miR-29a overexpresion induces apoptosis through targeting VEGF in hepatocellular carcinoma

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Abstract

Background : This study aimed to explore the role of miR-29a in hepatocellular carcinoma (HCC) cells. Further, to confirm whether miR-29a targetes vascular endothelial growth factor (VEGF) in HCC cells.

Methods: Cell counting Kit-8 (CCK8) and clone formation assay were used to analyze the proliferation of HCC cells. Flow cytometry was performed to analyze the apoptosis and cell cycle of HCC cells. Western blot was used to analyze the expression of Bax, B-cell lymphoma-2 (Bcl-2), cyclin dependent kinase 4 (CDK4), CyclinD1, Retinoblastoma (Rb), p53 and myeloid cell leukemia-1 (MCL1). The targeting relations between miR-29a and VEGF was measuered by dual luciferase assay.

Results : Overexpression of miR-29a could distinctly inhibit the proliferation and promote apoptosis of HCC cells. Overexpression of miR-29a obviously up-regulated the expressions of Bax and Rb, and reduced the expressions of CDK4, Bcl-2, Cyclin D1, p53, MCL1 and VEGF in HCC cells. Dual luciferase assay proved that VEGF was the target of miR-29a. Rescue experiments showed that miR-29a targeted VEGF to regulate the proliferation and apoptosis in HCC cells.

Conclusions : These results indicated overexpression of miR-29a inhibited the HCC cells proliferation through targeting VEGF.

Background

Hepatocellular carcinoma (HCC) is the fourth leading cause of death last year [1, 2] and the incidence of HCC is increasing year by year [3]. In the Pacific region, especially China, HCC is characterized by high prevalence and incidence [4]. Over the past several decades, many efforts have been made to elucidate the pathogenesis of liver cancer to obtain better clinical therapeutic effects. But the results were inadequate and unsatisfactory. About 70–80% of human gene expression products are non coding RNA (ncRNA). MicroRNAs(miRNAs) are included in ncRNA and play a crucial role in a variety of biological processes and diseases by binding to 3’-untranslational region of the targets [5–7].

Several studies have shown that miR-29a played an important role in HCC cells [8, 9]. MiR-29a could reduce the proliferation of HCC cells by targeting silent mating type information regulation 2 homolog- 1 (SIRT1) [8]. MiR-29a inhibited the expression of recombinant claudin 1 (CLDN1) to reduce the migration and proliferation of HCC cells [9]. After the overexpression of miR-29a in HCC cells, Dnmt3a was down-regulated to mediate epigenetic regulation of methylation [10]. These results suggested that miR-29a played an important role of antitumor activity in HCC.

In vascular endothelial growth factor (VEGF) family, VEGF-A is the first and most important member. Sometimes, VEGF-A would be presented as VEGF [11]. VEGF was reported as a downstream target of miR-29a in other diseases. In nasopharyngeal carcinoma, VEGF was proved to be one of the downstream genes of miR-29a by database prediction and dual luciferase reporter gene [12]. After transfection of miR-

29a, luminescence reactions of VEGF 3'-UTR luciferase reporter were statistically down-regulated [13]. Therefore, we wonder if similar regulatory effect exists in HCC.

In present study, we found miR-29a overexpression induced cell apoptosis and regulated cell cycle correlated proteins in HCC cells. Then, dual luciferase reporter assay was applied to testify the interaction of VEGF and miR-29a. Furthermore, we showed that miR-29a overexpression and the VEGF lowexpression reduced the proliferation of HCC cells..

Methods

Cell culture

Normal liver cell line LO_2 (BNCC100012) and HCC cell lines HepG2 (BNCC102171) and Bel-7402 (BNCC338237) were got from BNCC cell library. Cells were cultured in RPMI 1640 medium with 10% fetal bovine serum (Gibco, Rockville, MD, USA) in an incubator (Thermo Fisher Scientific, Waltham, USA) at 37 °C and 5% CO_2. Cells in logarithmic growth phase were chosen for experiments.

Cell groups and administration

HCC cell lines were taken for experiment. The experiment was classified to three groups: (1) blank control; (2) miR-29a mimic group: transfected miR-29a mimic into cells; (3) mimic-NC: add miR-29a mimic negative control reagent. miR-29a mimic (mir10000802-1-5) and miR-29a mimic control (mir01101-1-5) were purchased from Ribobio(Guangzhou, China).

After 0.25% trypsin digestion and passage, 2×10^5 cells at the logarithmic stage were put in 6-well plates. After 24 h, the cell growth was watched by microscope. When the cell density reached 30–50%, the HCC cells were transfected. Add 5μL miR-29a mimic and miR-29a mimic control into 250μL serum-free medium as liquid A. Add 5μL LipofectamineTM2000 into 250μL serum-free medium as liquid B. Liquid A and B were mixed at 25 °C for 15 min. After 24 h, replaced the mixture with a fresh medium containing 10% FBS. miR-29a expression was evaluated by RT-qPCR.

RT-qPCR

Total RNA isolation kit (A27828, MagMAX™ mirVana™ Total RNA Isolation Kit, Thermo Fisher Scientific, Waltham, USA) was used to extract total RNA from human normal liver cells, HCC cells and transfected HCC cells according to the instructions. RNAs reversely transcribed into cDNAs in reaction system using miR-29a primer (mirq0000802-2-1, Ribobio, Guangzhou, China). qPCR was subsequently performed using SYBR Green Qpcr Master Mix (MedChemExpress) and 2 μl cDNA as a template. The PCR reaction was performed according to the following conditions: every cycle was 10 min at 95 °C, 15 s at 95 °C and 1 min at 60 °C and 40 cycles were performed. With U6 and GAPDH genes (mqp-0202, Ribobio, Guangzhou, China) as internal reference, the relative expressions of miR-29a and VEGF were conducted by $2^{-\Delta\Delta Ct}$. Primers as follows:

miR-29a: F: 5'- TGTCTCGAGCAAGGGACGCCGTGGAAGA – 3', R: 5'- TGTGTCGACCCGCACACCGATATGGTT – 3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCACGAATTTGCGTGTCAT-3'; VEGF: F: 5'-CAACATCACCATGCAGATTATGC-3', R: 5'-CCCACAGGGATTTTCTTGTCTT-3'; GAPDH: F: 5'-GAAGGTGAAGGTCGGAGTC-3', R:5'-GAAGATGGTGATGGGATTTC-3'.

