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Tenacigenin B Has Anti-Tumor Effect in Lymphoma by *In Vitro* and *In Vivo* Study

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Background: The aim of this study was to examine the effects and mechanisms of tenacigenin B in lymphoma treatment by *in vitro* and *in vivo* experiment.

Material/Methods: Raji cells were treated by difference methods. Measuring the cell proliferation of difference groups was done by MTT assay; cell apoptosis and cell cycle of difference groups were evaluated by flow cytometer; relative mRNA expression was evaluated by real-time polymerase chain reaction (RT-PCR), and relative protein expressions were measured by western blot assay in an *in vitro* study. In an *in vivo* study, we used a nude mice model to explore the anti-tumor effects and mechanism of tenacigenin B. Cell apoptosis was measured by TUNEL assay; relative protein expressions were evaluated by immunohistochemistry assay, and relative mRNA expression was evaluated by RT-PCR. In addition, the blood components of difference groups were measured.

Results: Compared with the Normal control group, the cell proliferation rate was significantly downregulated, with cell apoptosis significantly increasing with G1 phase in the Drug group and the si-Aurora-A group ($P<0.05$, respectively). The PTEN, PI3K, AKT, P53, and P21 mRNA and protein expressions of the Drug group, the si-Aurora-A group, and the si-Aurora-A+Drug group were significantly different ($P<0.01$, respectively), The tumor volume and weight of the Drug group, the si-Aurora-A group, and the si-Aurora-A+Drug group were significantly suppressed compared with the Normal group ($P<0.01$, respectively). The positive apoptosis cell number in the Drug group, the si-Aurora-A group, and si-Aurora-A+Drug group were increased compared with that of Normal group ($P<0.01$, respectively).

Conclusions: Tenacigenin B had anti-tumor effects on lymphoma via regulation of Aurora-A *in vitro* and *in vivo*.

MeSH Keywords: Adenolymphoma • Apoptosis Inducing Factor • Aurora Kinase A • Cerebral Revascularization

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Background

Malignant lymphoma is a kind of tumor with abnormal differentiation of lymphoid stem cells and clonal proliferation. Malignant lymphoma is one of the most common malignant tumors in childhood, and its incidence is increasing year by year [1]. At present, traditional chemotherapy is still the main therapeutic approach for malignant lymphoma. However, drug resistance is one of the important factors affecting long-term survival. Therefore, it is of great significance to seek a more effective prevention and treatment measure to improve the prognosis and quality of life of affected children. Glaucescent fissistigma root, also known as Marsdenia tenacissima, is a plant from the genus of Marsdenia in the Asclepiadaceae family. Modern pharmacology suggests that glaucescent fissistigma root has several chemical constituents including steroidal glycosides, organic acids, and polysaccharides. Among them, C21 steroidal glycosides and polysaccharides have anti-tumor activity [2], have obvious inhibitory effects on many malignant tumor cells, and has been used to treat multiple digestive system tumors and achieved good results. Tenacigenin B is an important extraction of the glaucescent fissistigma root, and until now, the effects of tenacigenin B in malignant lymphoma treatment has been unclear.

The Aurora kinase family is a group of serine/threonine protein kinases composed of Aurora A kinase, Aurora B kinase, and Aurora C kinase. Its function is to carry out various mitotic activities and maintain genomic integrity, which is critical to the process of cell mitosis [3,4]. Studies have shown that Aurora A plays an important role in the anti-tumor effect of drugs [5,6]. However, it is still unclear with respect to the role Aurora A plays in inhibiting malignant lymphoma cells when using the glaucescent fissistigma root. In this study, the human Burkitt lymphoma cell line, Raji cells, were used as the lymphoma cell model to explore the mechanism of Aurora A *in vivo* and *in vitro*, so as to provide a theoretical reference for further enriching the understanding of the anti-tumor effect and mechanism of tenacigenin B.

Material and Methods

Materials

The following materials were used in this study: tenacigenin B (HP Bio Shanghai Technology Co., Ltd.); fetal bovine serum (FBS; ExCell Biology, FSS500, USA); RPMI-1640 culture medium (Gibco, 31800-105, USA); Annexin V-APC/7-AAD apoptosis detection kit (Jiangsu Keygen Biotech Corp., Ltd., KGA1024; China); MTT (Amresco, 0793, USA); DMSO (Sigma, D2650, USA); cell cycle detection kit (Jiangsu Keygen Biotech Corp., KGA511; China); TRIzol (Invitrogen, 15596-026, USA); first-strand cDNA synthesis kit (TaKaRa, RR036B, Japan); One Step TB Green™ PrimeScript™ RT-PCR Kit II (SYBR Green) (TaKaRa, RR086B, Japan); PTEN, PI3K, AKT, p-AKT, P53, and P21 (Abcam, Cambridge, UK).

