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rs11671784 G/A variation in miR-27a decreases chemo-sensitivity of bladder cancer by decreasing miR-27a and increasing the target RUNX-1 expression

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ABSTRACT

Single nucleotide polymorphism (SNP) rs11671784 is in the loop of pre-miR-27a and the G/A variation can significantly decrease mature miR-27a expression. This study explored the role of miR-27a in chemo-sensitivity of bladder cancer and how rs11671784 G/A variation affects the sensitivity. Blood and tumor samples from 89 bladder cancer cases were analyzed. In-vitro study was performed to explore the mechanism of chemo-sensitivity and the downstream target of miR-27a by using bladder cancer cell lines. This study identified a causative relationship between rs11671784 G/A variation, lowered miR-27a expression, increased RUNX-1 expression and following weakened chemo-sensitivity. rs11671784 G allele has significantly stronger effect over A allele in promoting chemo-sensitivity in bladder cancer. miR-27a mediates chemotherapy at least partially through reducing P-gp expression and increasing apoptosis. In addition, RUNX-1 is a novel direct target of miR-27a, which is involved in its regulation of chemo-sensitivity in bladder cancer.

1. Introduction

Bladder cancer is the second most common cancer in male around the world [1]. This cancer is highly refractory to chemo-therapy, since only about 30%–50% of the patients respond to chemotherapeutic agents [2]. Furthermore, once the cancer become chemo-resistant, the cancer cells are highly possible to develop multidrug-resistance (MDR), regardless the nature of previous drug exposure [3,4]. The mechanism of drug resistance is very complicated and varies drastically among the patients, making it difficult to predict chemo-resistance and to develop personalized chemotherapy.

miRNA can downregulate the expression of target gene through mRNA degradation or translational repression [5]. Multiple miRNAs are dysregulated in bladder cancer and some are involved in the regulation of chemo-sensitivity. For example, lower level of miR-34a is associated with weaker response to cisplatin [6], while higher miR-101 expression enhances cisplatin sensitivity [7]. Therefore, some miRNAs might be predictive markers of chemotherapy [8]. With the markers, it is possible to identify subgroups of patients who are sensitive to typical regimes. Single nucleotide polymorphisms (SNPs) in miRNAs or pre-miRNAs might alter miRNA expression and generate significant difference in disease susceptibility and drug sensitivity [9,10]. miR-27a expression is significantly lower in bladder cancer and might be involved in chemotherapy resistance [8,11]. rs11671784, which is in the loop of pre-miR-27a and with G/A polymorphism could affect miR-27a expression [12]. However, whether this SNP is associated with chemo-sensitivity and what are the downstream targets of miR-27a in bladder cancer is not well understood.

Findings of this study demonstrated that rs11671784 G allele has significantly stronger effect over A allele in promoting miR-27a expression and chemo-sensitivity in bladder cancer. RUNX-1 is a novel direct target of miR-27a mediating chemo-sensitivity.

2. Materials and methods

2.1. Study participants

The study design was approved by the ethics committee of Sichuan Provincial People's Hospital. 89 patients who were

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diagnosed as local advanced (Stage II & III) or metastatic (Stage IV) non-muscle invasive bladder cancer from 2011 to 2014 at Sichuan Provincial People's Hospital were recruited with their informed consent. All of the patients received first line platinum containing combination chemotherapy for locally advanced or metastatic carcinoma according to the NCCN Clinical Practical Guidelines. All participants recruited have no direct blood relationship. All of them received cisplatin-based combination chemotherapy for a maximum of 6 cycles unless progression or unacceptable toxicity appeared. Following chemotherapy, patients presented partial or complete responses were offered consolidating surgery when applicable. 5 ml blood samples were taken from each patient. Tumor samples from patients were obtained after surgery.

2.2. Genotyping

To extract genomic DNA from blood samples, NucleoSpin Blood kit (Macherey–Nagel, GmbH & Co. KG, Germany) was used based on recommend protocol. The concentration of extracted DNA was determined by using Nanodrop Spectrophotometer (ND-1000, USA) with full wavelength. Direct sequencing was performed for genotyping of rs11671784 (SNPseq™, GeneSky).

2.3. Cell culture

HEK293T cells and bladder cancer cell lines (UM-UC-3, H-bc, RT4, T24 and 5637) were obtained from ATCC and were cultured in T75 flasks in DMEM medium with 10% v/v foetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin and 2 mM glutamine in humidified air (5% CO_2) under 37 °C.

2.4. Chemotherapeutic agents

The clinic grade of Paclitaxel (Pa, Taiji, Sichuan), Rifampin (Amerigen, Jiangsu), Cisplatin (Ci, Haosen, Jiangsu) and Adriamycin (Ad, Pfizer, Jiangsu) are used.