CCK-8

Cells at the logarithmic stage were digested with trypsin. The concentration was 1×10^5/ml, and then put in 96-well plates with 100 μL per well and 4 parallels. Then the cells were cultured at 37 °C with 5% CO_2. 10 μL CCK-8 solution was added into each well at 24 h, 48 h, 72 h and 96 h respectively. After 4 h, the absorbance value (OD) was detected at 450 nm.

Clone Formation Assay

The cells in each group at logarithmic growth stage were digested and prepared to single cell suspension. About 2 mL cell suspension was respectively taken from each group and put in a 6-well culture plate with 500 cells per well. The cells were dispersed evenly by shaking the culture plate and cultured at 37 °C and 5% CO_2. When there were clones visible to the naked eye, the medium was discarded. Cells were washed by phosphate buffered solution (PBS) for 2 times and fixed by methanol at 25 °C for 15 min. Each well was added with 1 mL Giemsa Stain solution(G1015, Solarbio, Beijing, China) and stained for 30 min. After washed with ultra-pure water, the record was imaged by a camera. The clone formation number was directly counted by naked eye.

Flow Cytometry

The HCC cells were trypsinized and were collected by centrifugation at 999 x g for 5 min at 4˚C after transfection 48 h. Cells were subsequently thoroughly washed twice with pre-cooled sterile PBS at 4˚C. We chose single staining with PI for cell cycle dection. After washing, the cells were fixed overnight with 70% ethanol and precooled at 4 °C. After PBS washing, 100 μL RnaseA (Solarbio, Shanghai, China) was added to resuspend at 37 °C for 30 min. Then 400 μL propidium iodide (PI, Solarbio, Shanghai, China) was added and avoided light for 30 min. The cell cycle was detected by flow cytometer (Beckman Coulter, Brea, CA, USA). We chose AnnexinV-FITC apoptosis assay kit (BD Biosicences, San Diego, US) for apoptosis detection. After PBS washing, 300 μL 1 × binding buffer was added to the cells for suspension. Then 5 μL AnnexinV-TIFC was added. After mixed and incubated in dark for 15 min, 5 μL PI was added and mixed. CellQuest software (Version, 5.1, BD Biosicences, San Diego, USA) was used to analyze the data. The Q1 represented necrotic cells, the Q2 presented late apopotic cells, the Q3 represented living cells, the Q4 represented early apoptotic cells. The apoptosis rate = Q2 + Q4.

Western Blot

Total proteins were extracted with a total protein extraction kit (BC3640-50T, Solarbio, Beijing, China) according to the instruction. Protein concentration was measured by the BCA Protein Assay Kit (23225, Pierce™ BCA Protein Assay Kit, Thermo Fisher Scientific, Waltham, USA). Samples (40 μg) from each

group were isolated by 10% SDS-PAGE electrophoresis (Mini-Protean-3, Bio-Rad, Hercules, CA, USA) and then transferred to PVDF membrane (Millipore, Massachusetts, USA). 5% skim milk was used to seal for 1 h. The membranes were incubated overnight at 4˚C with the following primary antibodies diluted in 5% bovine serum albumin (BSA): rabbit anti-human Bcl-2 antibody (1:1000, ab32124, Abcam), rabbit anti-human Bax antibody (1:1000, ab32503, Abcam), rabbit anti-human CDK4 antibody (1:1000, ab108357, Abcam), rabbit anti-human Cyclin D1 antibody (1:1000, ab134175, Abcam), rabbit anti-human Rb antibody (1:1000, ab181616, Abcam), rabbit anti-human VEGF antibody (1:1000,AV202, Beyotime), rabbit anti-human p53 (1:1000,ab32389，Abcam),rabbit anti-human MCL1 (1: 1000，ab32087，Abcam) and rabbit anti-human beta-actin antibody (1:1000, ab8227, Abcam). After overnight at 4 °C, TBST(TBS, 1 ml/L tween-20) washed the membrane three times with 10 min each time. Then the membrane was incubated with goat anti-rabbit IgG HRP (1:2000, ab6721, Abcam) at room temperature for 2 h. Again, TBST washed the membrane three times with 10 min each time. ECL chemiluminescence was used for detection. ImageJ software (version 6;National Institutes of Health) was used for grayscale scanning and quantification.

Dual Luciferase assay

Wild-type (WT) and mutant (MUT) 3 ‘UTRs of VEGF were amplified in pGL3/ luciferase vector (Promega, Madison, WI, USA) and cloned into the downstream of luciferase gene. According to the instruction, luciferase activity of cells was detected after transfection with the dual luciferase reporting system (Promega) 48 h.

Rescue experiments

To further verify the effect of miR-29a on HCC cells via targeting VEGF, HCC cells were randomly divided into 6 groups: normal control group (Control), miR-29a mimic group (miR-29 mimic), VEGF siRNA group (VEGF siRNA), VEGF siRNA control group (si-NC), miR-29a mimic + VEGF mimic-NC group (miR-29a mimic + mi-NC), miR-29a mimic + VEGF mimic group (miR-29a mimic + VEGF mimic). These reagents were purchased from Ribobio(Guangzhou, China). Similarly, the above indexes were analyzed repeatedly.

Statistics

SPSS 19.0 (IBM Corp.) was used to analyze the statistical data. The data was expressed as mean ± SD. Statistical differences between two groups were analyzed by t test. Data analysis about multiple groups was performed by ANOVA followed by LSD and Turkey test for multiple comparisons.. $P < 0.05$ was considered statistically significant.

Results

miR-29a expression is lower in HCC cells

As indicated in Fig. 1, miR-29a expression in HepG2 and Bel-7402 was obviously lower than that in LO2 cells ($p < 0.05$, Fig. 1A). After transfected miR-29a mimic into two kinds of HCC cells, miR-29a expression

in HepG2 and Bel-7402 cells was obviously increased compared to control HCC cells ($p < 0.05$, Fig. 1B, C). After transfected miR-29a mimic-NC, there was no statistical difference between control group and mimic-NC group ($p > 0.05$, Fig. 1B, C) .