Cell grouping and treatment

Raji cells were purchased from ATCC; Raji cell were cultured by RPMI-1640 medium contained 10% FBS. Raji cells were divided into a Normal group, Drug group, si-Aurora-A group, si-Aurora-A+Drug group, Aurora-A group, and Aurora-A+Drug group. Raji cells in the Normal group were cultured in normal medium, and Raji cells in the Drug group were treated with 50 μg/mL tenacigenin B in the conventional medium. In the si-Aurora-A group, Raji cells were transfected with si-Aurora-A by lipofectamine 2000 and cultured in normal medium. Furthermore, in the si-Aurora-A+Drug group, Raji cells were transfected with si-Aurora-A by lipofectamine 2000 and cultured in medium containing tenacigenin B 50 μg/mL. In the Aurora-A group, Raji cells were transfected with Aurora-A by lipofectamine 2000 and cultured in normal medium. Besides, Raji cells in the Aurora-A+Drug group were transfected with Aurora-A by lipofectamine 2000 and cultured in medium containing 50 μg/mL tenacigenin B.

Animal grouping and treatment [3]

Thirty-six Balb/c nude mice (18–22 g, 4–5 weeks of age, male) were purchased from Shanghai Lingchang Biotechnology Co., Ltd. The study nude mice were randomly divided into the following groups: Normal, Drug, si-Aurora-A, si-Aurora-A+Drug, Aurora-A, and Aurora-A+Drug groups. There were 6 mice in each group.

The Raji cells were treated by difference methods. The cell concentration was adjusted to 1×10^7/mL; each nude mouse was inoculated with 0.1 mL cell solution under the right axilla.

Nude mice in the Normal group were inoculated with Raji cells, followed by tail vein injection of normal saline (50 mg/kg per day, every day) for 2 weeks. In the Drug group, mice were inoculated with Raji cells, followed by tenacigenin B through tail vein (50 mg/kg per day, every day) for 2 weeks. In the si-Aurora-A group, mice were inoculated with Raji si-Aurora-A for the interference of cells, and then injected with normal saline through tail vein (50 mg/kg per day, every day) for 2 weeks. In the si-Aurora-A+Drug group, following the inoculation of Raji Aurora-A for interfering cells, mice received tenacigenin B via tail vein (50 mg/kg per day, every day) for 2 weeks. In the Aurora-A group, mice were inoculated with Raji Aurora-A to over-express cells and then injected with normal saline via tail vein (50 mg/kg per day, every day) for 2 weeks. In addition, the mice in the Aurora-A+Drug group were inoculated with Raji Aurora-A to over-express cells and then injected

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with tenacigenin B via tail vein (50 mg/kg per day, every day) for 2 weeks. Two weeks later, serum samples were extracted from the nude mice after 12-hour fasting in each group. After that, the nude mice were sacrificed, and tumors were removed and weighed.

The tumor tissues were taken out and divided into 2 parts, 1 part was stored in –80°C until used to measure relative mRNA expression, and 1 part was fixed in 4% paraformaldehyde until used.

This animal experiment was approved by the ethics committee of Nanjing University of Traditional Chinese Medicine (No. 2017060301),

MTT assay

Raji cells at logarithmic growth phase were inoculated into 24-well cell culture plate at an adjusted cell density of 1×10^5/mL, with 100 μL cell suspension added to each well, followed by the application of different treatments. At the same time, the control group was set up; each group had 3 duplicate wells, and the experiment was repeated 3 times. Following 48-hour cell incubation, 10 μL MTT (5 mg/mL) were added to each well, 4 hours before the end of incubation, followed by centrifugation at 1500 rpm for 10 minutes. After discarding the supernatant, 100 μL of DMSO was added to each well and then the plates were shaken for 15 minutes to dissolve crystallization. The absorbance per well was determined by automatic Microplate Reader at 490 nm, and the cell proliferation rate was then calculated.