2.5. Cell transfection

The wildtype lentiviral miR-27a expression construct (Lenti-miR-27) with rs11671784 G allele was purchased from System Biosciences. The variant miR-27a expression construct with rs11671784 A allele was generated through introducing a G/A substitution at the SNP site by using QuikChange Multi Site-Directed Mutagenesis Kit (Agilent Technologies) according to instruction. The widetype and mutant constructs were designed as Lenti-miR-27a-G and Lenti-miR-27a-A respectively. Sequencing of the two constructs was performed to confirm substitution. Lentiviral RUNX-1 expression vector (Lenti-RUNX-1) was purchased from Origene. The lentiviral vectors and corresponding packaging kit were co-transfected to HEK-293T cells respectively according to manufacturer's instruction. 48 h after transfection, viral titers of the culture supernatants were measured and the supernatants were harvested if the titers were between 10^6–10^7 TU/mL. UM-UC-3 cells were then treated with the viral supernatants containing 5 μg/ml polybrene (Sigma–Aldrich). RUNX-1 siRNA and siRNA control were purchased from Santa Cruz and were transfected to UM-UC-3 cells by using Oligofectamine (Invitrogen) according to the instruction.

2.6. Chemo-resistance profiling (IC50 measurements)

Cells in the logarithmic phase were plated in 96-well plates at the density of 5×10^3/well and were treated with four-fold serially diluted drugs for 72 h. Cell viability was measured with MTT (Sigma Aldrich) assay according to recommend protocol. The O.D. value was measured at 490 nm with a micro plate reader (Lab systems Multiscan MCC/340). The drug concentration resulted in 50% growth inhibition (IC50) was determined from the corresponding dose response curve.

2.7. Analysis of intracellular Ad concentrations

Fluorescence intensity of intracellular Ad was measured by flow cytometry as described previously [13]. Generally, cells were plated at 10^6 cells/well in 6-well plates and were further cultured overnight. On the second day, Ad (5 μg/ml) was added to cultures. Cells were then incubated for 1 h and then collected to detect Ad accumulation or further maintained in a drug-free medium for another 2 h to measure Ad retention by measuring fluorescence intensity. The Ad release index was calculated by using the following formula: releasing index = (accumulation value-retention value)/accumulation value. Experiments were performed in triplicate.

2.8. Quantitative RT-PCR

Total RNAs from tissue, blood and cell sample were extracted with TRIzol isolation reagent (Invitrogen) according to recommend protocols. Purity and concentration of RNA samples were determined with a UV–visible spectrophotometer (NanoDrop Technologies). Taqman miRNA Assays was applied to quantify mature miR-27a expression. RNU6B was used as an internal control. β-Actin gene was used to normalize the expression of pri- and pre-miRNAs. pri- and pre-miRNA levels were determined based on qRT–PCR in triplicate, with gene-specific primers and Power SYBR Green PCR Master Mix. The primer sequences used for pri-miR-27a were: (F) 5′-ATATGAGAAAAGAGCTTCCCTGTG-3′ and (R) 5′-CAAGGCCA-GAGGAGGTGAG-3′; for both pre- and pri-miR-27a: (F) 5′-AGGGCTTAGCTGCTTGTGAG-3′ and (R) 5′-CAAGGCCA-GAGGAGGTGAG-3′ [12]; for RUNX-1, (F) 5′- CCGAGAACCTCGAA-GACATC-3′ and (R) 5′-GATGGTTGGATCTGCCTTGT-3′. All qRT-PCRs were performed with ABI Prism 7300 sequence detection system (Applied Biosystems Inc.).

2.9. Western blot analysis

Cells were collected 48 h after transfection/drug treatment and were lyzed with lysis buffer (Beyotime, Shanghai, China). The extract samples were separated by 10% SDS–PAGE gel and transferred to NC membrane for a conventional western blotting analysis. The membranes were probed with primary antibodies to Bcl-2 (1:1000, ab18210, Abcam), Bax (1:1000, ab32503, Abcam), P-gp (1:500, ab10333, Abcam), active caspase-3 (1:1000, ab77973, Abcam) and RUNX-1 (1:1000, ab23980, Abcam) respectively and then incubated with corresponding HRP-conjugated secondary antibody (1: 5000, anti-rabbit IgG, HRP, ab6721; 1: 5000, anti-mouse IgG, HRP, ab6728, Abcam). Signals were visualize by using an ECL kit (Pierce, IL, USA). The band intensity was quantified by using Image-J software. Experiments were performed in triplicate.