miR-29a overexpression promotes apoptosis and inhibit proliferation of HCC cells

Results as processed in Fig. 2, the activities of HCC cells were significantly reduced after transfected by miR-29a mimic ($p < 0.05$, Fig. 2A). Meanwhile, miR-29a mimic transfection obviously decreased the proliferation of two types cells ($p < 0.05$, Fig. 2B). Further, the cell apoptosis and cell cycle were analyzed by flow cytometric analysis (Fig. 2C, D). In Fig. 2C, the cell apoptosis was notably increased in miR-29a mimic group when compared to control group ($p < 0.05$). The cell cycle was prevented the transition from G0/G1 to S in miR-29a mimic group, compared to control group mimic-NC group ($p < 0.05$, Fig. 2D). In Fig. 2C showed that, in HepG2 cells, overexpression of mir29a caused a significant increment of necrotic cells in Q2 and a small increment of early apoptotic cells in Q4, compared to control. Conversely, in Bel-7402 cells, overexpression of mir29a caused a significant increment of apoptotic cells in Q4, but a small change for necrotic cells in Q2, compered to control. These data was revealed that miR-29a miminc could statisticlly reduce proliferation of HCC cells.

miR-29a overexpression promotes apoptosis proteins in HCC cells

Results as expressed in Fig. 3, miR-29a overexpression could significantly promote Bax and Rb proteins expressions in HepG2 and Bel-7402 cells, and down-regulate Bcl-2, CDK4, Cyclin D1, p53 and MCL1 proteins expressions ($p < 0.05$), compared to control group and mimic -NC group. In mimic-NC group, there didn’t exist statisticlly difference about the expressions of the above proteins compared to control group ($p > 0.05$). A more significant increment of Bax protein in Bel-7402 cells compared to HepG2 cells. A more significant increment of Rb protein HepG2 cells compared to Bel-7402 cells. It revealed that miR-29a could regulate apoptosis and cell cycle related proteins expression in HCC cells.

VEGF is a downstream molecule of miR-29a in HCC cells

As shown in Fig. 4A, western blot revealed that miR-29a could significantly reduce VEGF expression in HepG2 and Bel-7402 cells. Furthermore, we identified VEGF as a downstream molecule of miR-29a through bioinformatics retrieval (Fig. 4B). Dual luciferase report assay was applied to testify targeting relation between miR-29a and VEGF. The results indicated that miR-29a inhibited luciferase activity of VEGF with WT 3’UTR, but did not decrease luciferase activity of VEGF with Mut 3’UTR (Fig. 4C).

miR-29a targetes VEGF to affect proliferation and apoptosis in HCC cells

In order to confirm whether miR-29a targetes VEGF to affect proliferation of HCC cells, the rescue experiments were carried out. As shown in Fig. 5A, the relative expression of VEGF mRNA was measured in groups, and the highest expression of VEGF mRNA was showed in miR-29a mimic + VEGF mimic group when contrast to the other groups ($p < 0.05$). There was no significantly difference for VEGF mRNA expression among miR-29a mimic group, VEGF siRNA and miR-29a mimic + mi-NC group ($p > 0.05$). Interesingly, the VEGF mRNA levels were obviously lower in above three groups compared to control group or si-NC group ($p < 0.05$). Further, the cell activites were showed in Fig. 5B. Compared to NC or si-NC group, the HCC cell activites were significantly reduced in miR-29a mimic group and VEGF siRNA group ($p < 0.05$). Compared with miR-29a mimic group, the HCC cell activites were significantly increased in miR-29a mimic + VEGF mimic group ($p < 0.05$). The results of clone formation assy were further confirmed above result (Fig. 5C). The results of cell apoptosis were showed in Fig. 6. Contrasted to control group, apoptosis rate of cells was statistically increased in miR-29a mimic group and VEGF siRNA group ($p < 0.05$). Compared to miR-29a mimic group, apoptosis rate of cells was statistically decreased in miR-29a mimic + VEGF mimic group ($p < 0.05$). Interestingly, in HepG2 cells, overexpression of mir29a caused a significant increment of necrotic cells in Q2 and a small increment of early apoptotic cells in Q4, compared to control. Conversely, in Bel-7402 cells, overexpression of mir29a caused a significant increment of apoptotic cells in Q4, but a small change for necrotic cells in Q2, compered to control (Fig. 6). These results revealed miR-29a could effevtively reduce the proliferation of HCC cells by targeting VEGF.

miR-29a targetes VEGF to module the expression of apoptosis and related proteins in HCC cells

Results as expressed in Fig. 7, compared with contol group and si-NC group, miR-29a mimic administration or VEGF siRNA administration could significantly up-regulate the expressions of Bax and Rb proteins, and down-regulate the expressions of Bcl-2, CDK4 and Cyclin D1 proteins in HCC cells ($p < 0.05$). Noteworthly, expressions of the above proteins in miR-29a mimic + VEGF mimic group were obviously changed ($p < 0.05$),when compared with compared to miR-29a mimic + mimic-NC group. These dats indicated that miR-29a could regulate apoptosis and cycle related proteins expression via targeting VEGF in HCC cells.

Discussion

miR-29a plays an inhibitory role in HCC. Our research confirmed the lower expression of miR-29a in HCC cell lines [9]. It was found that the miR-29a overexpression could effectively reduce the proliferation, block cell cycle and induce apoptosis in HCC cells. Further, this study found that miR-29a regulated the proliferation and apoptosis and cycle related proteins expression via targeting VEGF in HCC cells.

Apoptosis is a form of programmed cell death. There are intrinsic and extrinsic apoptosis pathways. This selective cell suicide is essential for many physiological and pathological process [14]. The balance of this biological process is moduled by many factors, including Bcl-2 family. Given that evasion of

apoptosis is a hallmark of cancer, it is not surprising that anti-apoptotic proteins Bcl-2, p53 and MCL1 are dysregulated in numerous malignancies [15]. Bcl-2 and Bax are the primary members of the Bcl-2 family which regulate apoptosis in cancer cells [16]. Overexpression of Bcl-2 enhanced resistance of cancer cells to most cytotoxins [17]. Overexpression of Bax may antagonize the protective effect of Bcl-2 [18]. Bax and Bcl-2 could form isodimer to down-regulate the activity of Bcl-2. In our experiments, miR-29a ovexpression promoted Bax and Rb proteins expressions, and down-regulated Bcl-2, p53 and MCL1 proteins expressions in HCC cells.