Detection of cell apoptosis and cell cycle distribution by flow cytometry

Raji cells at logarithmic growth phase were inoculated into 24-well cell culture plates at the adjusted cell density of 1×10^6/mL, with 1 mL cell fluid added in each well, followed by different treatments. The control group was set up simultaneously; each group had 3 duplicate wells, and the experiment was repeated 3 times. Cells were incubated for 48 hours, and then centrifuged, collected, and fixed with 70% ethanol. After overnight culture at 4°C, cells were then washed with phosphate-buffered saline (PBS) 2 times, and supplemented with 1 mL propidium iodide (PI). After cell incubation in the dark at 4°C for 30 minutes, cell apoptosis was detected by flow cytometry.

Detection of related protein expression by western blotting

Protein concentration was determined by bicinchoninic acid (BCA) method after cell protein samples were collection from each group. Protein at 80–100 μg was sampled and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis was performed. Afterwards, electrorotation was carried out for 60 minutes under constant current (250–350 mA), and then samples were sealed with skim milk powder for 1–2 hour. Following the addition of primary antibody and overnight incubation in a shaking table at 4°C, samples were washed with TBST 3 times (5–10 minutes each time). Subsequently, horseradish peroxidase conjugated secondary antibody was supplemented and incubated at 37°C for 2 hours. The membrane was washed with TBST another 3–5 times (5~10 minutes each time) and then chemiluminescent solution was added, and the gel image processing system was used to observe and analyze the results.

Table 1. The relative gene primer sequence.

Gene name	Primer sequence	Size
PTEN	Forward: 5'-GGTCTGAGTCGCCTGTCACCAT-3' Reverse: 5'-CCGTGTTGGAGGCAGTAGAAGG-3'	80bp
PI3K	Forward: 5'-TGCGACAAGACTGCCGAGAG-3' Reverse: 5'-GCCTGAAGCTGAGCAACATCCT-3'	97bp
AKT	Forward: 5'-CAGGATGTGGACCAACGTGAGG-3' Reverse: 5'-GGCAGCGGATGATGAAGGTGTT-3'	106bp
P53	Forward: 5'-TGCTTTCCACGACGGTGACAC-3' Reverse: 5'-ATCTGACTGCGGCTCCTCCAT-3'	85bp
P21	Forward: 5'-CTCGTTCTCGCCCGCAATTTAG-3' Reverse: 5'-AGCTCATCTGCTGCCTGTATCA-3'	82bp
GAPDH	Forward: 5'-AGATCATCAGCAATGCCTCCT-3' Reverse: 5'-TGAGTCCTTCCACGATACCAA-3'	90bp

Blood component detection

Blood components in nude mice of each group were detected using a blood cell analyzer.

Real-time polymerase chain reaction (RT-PCR)

We used RT-PCR to evaluate relative mRNA expression in cells and tumor tissues. Total RNA was extracted using the TRIzol

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reagent (Invitrogen, USA) according to the manufacturer's instruction. RNA concentration was quantitated by Nanodrop (Thermo Fisher, USA). RNA was converted to cDNA by reverse transcription, which was subjected to qRT-PCR with SYBR Green kit (Takara, Japan). After that, the PCR amplification was performed: 95°C for 5 minutes, followed by 40 cycles of 95°C for 5 seconds, and 61°C for 30 seconds. The PCR primer information are presented in Table 1. The internal reference was GAPDH, and the calculation of qRT-PCR results was determined using $2^{-\Delta\Delta ct}$ method. Primer sequence are shown in Table 1.

Paraffin section

Dewaxing and hydration were carried out according to the conventional method. The slices were soaked in xylene for 5 minutes, then replaced with xylene for another 5 minutes. Slices were dehydrated in ascending series of ethanol, including absolute ethanol for 5 minutes, 95% ethanol for 5 minutes, 85% ethanol for 5 minutes, and 70% ethanol for 5 minutes, respectively, followed by PBS washing for 3 times (3 minutes each time).

TUNEL staining of tumor-bearing tissues

Tumor tissue specimens were fixed with 4% paraformaldehyde, followed by embedding in paraffin blocks, sectioning onto slides, then slide sections soaked in xylene and dehydration with ethanol. After washing with PBS 3 times, tissue sections were treated with proteinase K working fluid for 15 minutes at 37°C. Following PBS washing, 3 times, the tissue specimens had 50 μL TUNEL reaction mixture added, and were incubated at 37°C for 1 hour with a cover glass. After PBS washing 3 times, 50 μL converter-POD was added, followed by incubation at 37°C for 30 minutes with a cover glass. Then the reacted specimens were washed with PBS 3 times, and 100 μL DAB substrate was added, and the reaction was continued for 10 minutes at 25°C. Following PBS washing, 3 times, tissue sections were re-stained with hematoxylin for 30 seconds. Tissue sections were mounted and photographed after dehydration in an ascending series of ethanol concentrations.