2.10. Flow cytometry analysis of apoptosis

48 h after transfection, UM-UC-3 cells were plated in six-well plates at 4×10^5 cells/well. The proportion of apoptotic cells was determined by using Fluorescein active caspase 3 staining kit (88-7004, eBioscience) with a flow cytometry (FACSCalibur, BD Biosciences). The results were analyzed by using ModFit (BD Biosciences)

2.11. Dual luciferase analysis

DNA oligonucleotides containing the wild-type or mutated human RUNX-1 3′-UTR regions were chemically synthesized and were cloned into the downstream of the firefly luciferase gene in pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega) between PmeI and XbaI sites. The sequence details were: WT: F, 5′-aaacAGGAAGATTCCCGGAGGGAAACTGTGAATGCTTCTGATTTAGCAATGCt-3′; R, 5′-ctagaGCATTGCTAAATCAGAAGCATTCACAGTTTCCCTCCGGGAATCTTCCTgttt-3′; MUT: F, 5′-aaacAGGAAGATTCCCGGAGGGAAACACACTATGCTTCTGATTTAGCAATGCt-3′; R, 5′-ctagaGCATTGCTAAATCAGAAGCATAGTGTGTTTCCCTCCGGGAATCTTCCTgttt-3′. The reconstructed vectors were designated as pmirGLO-RUNX-1-WT and pmirGLO-RUNX-1-MUT respectively. Insertion was confirmed by sequencing. HEK-293T cells/UM-UC-3 cells were co-transfected with 150 ng reporter plasmids and 400 ng miR-27a-G or miR-27a-A plasmids respectively. 18 h after transfection, cells were lyzed to measure both firefly and Renilla luciferase activities by the Dual-Luciferase Reporter Assay System (Promega) using a Promega GloMax 20/20 luminometer. The firefly luciferase activity was normalized to the renilla luciferase activity.

2.12. Statistical analysis

Quantitative variables with normal distribution were reported as median ± SD. Between group difference was compared by using t-test (Mann–Whitney rank sum test). Odds ratios (OR) and 95% confidence intervals (CI) was used to estimate the association between SNP rs11671784 variants (GG vs. GA + AA) and chemotherapy sensitivity. $p < 0.05$ was considered as statistically significant. All statistical analyses were performed using SPSS for Windows 17.0 (SPSS Inc).

3. Results

3.1. Higher miR-27a expression and rs11671784 G allele are associated with better response to chemotherapy in bladder cancer patients

Through analyzing blood and tumor samples of the 89 patients, it was observed that the patients with progressive disease (PD) or stable disease (SD) had significantly lower mature miR-27a expression in both tumor tissue (median difference: 1.55 fold lower, $p < 0.0001$) ((Fig. 1A) and serum (median difference: 0.58 fold lower, $p < 0.0001$) than patients with complete response (CR) or partial response (PR) (Fig. 1B). Considering rs11671784 is an important SNP affecting miR-27a expression, we further explored the association between this SNP and chemotherapy resistance. Patients with GA and AA genotypes had significantly higher rate of PD and SD compared with GG carriers (OR: 9.49, 95%CI: 3.57-25.20, $p < 0.00001$) (Fig. 1C).

3.2. rs11671784 A allele interrupts miR-27a expression

To identify how rs11671784 G/A variation interrupts miR-27a expression, expression of mature miR-27a, pri-miR-27a and both pri- and pre-miR-27a in rs11671784 GG, GA and AA patients and in in-vitro models were measured. miR-27a expression in bladder tumor tissues from patients with GA or AA genotypes was significantly lower than in that from patients with GG genotype (Fig. 2A). To explore the biogenesis process in which the expression of miR-27a is altered, expression of pri-miR-27a alone and the total amount of both pri-miR-27a and pre-miR-27a were measured in tumor samples. Results confirmed similar expression of pri-miR-27a and both pri-miR-27a and pre-miR-27a in AA, AG and GG sample groups ($p > 0.05$) (Fig. 2B and C). To further verify the altered expression is caused by this SNP, miR-27a expression was measured in RT4 cells transfected with Lenti-miR-27a-G or Lenti-miR-27a-A. miR-27a-G RT4 cells had significantly higher miR-27a expression compared with miR-27a-A RT4 cells ($p < 0.01$) (Fig. 2D). However, expression of pri-miR-27a and both pri-miR-27a and pre-miR-27a were similar in these two cell lines ($p = 0.8$ and $p = 0.7$ respectively) (Fig. 2E and F). These findings suggest that lower expression of mature miR-27a in rs11671784 A allele group is caused by interrupted processing of pre-miR-27a to its mature form, implying that the G allele is critical for efficient expression and/or processing of miR-27a.

3.3. miR-27a overexpression increases sensitivity of bladder cell lines to chemotherapy in a dose dependent manner

The relative IC50 values of five bladder cancer cell lines (UM-UC-3, H-bc, RT-4, T24 and 5637) to three chemotherapeutic agents (Ci, Pa and Ad) were determined. Judged by the fold difference over the lowest IC50, 5637 was the most multi-chemo-sensitive cell line that had the lowest IC50 to all of the three drugs. UM-UC-3 is the