Retinoblastoma (Rb), a tumor suppressor protein, has been found to be under expressed in a variety of tumors. It is responsible for inducing cell cycle arrest when cells are unready to divide. When cells are prepared to divide, Rb is phosphorylated as pRb and enters an inactive state [19]. Cyclin D1 regulates the activity of Cyclin-dependent kinase (CDKs) [20, 21]. Cyclin D1 is the functional subunit of CDK4 and regulates the G1/S transition of cells [22]. CDK4 belongs to the Ser/Thr protein kinase family. It's essential for cell cycle G1. And its activity is limited to the G1-S. CDK4 has the function of phosphorylation to inhibit the biological activity of Rb1, thus mediating the transformation of G1/S phase [23]. In this study, miR-29a ovexpression promoted Rb proteins expressions, and down-regulated up-regulate the expressions of CDK4 and Cyclin D1 proteins in HCC cells, which suggested that miR-29a regulated the HCC cell cycle.

A recent study demonstrated that p53 indirectly downregulated VEGF expression through Rb pathway in a p21-dependent fashion [24]. In this study, VEGF was one of the downstream target of miR-29a and they are negative correlation. When miR-29a and VEGF were both overexpression, the inhibitory effect caused by miR-29a mimic was reversed. Rb related protein p130 inhibited angiogenesis in vivo, which was correlating with the downregulating of VEGF expression [24]. Consistent with previous researches, our data showed miR-29a regulated Rb, p53 exprssion via targeting VEGF in HCC cells.

Conclusion

Compared with human normal liver cells, miR-29a was reduced in HCC cells, indicating that miR-29a was closely revalent to growth of HCC. Forced expression of miR-29a could statisticlly reduce the proliferation,block cell cycle, and increase apoptosis of HCC cells. VEGF was one of the downstream target of miR-29a and they are negative correlation. The mechanism may be related to the negative regulation of targeted VEGF.

Abbreviations

Hepatocellular carcinoma (HCC), Vascular endothelial growth factor (VEGF), Cell counting Kit-8 (CCK8), B-cell lymphoma-2 (Bcl-2), Cyclin dependent kinase 4 (CDK4), CyclinD1, Retinoblastoma (Rb), Myeloid cell leukemia-1 (MCL1), regulation 2 homolog- 1 (SIRT1), Recombinant claudin 1 (CLDN1), Phosphate buffered solution (PBS).

Declarations

Ethics approval and consent to participate

Not application

Consent to publish

All co-authors agree with the contents to publish.

Availability of data and materials

The data used during the current study are availbale from corresponding author.

Competing interests

The authors declare that there are no conflicts of interest.

Funding

No.

Authors' contributions

AJW carried out the experimental work and the data collection and interpretation. HJW and HHH participated in the design and coordination of experimental work, and acquisition of data. CHY, KN and H HH participated in the study design, data collection, analysis of data and preparation of the manuscript. CW and XBY carried out the study design, the analysis and interpretation of data and drafted the manuscript. All authors read and approved the final manuscript.

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Figures

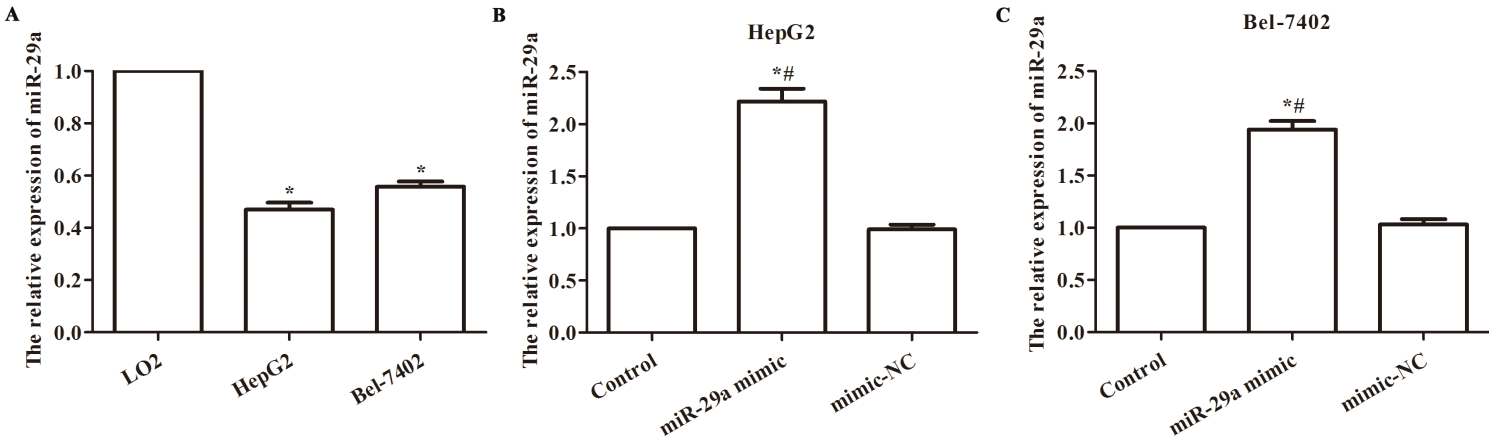

Figure 1

RT-qPCR was performed to analyze miR-29a expression. (A) miR-29a expression in normal hepatocytes and HCC cells. Compared to LO2 cells, *p<0.05. (B) miR-29a expression in HepG2 and Bel-7402 cells which were transfected by miR-29a, respectively. The results which were compared to control were presented as *p<0.05; Compared to mimic-NC, the data were showed as #p<0.05. The results were expressed as mean±SD and analyzed using one-way ANOVA followed by LSD test.