Immunohistochemistry of tumor-bearing tissues

Tumor tissue specimens were fixed with 4% paraformaldehyde, embedded into paraffin blocks, sectioned onto slides, and soaked in xylene twice (5 minutes each time), then dehydration with gradient ethanol (100%, 95%, 90%, 80%, and 70%) at 1 minute for each ethanol concentration. After washing in PBS, 0.3% hydrogen peroxide methanol solution was added for 30 minutes, then the specimens were washed in PBS 3 times (5 minutes each time); then 0.3% Triton X-100 was added and specimens treated for 30 minutes, then washed 3 times (5 minutes each time). After that, diluted mouse anti-human TRAIL monoclonal antibody (1: 500) was added and specimens were stored at 4°C for 24 hours. After absorption of the antibody, specimens were washed in PBS 3 times (5 minutes each time). Subsequently, PBS diluted biotin-labeled rabbit anti-mouse secondary antibody was added, and the specimens incubated at room temperature for 2 hours. Specimens were then washed 3 times in PBS (5 minutes each time), and ABC complex was added, and the specimens were incubated at room temperature for 2 hours, followed by washing 3 times in PBS (5 minutes each time) and then rapid flushing with distilled water, 3 times. DAB was added to samples to develop for 10 minutes, then samples were rinsed quickly with distilled water, and re-stained with hematoxylin for 30 seconds. Samples were mounted and photographed after dehydration in an ascending series of ethanol concentrations.

Statistical analysis

The data were analyzed using SPSS 20.0 software, the data are shown as mean ±SD (standard deviation), the differences between groups were analyzed by one-way ANOVA with least significant difference (LSD) test; $P<0.05$ was considered to indicate statistically significant differences.

Results

Tenacigenin B had effects on Raji cell proliferation, cell apoptosis, and cell cycle

Figure 1A shows the cell proliferation rate of the Drug group, the si-Aurora-A group, and the si-Aurora-A+Drug group were significantly depressed compared with the Normal group ($P<0.05$, respectively), meanwhile, compared with the Drug group, the cell proliferation rate of the si-Aurora-A+Drug group was significantly suppressed ($P<0.05$). By flow cytometer, the cell apoptosis of the Drug group, the si-Aurora-A group, and the si-Aurora-A+Drug group were significantly upregulated (Figure 1B) with the G1 phase significantly increasing compared with the Normal group ($P<0.05$, respectively) (Figure 1C). Meanwhile, the cell apoptosis of the si-Aurora-A+Drug group was significantly inhibited with the G1 phase upregulated compared to the Drug group ($P<0.05$, respectively). The relative data are shown in Figure 1.

Tenacigenin B affected relative mRNA and protein expression *in vitro*

By RT-PCR, the PTEN, P53, and P21 gene expression in the Drug group, the si-Aurora-A group, and the si-Aurora-A+Drug group were significantly upregulated, and the PI3K and AKT mRNA expression of the Drug, the si-Aurora-A, and the si-Aurora-A+Drug groups were significantly downregulated compared with the Normal group ($P<0.05$, respectively, Figure 2A, 2B). The PTEN, P53, and P21 gene expression in the si-Aurora-A+Drug group