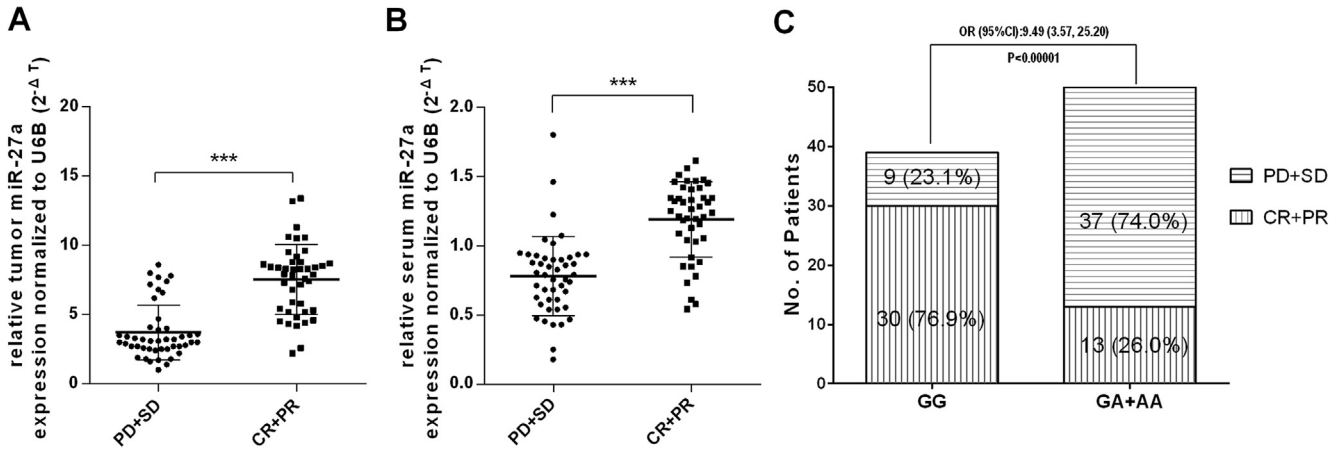

Fig. 1. miR-27a expression is lower in chemotherapy insensitive bladder cancer patients. (A) Real-time PCR analysis of relative miR-27a expression in tumor tissues obtained from patients with progressive disease (PD)/stable disease (SD) and complete response (CR)/partial response (PR). (B) Real-time PCR analysis of relative miR-27a expression in serum of patients with PD/SD and CR/PR. (C) The number and proportion of patients with PD or SD and CR or PR in rs11671784 GG and GA/AA group respectively. Data are showed as mean ± S.D. by three independent experiments. *P < 0.05, **P < 0.01. $^{NS}P > 0.5$.