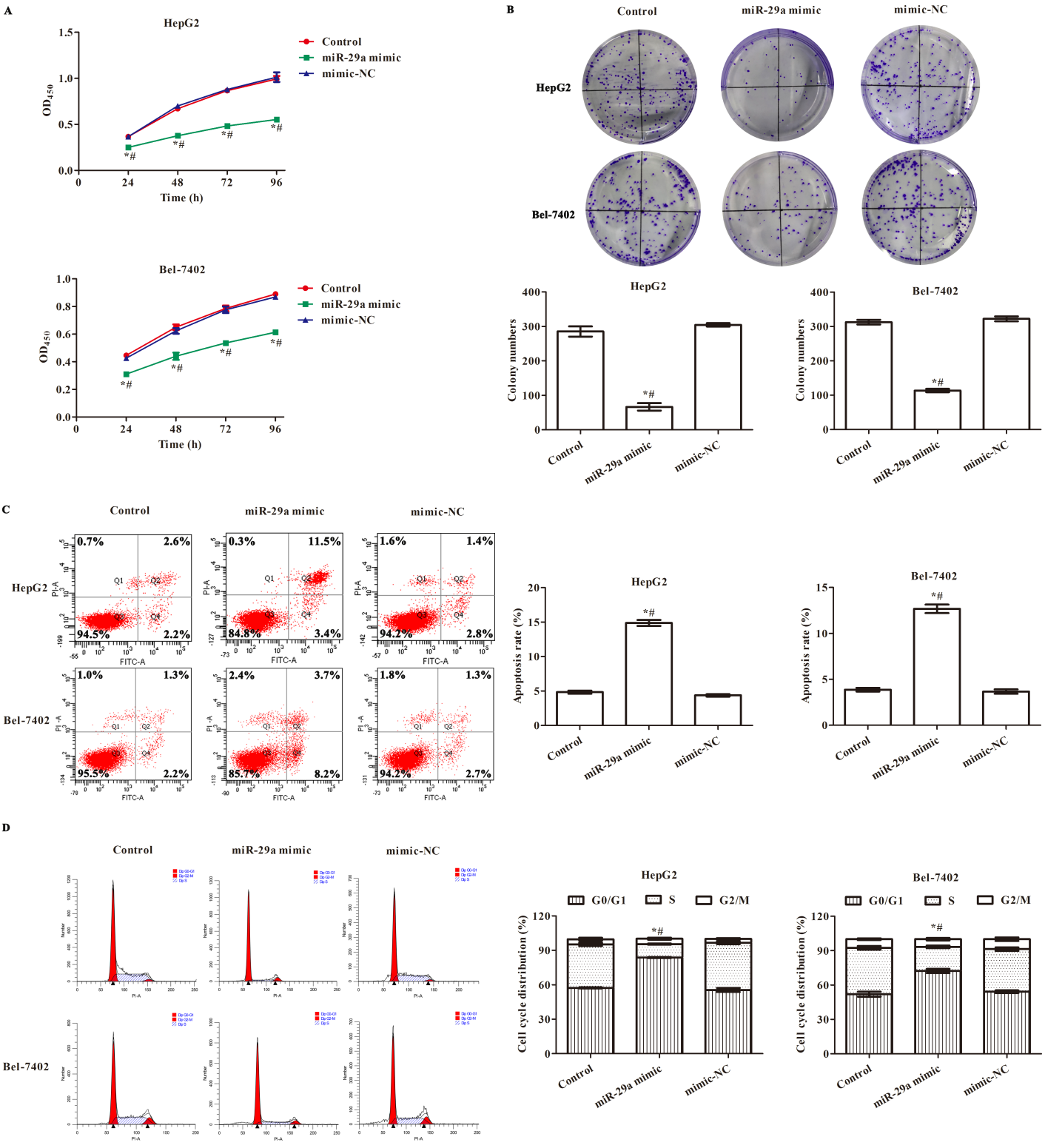

Figure 2

Effects about miR-29a overexpression modules proliferation and apoptosis in HepG2 and Bel-7402 cells. (A) The cell acitivites were measured by CCK8, and miR-29 overexpression obviously decreased the cell activities; (B) The cell proliferation was tested by clone formation assay, and miR-29a overexpression notably inhibited cells proliferation; (C) The cell apoptosis was analyzed by flow cytometry, and miR-29a overexpression notably increased cells apoptosis rate; (D) the cell cycle was detected by flow cytometry,

and miR-29a overexpression significantly prevented the transition from G0/G1 to S. The results were expressed as mean±SD and analyzed using one-way ANOVA followed by LSD test. *p<0.05, compared with control group. #p<0.05, compared to mimic-NC.