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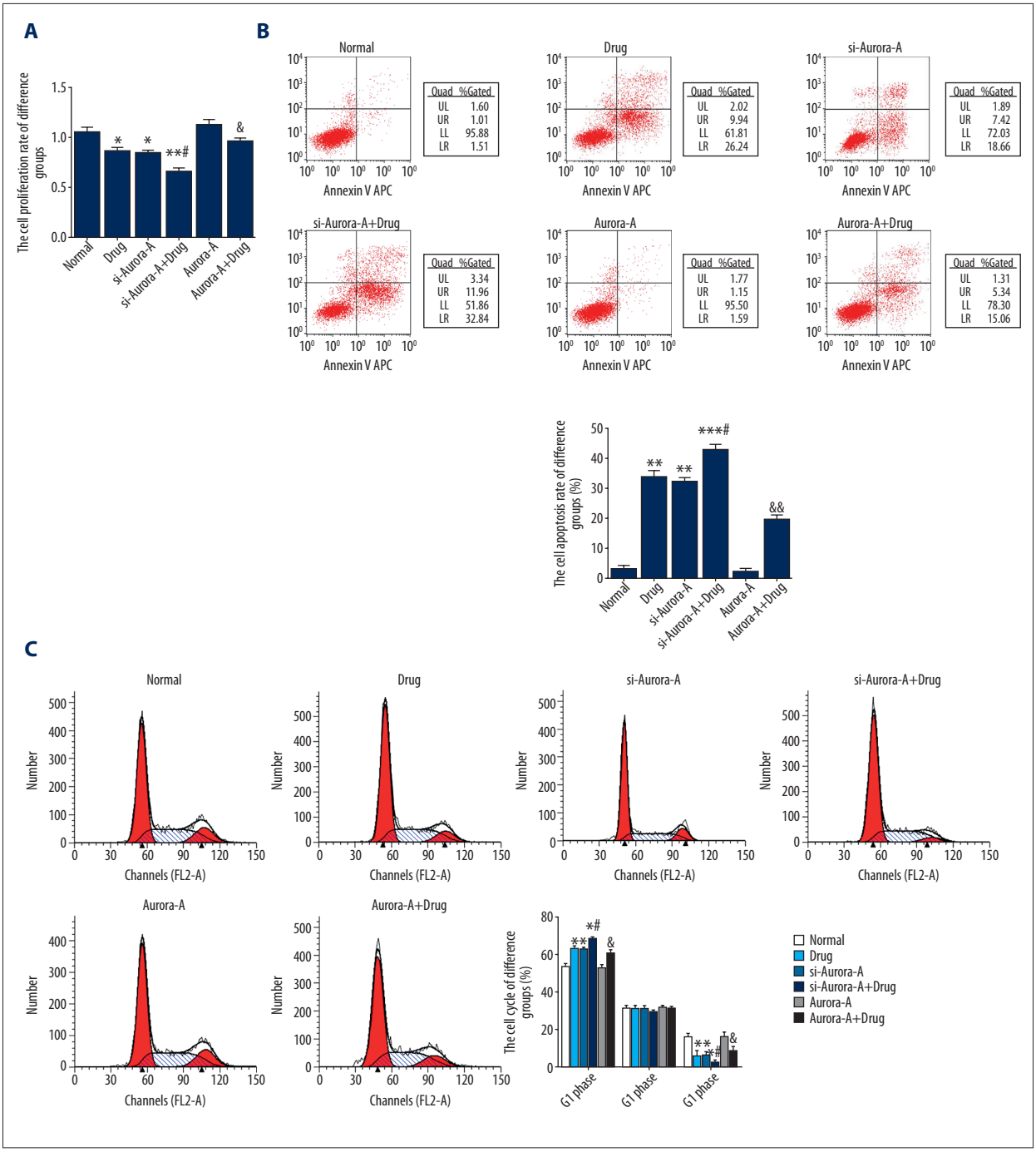

Figure 1. Tenacigenin B had effects on Raji cell proliferation, cell apoptosis, and cell cycle. (**A**) Tenacigenin B affected cell proliferation as shown by MTT assay. * $P<0.05$, ** $P<0.01$ versus Normal group; # $P<0.05$ versus Drug group; & $P<0.05$ versus Aurora-A group. (**B**) Tenacigenin B affected cell apoptosis as shown by flow cytometer. ** $P<0.01$, *** $P<0.001$ versus Normal group; # $P<0.05$ versus Drug group; & $P<0.01$ versus Aurora-A group. (**C**) Tenacigenin B affected cell cycle as shown by flow cytometer. $P<0.05$, versus Normal group; # $P<0.05$ versus Drug group; & $P<0.05$ versus Aurora-A group.