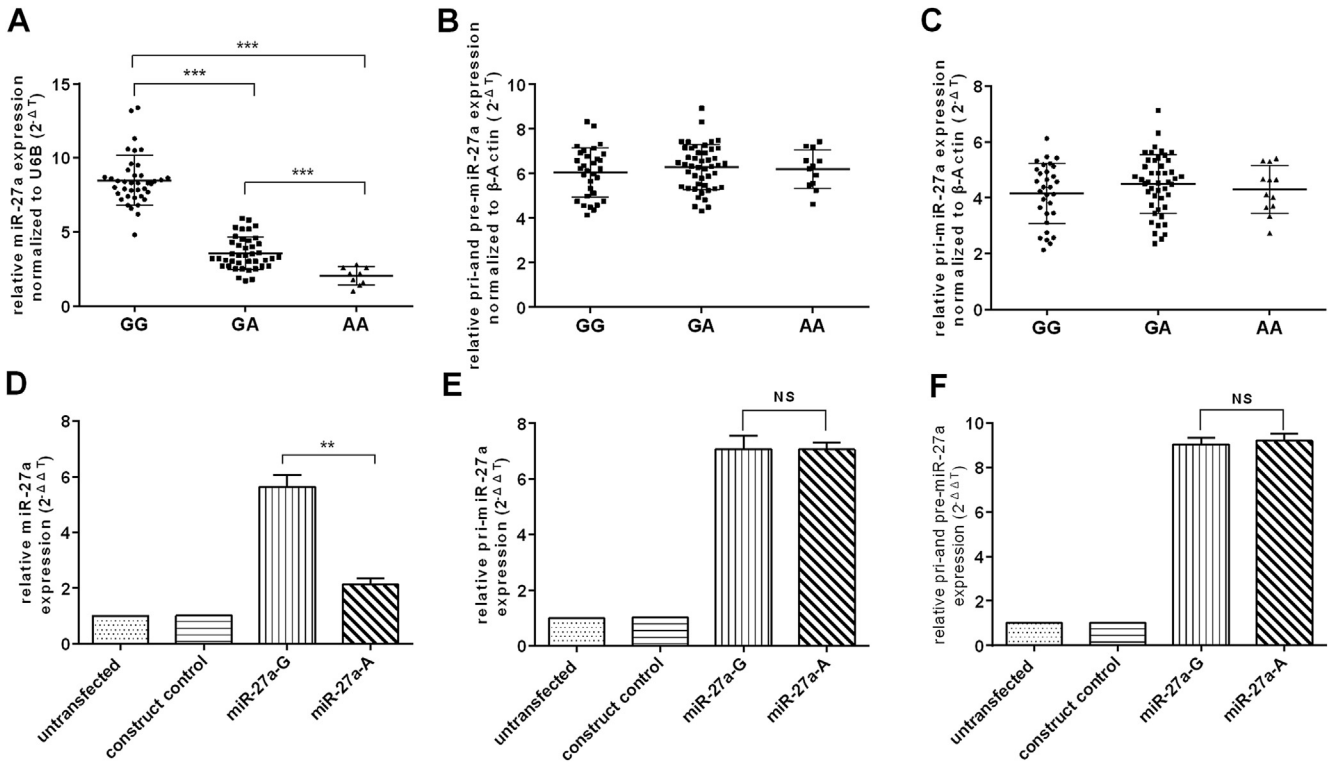

Fig. 2. rs11671784 A allele interrupts expression of miR-27a. (A–C) Real-time PCR analysis of mature miR-27a, pri-miR-27a and both pri- and pre-miR-27a expression in rs11671784 GG, GA and AA patients. (D–F) Real-time PCR analysis of mature miR-27a, pri-miR-27a and both pri- and pre-miR-27a expression in untransfected, construct control, miR-27a-G and miR-27a-A RT-4 cells. Data are showed as mean ± S.D. by three independent experiments. *P < 0.05, **P < 0.01. ^{NS}P > 0.5. Error bars depict S.D.

most multi-chemo-resistant cell line, with the highest IC50 to Ad and second-highest IC50 to Pa and Ci (Fig. 3A). Interestingly, chemo-sensitivity of the cell lines was positively related to miR-27a expression. 5637 had the highest, while UM-UC-3 had the lowest miR-27a expression (Fig. 3B). We further studied the influence of rs11671784 G/A variation on chemo-sensitivity by using UM-UC-3 cells transfected with miR-27a-G/miR-27a-A expression constructs (Fig. 3C). Higher miR-27a expression significantly promoted chemo-sensitivity to Ci, Pa and Ad, while the G allele had stronger effect than the A allele (Fig. 3D).

To explore the role of rs11671784 G/A variation in drug efflux of bladder cancer cells, Ad release index in UM-UC-3 cells transfected with miR-27a-A or miR-27a-G were determined. Both miR-27a-G and miR-27a-A transfection reduced efflux of Ad, but miR-27a-G had stronger effect (Fig. 3E). In consistent with Ad release index, miR-27a-G and miR-27a-A transfection reduced P-glycoprotein (P-gp) expression at protein level (Fig. 3F). To further explore the effect of rs11671784 G/A variation on P-gp release, we treated UM-UC-3 cells with P-gp activator Rifampin. Western blot analysis confirmed a dose dependent P-gp increase after Rifampin treatment (Fig. 3G). Rifampin supplement reversed the increased chemo-sensitivity induced by overexpressing miR-27a (Fig. 3H). These results suggest miR-27a expression is associated with reduced efflux of ADR via P-gp downregulation, while rs11671784 G allele has stronger effect than A allele.

We further explored the effect of the two alleles on drug-induced apoptosis of UM-UC-3 cells. Flow cytometry analysis demonstrated that apoptosis of the cells were enhanced by miR-27a expression, while rs11671784 G allele was associated with stronger apoptosis than A allele (Fig. 3I and J). In addition, the expression of proapoptosis markers (Bax and active Caspase 3) and anti-apoptosis protein Bcl-2 were examined after treatment with Ad. miR-27a enhanced the expression of Bax and active Caspase 3, while reduced the expression of Bcl-2. Consistently, rs11671784 G allele had stronger effect than A allele. These results suggest that higher miR-27a expression is associated with higher susceptibility to drug-induced apoptosis.

3.4. miR-27a modulates sensitivity to chemotherapy through directly inhibiting RUNX-1 expression

Database searching identified a putative pairing between Has-miR-27a and 3′-UTR of RUNX-1. Therefore, two dual luciferase reporters carrying widetype and mutant binding sequences were designed (Fig. 4A). Dual luciferase assay showed that both miR-27a-A and miR-27a-G could inhibit luciferase activity of reporter with widetype binding sequence, but miR-27a-G construct had stronger inhibiting effect. However, none of the constructs could inhibit the luciferase activity of the reporter with mutant binding sequence (Fig. 4C and D). Besides, miR-27a expression was negatively correlated with RUNX-1 expression at both mRNA and protein level (Fig. 4E and F). We further verified the influence of RUNX-1 on chemo-sensitivity. RUNX-1 overexpression significantly increased chemo-resistance, while its downregulation significantly decreased chemo-resistance of UM-UC-3 cells to Ci, Ad and Pa simultaneously (Fig. 4H). In addition, RUNX-1 overexpression could reverse the increased chemo-sensitivity due to miR-27a overexpression in UM-UC-3 cells (Fig. 4I). These results suggest that miR-27a modulates sensitivity of chemotherapy through directly inhibiting RUNX-1 expression.