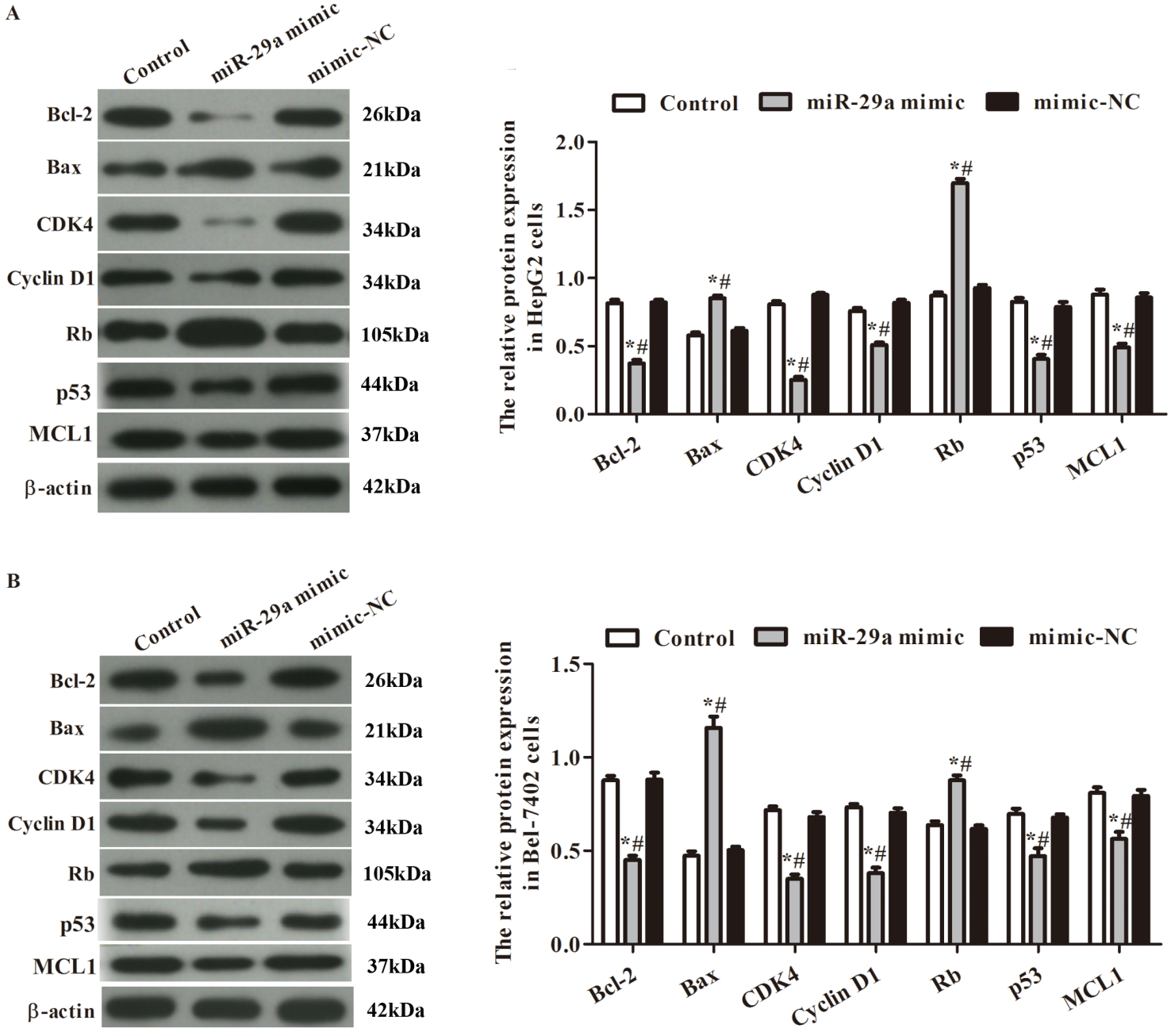

Figure 3

Western blot was applied to detect whether miR-29a overexpression affected proliferation and expressions of apoptosis and cell cycle related protein in HepG2 (A) and Bel-7402 (B). The full-length are presented in Supplementary Figure 1 and Figure 2. The results were expressed as *p<0.05, which were compared to NC; The data were compared to mimic-NC, presented as p<0.05. The results were expressed as mean±SD and analyzed using one-way ANOVA followed by LSD test.

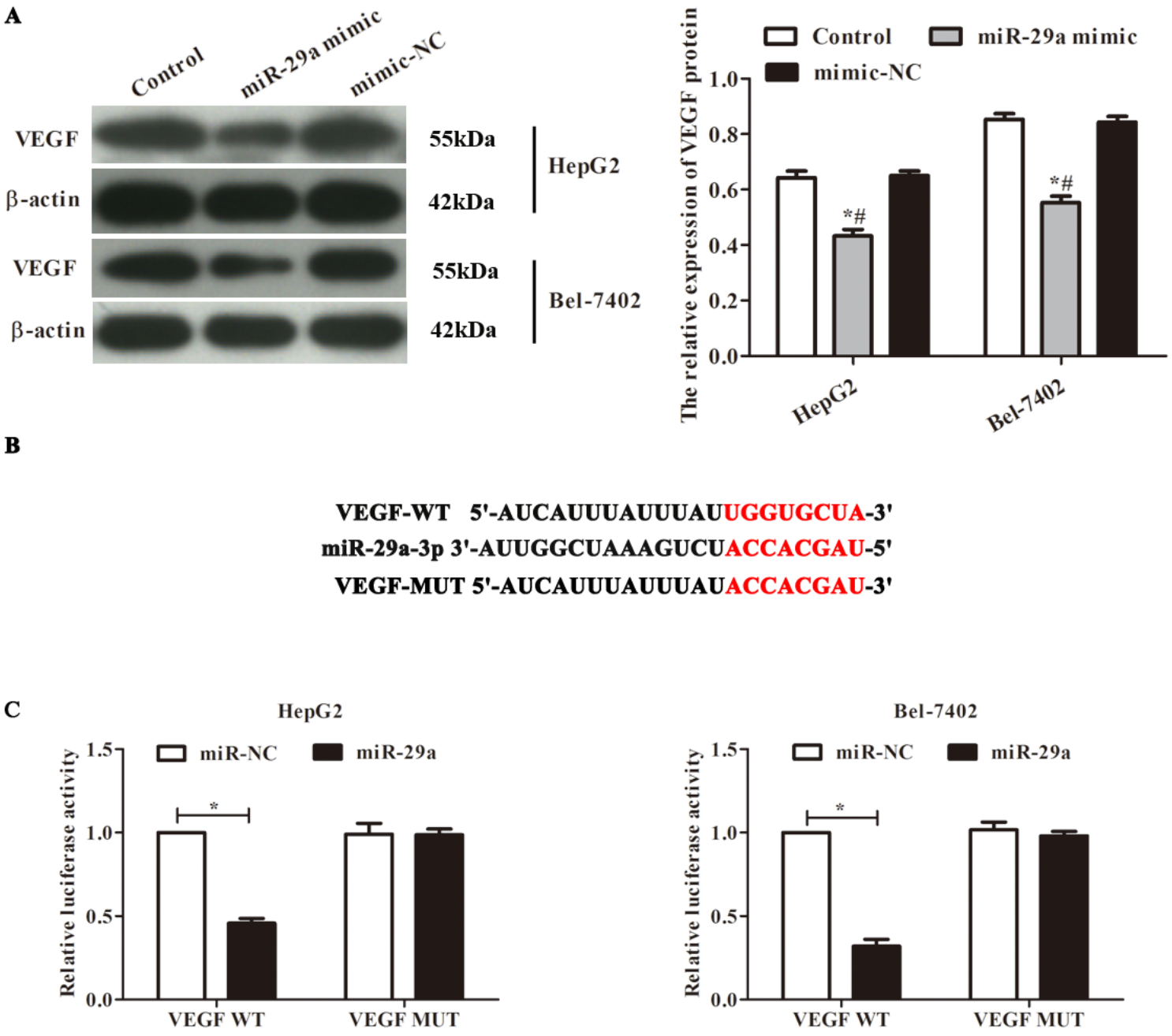

Figure 4

VEGF is the downstream gene of miR-29a. (A) Western blot analysis of miR-29a effect on VEGF protein expression.(B) At the complementary sites in miR-29a, 3 'UTR sequences of the VEGF mRNAs were generated mutations; (C) the effect about that miR-29a overexpression affected the wild-type and mutant 3'UTR activity of VEGF was analyzed by dual luciferase assay.* $p < 0.05$. The full-length are presented in Supplementary Figure 3. The results were expressed as mean±SD and analyzed using t test between two group, one-way ANOVA followed by LSD test among multiple groups.