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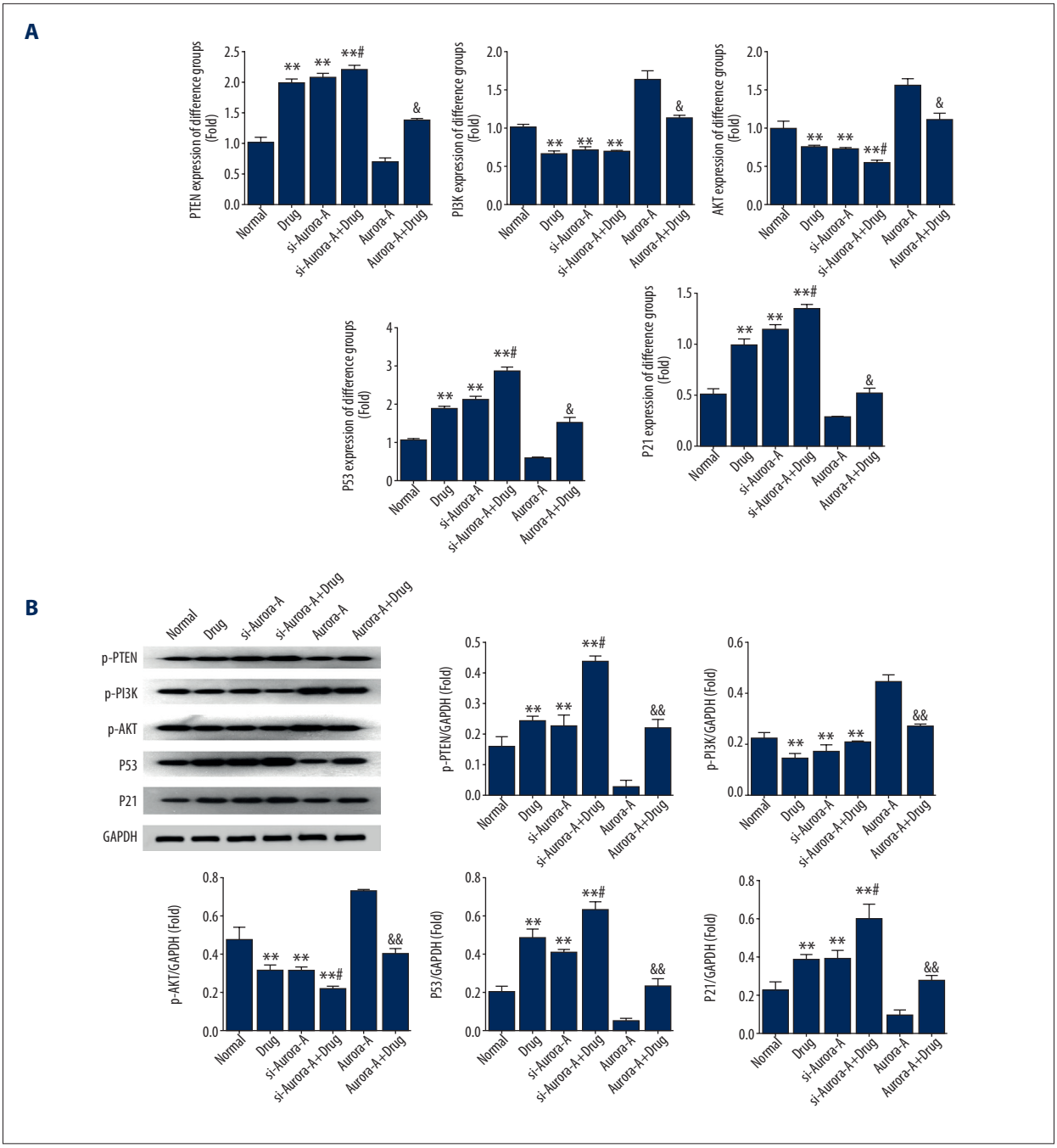

Figure 2. Tenacigenin B affected relative mRNA and protein expressions. (**A**) Tenacigenin B had effects on relative gene expressions as shown by RT-PCR. ** *P*<0.01 versus Normal group; # *P*<0.05 versus Drug group; & *P*<0.05 versus Aurora-A group. (**B**) Tenacigenin B had effects on relative proteins expressions as shown by western blot assay. ** *P*<0.01 versus Normal group; # *P*<0.05 versus Drug group; & *P*<0.05 versus Aurora-A group. RT-PCR – real-time polymerase chain reaction.

