-148a attenuates paclitaxel resistance of hormone-refractory, drug-resistant prostate cancer PC3 cells by regulating MSK1 expression. J Biol Chem 285: 19076-19084, 2010.
9. Puhr M, Hoefer J, Schäfer G, Erb HH, Oh SJ, Klocker H, Heidegger I, Neuwirt H and Culig Z: Epithelial-to-mesenchymal transition leads to docetaxel resistance in prostate cancer and is mediated by reduced expression of miR-200c and miR-205. Am J Pathol 181: 2188-2201, 2012.
10. Shi GH, Ye DW, Yao XD, Zhang SL, Dai B, Zhang HL, Shen YJ, Zhu Y, Zhu YP, Xiao WJ and Ma CG: Involvement of microRNA-21 in mediating chemo-resistance to docetaxel in androgen-independent prostate cancer PC3 cells. Acta Pharmacol Sin 31: 867-873, 2010.
11. Kojima K, Fujita Y, Nozawa Y, Deguchi T and Ito M: MiR-34a attenuates paclitaxel-resistance of hormone-refractory prostate cancer PC3 cells through direct and indirect mechanisms. Prostate 70: 1501-1512, 2010.
12. Wang Y, Lieberman R, Pan J, Zhang Q, Du M, Zhang P, Nevalainen M, Kohli M, Shenoy NK, Meng H, *et al*: miR-375 induces docetaxel resistance in prostate cancer by targeting SEC23A and YAP1. Mol Cancer 15: 70, 2016.
13. Card DA, Hebbar PB, Li L, Trotter KW, Komatsu Y, Mishina Y and Archer TK: Oct4/Sox2-regulated miR-302 targets cyclin D1 in human embryonic stem cells. Mol Cell Biol 28: 6426-6438, 2008.
14. Liu H, Deng S, Zhao Z, Zhang H, Xiao J, Song W, Gao F and Guan Y: Oct4 regulates the miR-302 cluster in P19 mouse embryonic carcinoma cells. Mol Biol Rep 38: 2155-2160, 2011.
15. Lin SL, Chang DC, Chang-Lin S, Lin CH, Wu DT, Chen DT and Ying SY: Mir-302 reprograms human skin cancer cells into a pluripotent ES-cell-like state. RNA 14: 2115-2124, 2008.
16. Bourguignon LY: Matrix hyaluronan promotes specific MicroRNA upregulation leading to drug resistance and tumor progression. Int J Mol Sci 17: 517, 2016.
17. Bourguignon LY, Wong G, Earle C and Chen L: Hyaluronan-CD44v3 interaction with Oct4-Sox2-Nanog promotes miR-302 expression leading to self-renewal, clonal formation, and cisplatin resistance in cancer stem cells from head and neck squamous cell carcinoma. J Biol Chem 287: 32800-32824, 2012.
18. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
19. Wang Y, Zhao L, Xiao Q, Jiang L, He M, Bai X, Ma M, Jiao X and Wei M: miR-302a/b/c/d cooperatively inhibit BCRP expression to increase drug sensitivity in breast cancer cells. Gynecol Oncol 141: 592-601, 2016.
20. Zhao L, Wang Y, Jiang L, He M, Bai X, Yu L and Wei M: MiR-302a/b/c/d cooperatively sensitizes breast cancer cells to adriamycin via suppressing P-glycoprotein(P-gp) by targeting MAP/ERK kinase kinase 1 (MEKK1). J Exp Clin Cancer Res 35: 25, 2016.
21. Subramanyam D, Lamouille S, Judson RL, Liu JY, Bucay N, Derynck R and Blelloch R: Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. Nat Biotechnol 29: 443-448, 2011.
22. Lipchina I, Studer L and Betel D: The expanding role of miR-302-367 in pluripotency and reprogramming. Cell Cycle 11: 1517-1523, 2012.
23. Lin SL and Ying SY: Mechanism and method for generating tumor-free iPS cells using intronic microRNA miR-302 induction. Methods Mol Biol 936: 295-312, 2013.
24. Wang L, Yao J, Shi X, Hu L, Li Z, Song T and Huang C: MicroRNA-302b suppresses cell proliferation by targeting EGFR in human hepatocellular carcinoma SMMC-7721 cells. BMC Cancer 13: 448, 2013.
25. Wang L, Yao J, Zhang X, Guo B, Le X, Cubberly M, Li Z, Nan K, Song T and Huang C: miRNA-302b suppresses human hepatocellular carcinoma by targeting AKT2. Mol Cancer Res 12: 190-202, 2014.
26. Liang Y, Ahmed M, Guo H, Soares F, Hua JT, Gao S, Lu C, Poon C, Han W, Langstein J, *et al*: LSD1-mediated epigenetic reprogramming drives CENPE expression and prostate cancer progression. Cancer Res 77: 5479-5490, 2017.
27. Gupta S, Weston A, Bearrs J, Thode T, Neiss A, Soldi R and Sharma S: Reversible lysine-specific demethylase 1 antagonist HCI-2509 inhibits growth and decreases c-MYC in castration- and docetaxel-resistant prostate cancer cells. Prostate Cancer Prostatic Dis 19: 349-357, 2016.
28. Noguchi K, Katayama K, Mitsuhashi J and Sugimoto Y: Functions of the breast cancer resistance protein (BCRP/ABCG2) in chemotherapy. Adv Drug Deliv Rev 61: 26-33, 2009.
29. van der Kolk DM, Vellenga E, Scheffer GL, Müller M, Bates SE, Scheper RJ and de Vries EG: Expression and activity of breast cancer resistance protein (BCRP) in de novo and relapsed acute myeloid leukemia. Blood 99: 3763-3770, 2002.
30. Breedveld P, Beijnen JH and Schellens JH: Use of P-glycoprotein and BCRP inhibitors to improve oral bioavailability and CNS penetration of anticancer drugs. Trends Pharmacol Sci 27: 17-24, 2006.
31. Benadiba M, Dos Santos RR, Silva Dde O and Colquhoun A: Inhibition of C6 rat glioma proliferation by [Ru2Cl(Ibp)4] depends on changes in p21, p27, Bax/Bcl2 ratio and mitochondrial membrane potential. J Inorg Biochem 104: 928-935, 2010.
32. Sa G and Das T: Anti cancer effects of curcumin: Cycle of life and death. Cell Div 3: 14, 2008.