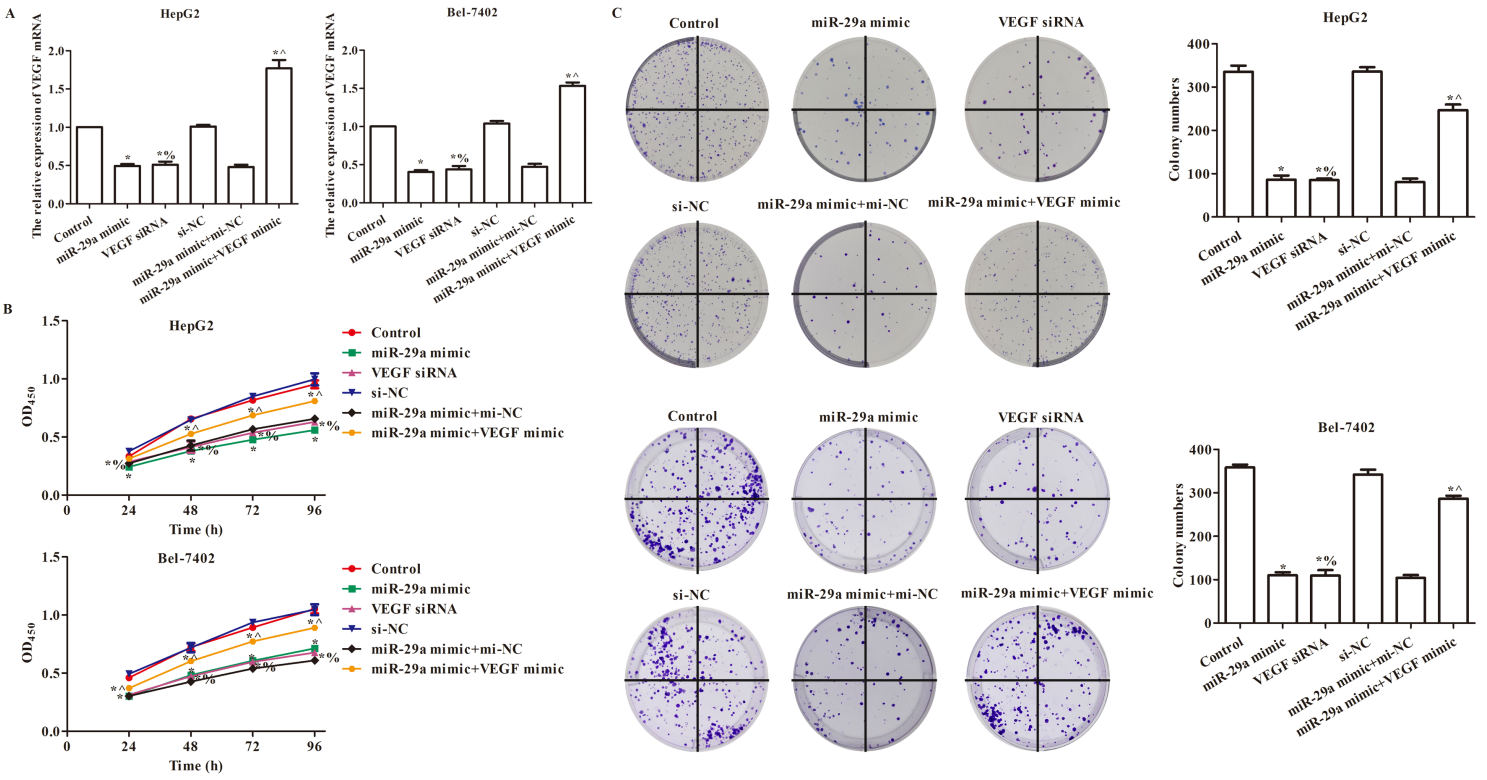

Figure 5

Effect about miR-29a targeted VEGF to module proliferation and apoptosis of HCC cells. (A) PCR detected the relative expression of VEGF mRNA in HepG2 and Bel-7402 cells. MiR-29a regulated VEGF mRNA expression; (B) CCK-8 was used to measured the cell activities, and miR-29a overexpression inhibited cell activities via regulating VEGF; (C) Clone formation assay was performed to analyze cells proliferation, and miR-29a overexpression restrained cells proliferation through targeting VEGF. The results were expressed as mean±SD, and analyzed using one-way ANOVA followed by Turkey test.The data were compared to control, eapressed as *p<0.05; The results which compared to si-NC were presented as %p<0.05; And the statistics which compared to VEGF mimic-NC were expressed as &p<0.05.