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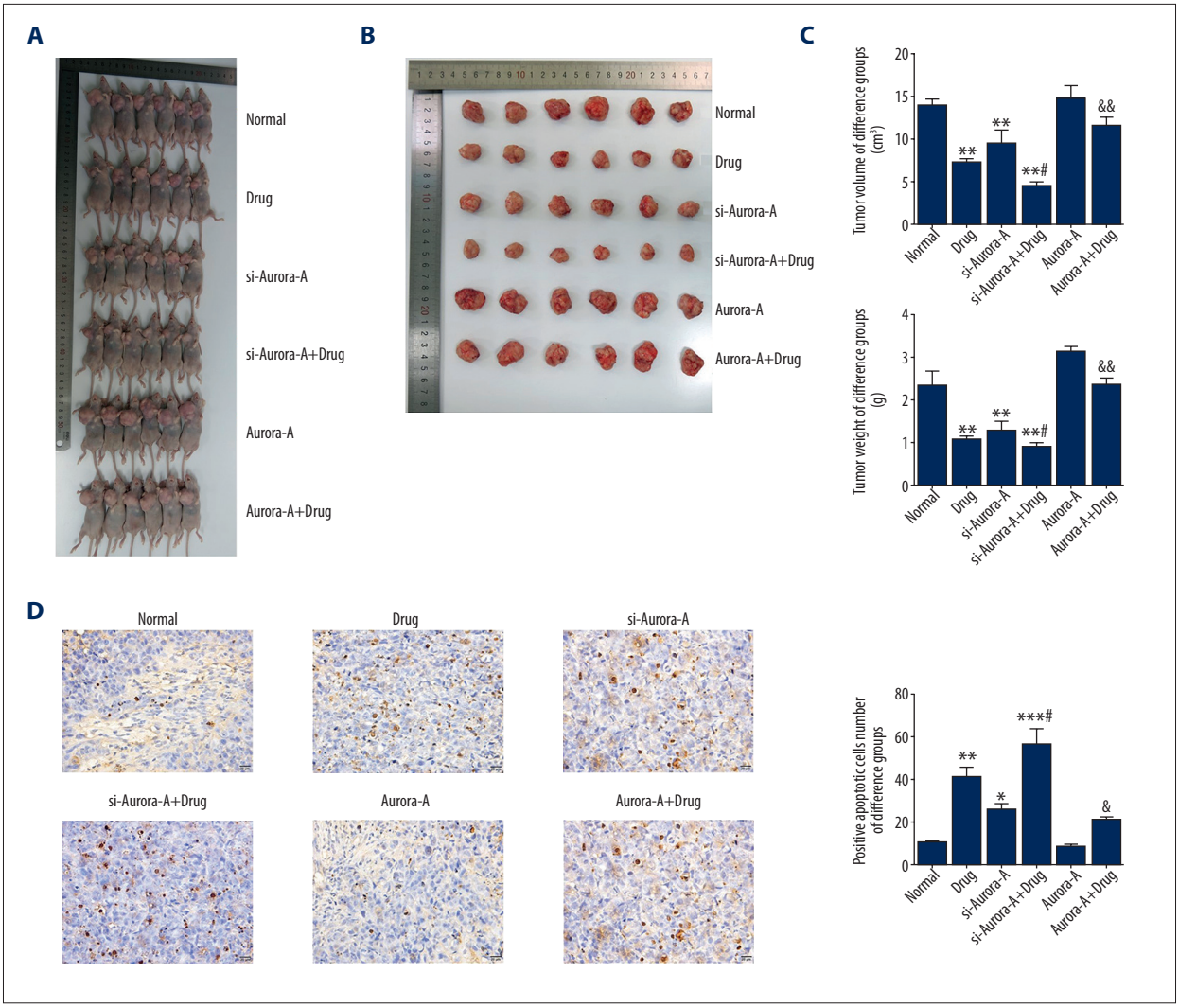

Figure 3. Tenacigenin B had anti-tumor effects *in vivo*. (**A**) The tumor in intact body. (**B**) The tumor tissue removed. (**C**) Tenacigenin B had effects on tumor volume and weight *in vivo*. ** $P<0.01$ versus Normal group; # $P<0.05$ versus Drug group; & $P<0.01$ versus Aurora-A group. (**D**) Tenacigenin B had effects on cell apoptosis as shown by TUNEL assay *in vivo*. ** $P<0.01$, *** $P<0.001$ versus Normal group; # $P<0.05$ versus Drug group; & $P<0.05$ versus Aurora-A group.

were significantly upregulated, and the PI3K and AKT mRNA expressions in the si-Aurora-A+Drug group were significantly downregulated compared with the Drug group ($P<0.05$, respectively, Figure 2A, 2B).