4. Discussion

SNPs in miRNA precursors can affect the maturation process of miRNAs and thus might have significant influence on the biological process in which the miRNAs are involved [9,14]. One recent study

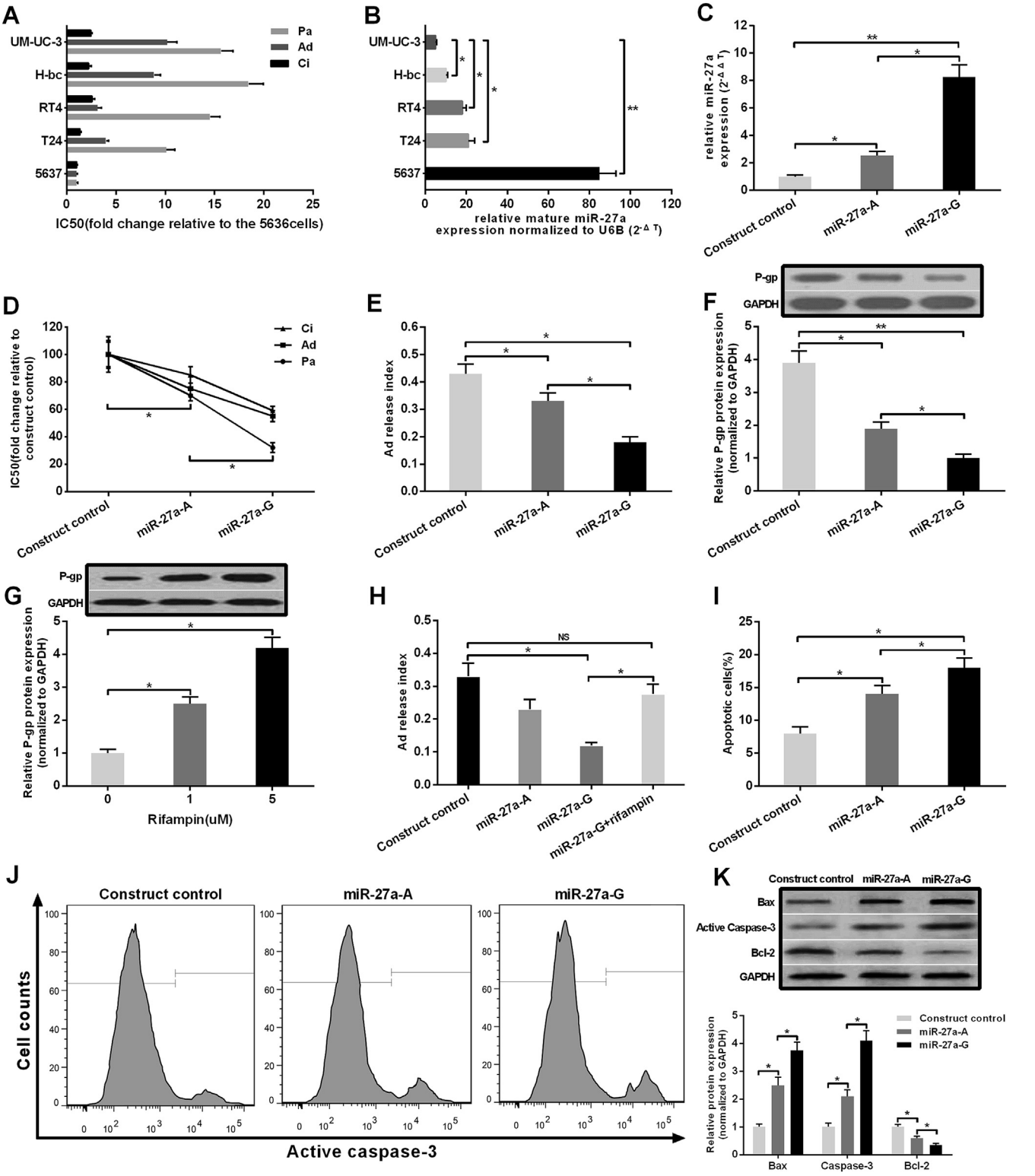

Fig. 3. Overexpression of miR-27a increases sensitivity of bladder cell lines to chemotherapy in a dose dependent manner. (A) MTT analysis, relative IC50 values (fold) of bladder cancer cell lines to three therapeutics with the lowest IC50 (5637 cell line) as a reference. (B) Real-time PCR analysis of relative miR-27a level (fold) in bladder cancer cell lines. (C) rs11671784 A vs. G allele on the expression of mature miR-27a in UM-UC-3 bladder cancer cells. (D) Compared with rs11671784 A allele, miR-27a expression was significantly higher with rs11671784 G allele construct. MiR-27a expression caused remarkably decreased IC50 of three chemotherapy drugs in UM-UC-3 cells. (E) The Ad release index of miR-27a-A and miR-27a-G transfected UM-UC-3 cells was detected 24 h after incubation with 5 ug/ml Ad. (F) The P-gp protein expression level in UM-UC-3 was determined by western blot after transfection of miR-27a-A and miR-27a-G construct. GAPDH was used as an internal control. (G) Rifampin had a dose-dependent effect on P-gp protein level in UM-UC-3 cells. (H) Rifampin reversed the increased chemotherapeutic sensitivity caused by miR-27a overexpression. (I) Quantification of apoptotic cells with active caspase-3 signal in Figure-J (J) miR-27a-A or miR-27a-G transfected UM-UC-3 cells were treated with Ad for 48 h. Then, flow cytometry analysis was performed to measure apoptotic cells by active caspase-3 staining. (K) The proapoptosis marker (Bax and active Caspase 3) and anti-apoptosis protein Bcl-2 were examined by western blot 48 h after Ad treatment. GAPDH was used as an internal control. Data are showed as mean ± S.D. by three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{NS}P > 0.5$.