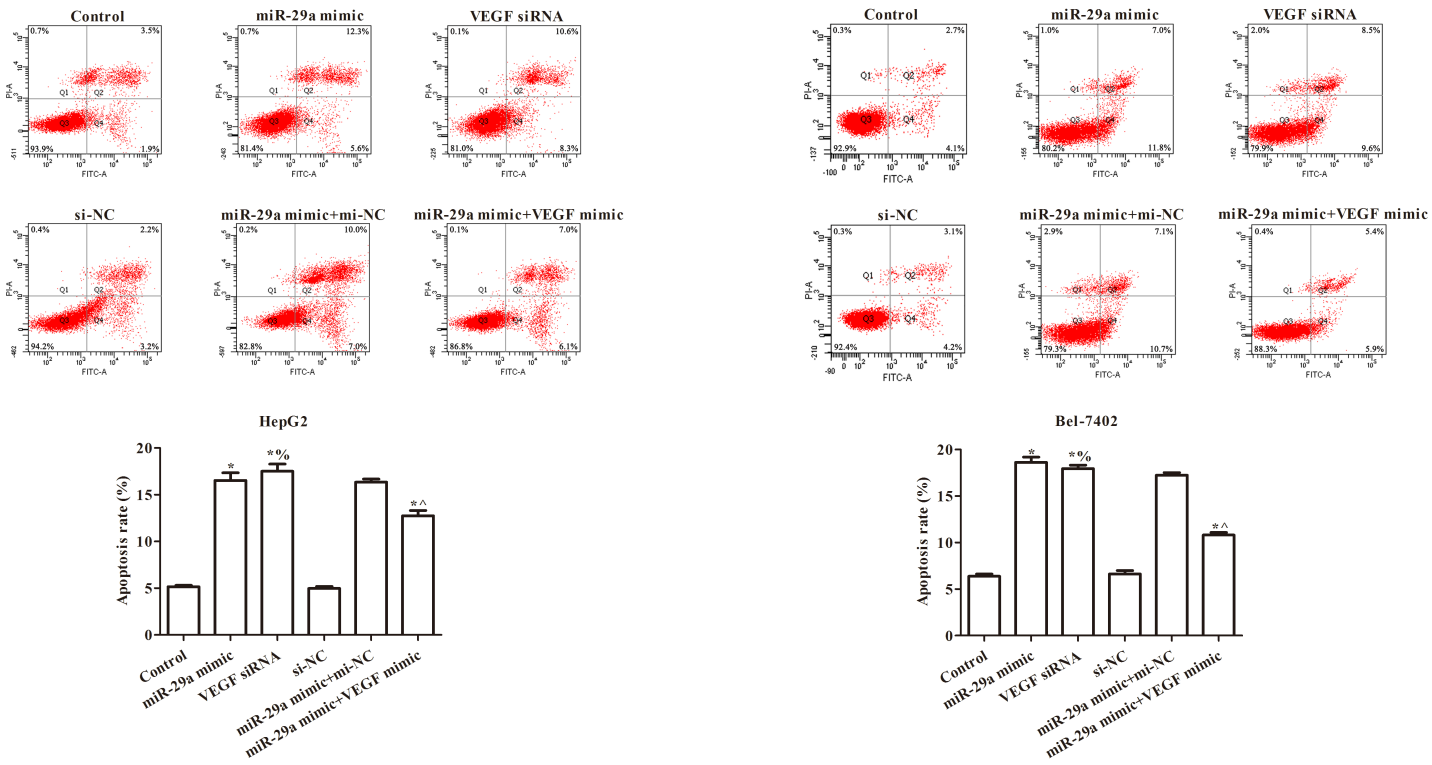

Figure 6

Effect about that miR-29a targeted VEGF to module apoptosis of HepG2 (A) and Bel-7402 (B). The results were detected by flow cytometry, and miR-29a overexpression increased cells apoptosis by targeting VEGF. Q1 represented necrotic cells, Q2 presented late apopotic cells, Q3 represented living cells, Q4 represented early apoptotic cells. The apoptosis rate was ananlyzed by Q2 and Q4. The results were expressed as mean±SD, and analyzed using one-way ANOVA followed by Turkey test. The data which compared to control, presented as *p<0.05 and which compared to si-NC, expressed as %p<0.05; The results expressed as ^p<0.05 which compared to miR-29a mimic+ mimic-NC.

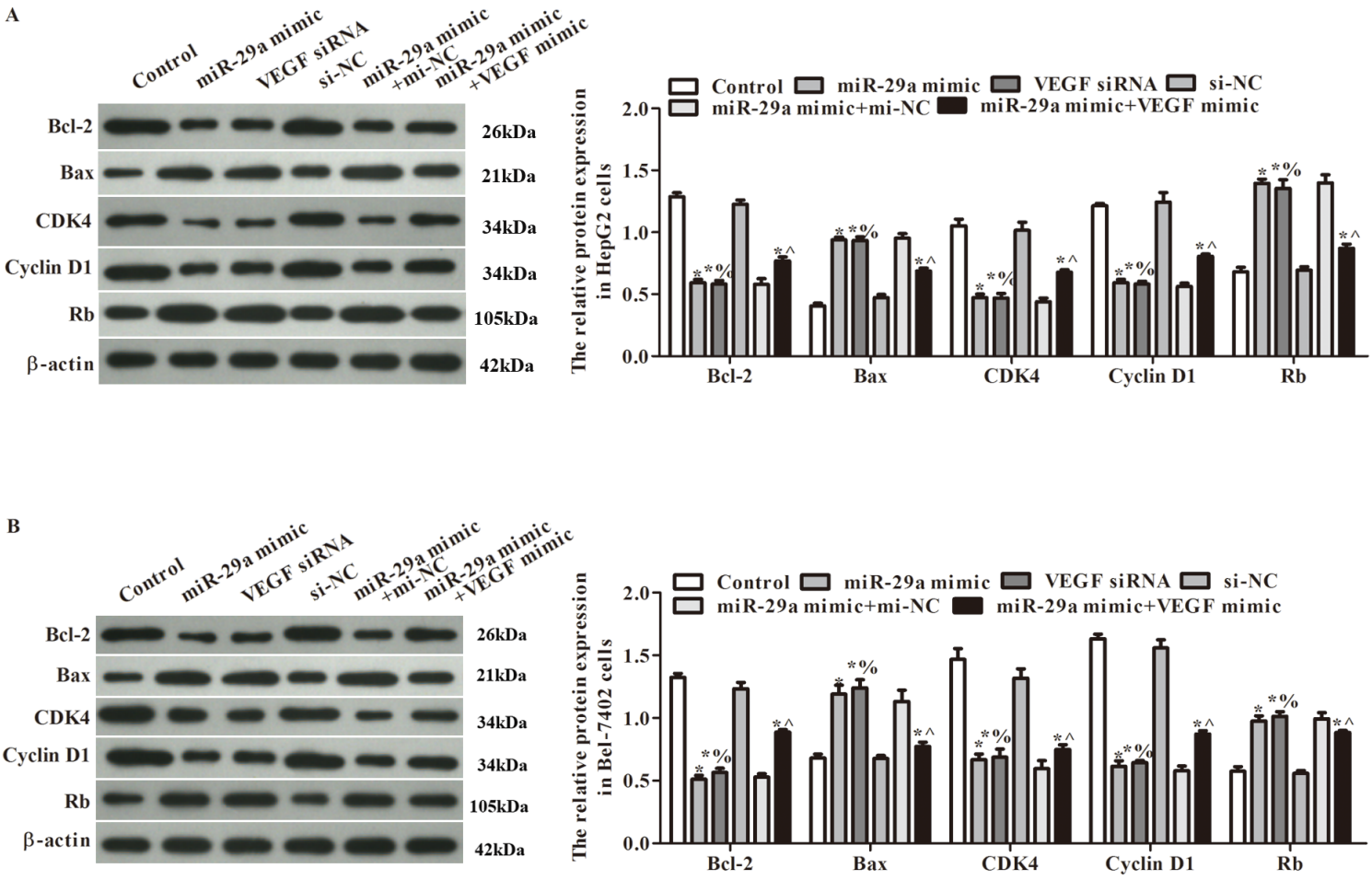

Figure 7

Western blot detection of the effect about that miR-29a targeted VEGF to module cell cycle and apoptosis related protein expression of HepG2 (A) and Bel-7402 (B). The full-length are presented in Supplementary Figure 4 and Figure 5. The results compared to control which were expressed as *p<0.05 and compared to si-NC which were presented as %p<0.05; And the data which compared to mir-29a mimic+ mimic-NC were expressed as ^p<0.05. The results were expressed as mean±SD, and analyzed using one-way ANOVA followed by Turkey test.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure3.pdf
- SupplementaryFigure5.pdf
- SupplementaryFigure2.pdf
- SupplementaryFigure4.pdf
- SupplementaryFigure1.pdf