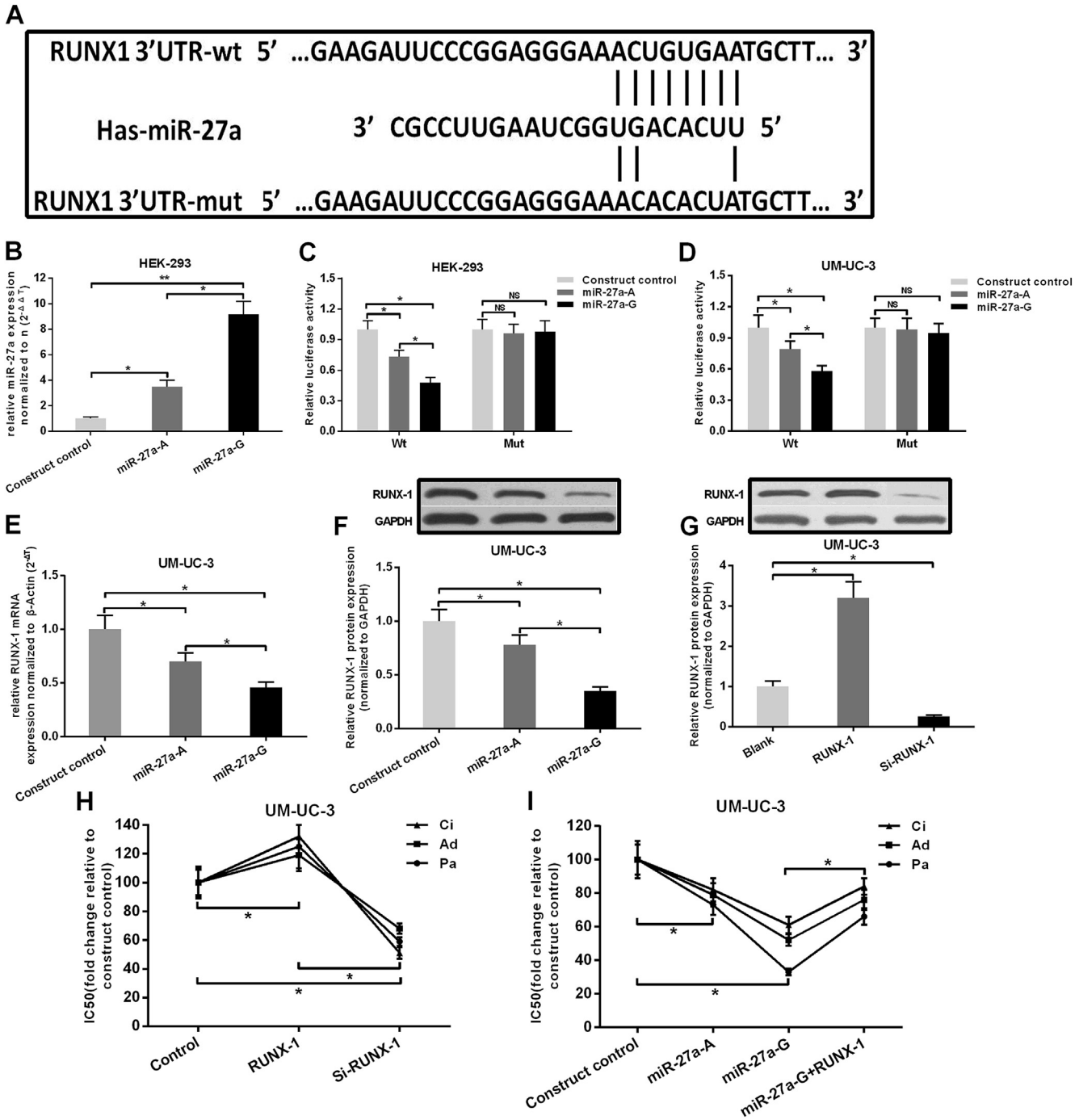

Fig. 4. miR-27a modulates sensitivity of chemotherapy through inhibiting its new direct downstream target, RUNX-1 expression. (A) Schematic description of direct interaction between miR-27-a and 3′-UTR of RUNX-1 according to the bioinformatics prediction. (B) The level of mature miR-27a was significantly increased after transfection of miR-27a-A and miR-27a-G construct in HEK-293 cells. (C) 48 h after co-transfected with either 400 ng miR-27a construct or control vector and 150 ng plasmid carrying either WT or MUT 3′-UTR of RUNX-1, the relative firefly luciferase activity normalized with Renilla luciferase was measured in HEK-293T cells. (D) The relative luciferase activity examination was further performed in UM-UC-3 cells. (E) Real-time PCR analysis of relative RUNX-1 mRNA and (F) western blot analysis of relative RUNX-1 protein level 48 h after transfection of either 600 ng miR-27a construct or control vector. (G) Western blot analysis of the overexpression and knockdown of RUNX-1 gene after transfecting RUNX-1 vector or siRNA targeting RUNX-1 respectively for 48 h. (H) The effects of overexpressing and knockdown of RUNX-1 gene on the IC50 of three anticancer drugs in UM-UC-3 cells. (I) Overexpression of RUNX-1 could reverse the chemo-sensitivity induced by miR-27a construct transfection in UM-UC-3 cells. Data are showed as mean ± S.D by three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{NS}P > 0.5$.

observed that rs11671784 A allele was significantly associated with decreased mature miR-27a expression in gastric cancer patients [12]. However, as an important SNP in miR-27a, how it influence miR-27a's regulative roles is not clear. One recent study observed that miR-27a expression in serum samples of bladder cancer patients were significantly lower compared with healthy individuals [15]. In addition, several studies also observed that miR-27a expression is related to cisplatin resistance in bladder cancer. Nordentoft et al. observed that bladder cancer patients with progressive disease after chemotherapy had significant lower

expression of miR-27a compared with patients with complete response [8]. In in-vivo study, they also confirmed that up-regulation of miR-27a could decrease tumor cell viability under cisplatin treatment [8].

Due to the important role of rs11671784 on miR-27a expression and the possible regulative role of miR-27a on chemo-sensitivity, we explored the influence of this SNP on chemo-sensitivity. We found higher miR-27a expression was associated with better response to chemotherapy. In addition, patients with rs11671784 homozygote GG also had significantly better responses to chemotherapy than patients carrying A allele. Then, we further explored the mechanism of miR-27a in MDR. By using MDR cell lines, we demonstrated that miR-27a transfection could remarkably decrease P-gp expression, a ATP-binding cassette (ABC) transporters involved in the development of multidrug resistance [16]. In addition, supplement of P-gp activator, rirampin, could reverse the Ad release index decrease induced by miR-27a, suggesting that P-gp downregulation might partially account for the effect of miR-27a on promoting drug sensitivity. Chemo-sensitivity might also be related to altered apoptosis of cancer cells. In the current study, we also demonstrated that miR-27a promoted apoptosis of bladder cancer cells. miR-27a expression was positively related to pro-apoptotic molecule Bax and active caspase-3 expression and negatively related to anti-apoptotic molecule Bcl2 expression. These results suggest that miR-27a can promote chemo-sensitivity through increasing the susceptibility of cancer cells to apoptosis. In all of the studies, rs11671784 G allele showed stronger effect than A allele in promoting chemo-sensitivity.

Till now, the downstream targets of miR-27a in chemo-sensitivity in bladder cancer are not quite clear. Drayton et al. explored the regulative role of miR-27a in chemotherapy resistance in bladder cancer and found it could directly and reversely regulate the expression of SLC7A11, a Cystine/Glutamate Exchanger regulating GSH biosynthesis [11]. Actually, upregulation of SLC7A11 was already confirmed as a mechanism of cisplatin resistance in ovarian cancer [17]. However, considering the complex regulative network of miRNAs, there might be some other unidentified targets. Therefore, this study further explored the downstream targets of miR-27a in chemo-sensitivity. Runt-related transcription factor 1 (RUNX-1) is identified as an oncogene in several cancers, including T-cell lymphoma [18], ovarian cancer [19], skin and head/neck squamous cell carcinomas [20]. In bladder cancer, RUNX-1 upregulation was also observed [21]. However, how it is regulated and its biological function is still not clear. This study confirmed that higher RUNX-1 expression is associated with decreased chemo-sensitivity in bladder cancer and miR-27a can directly target and regulate RUNX-1 expression through binding to its 3′-UTR. Therefore, the miR-27a-RUNX-1 axis plays an important role in chemo-sensitivity of bladder cancer.

Findings of this study have important clinical implications for more individualized therapy. Firstly, rs11671784 G/A variation could be a potential powerful indicator of chemotherapy sensitivity among bladder cancer patients. Therefore, through genotyping of this SNP, chemo-sensitivity in selected patients might be predicted. Secondly, some cases of chemo-resistance might be reversed by reinstatement of miR-27a, or by inhibiting RUNX-1 with small-molecule inhibitors. Therefore, if chemotherapy resistance becomes predicative, drug regimens should consider a combination of chemotherapeutic agents with other small-molecule inhibitors.

Conflict of interest

None.

Transparency document

The transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrc.2015.01.109.

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