Tenacigenin B had anti-tumor effects *in vivo*

In order to study the anti-tumor effects of tenacigenin B *in vivo*, we designed a nude mice experiment. Figure 3A shows the nude mice of difference groups before treatment, and Figure 3B shows the tumors of the difference groups. We measured the tumor volume and weight of the difference groups, and the results showed the tumor volume and weight of the Drug group, the si-Aurora-A group, and the si-Aurora-A+Drug group were significantly suppressed compared with the Normal group ($P<0.01$, respectively, Figure 3C). The tumor volume and weight of the si-Aurora-A+Drug group were significantly depressed compared with the Drug group ($P<0.05$, respectively, Figure 3C). In order to measure cell apoptosis *in vivo*, we evaluated cell apoptosis by TUNEL assay in tumor tissues of the difference groups. The positive apoptosis cell number in the Drug group the si-Aurora-A group, and the si-Aurora-A+Drug group were significantly upregulated compared with the Normal group ($P<0.05$, $P<0.01$ or $P<0.001$, respectively, Figure 3D). The positive apoptosis cell number in the si-Aurora-A+Drug group was significantly downregulated compared with the Drug group ($P<0.05$, Figure 3D).

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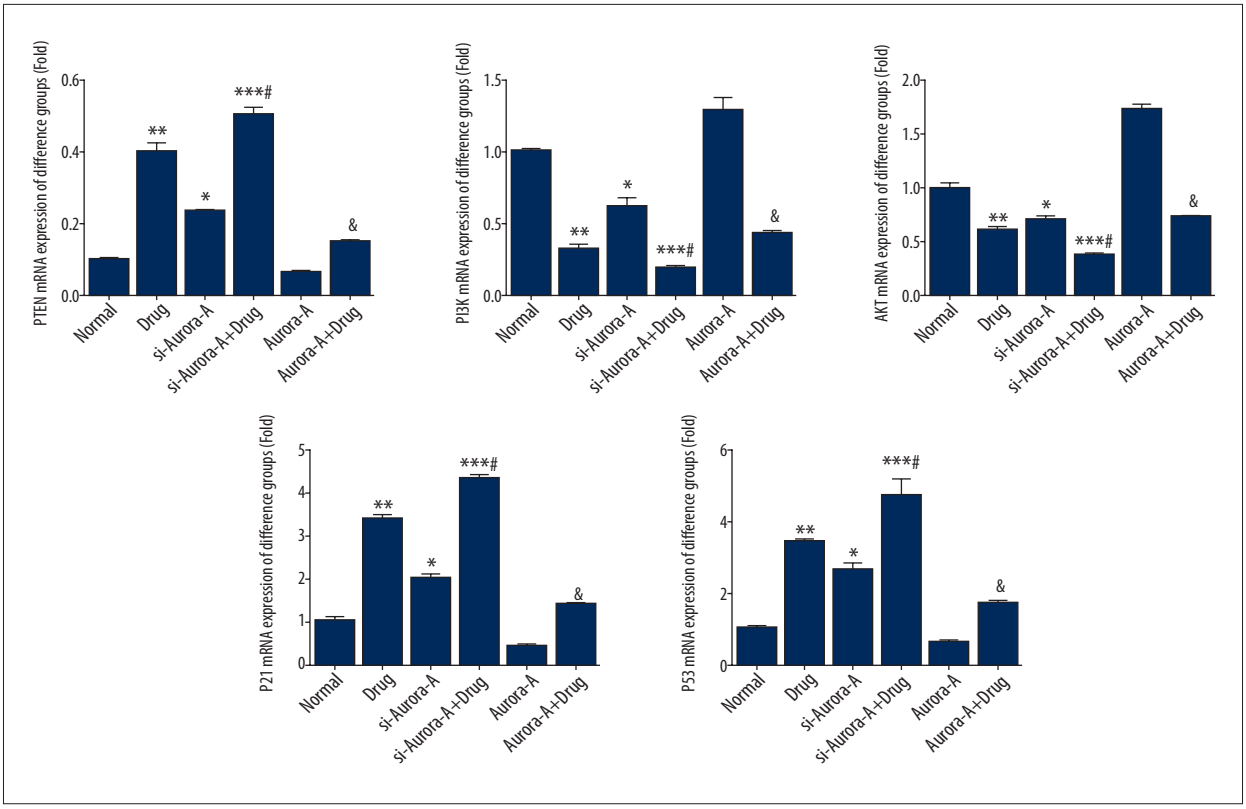

Figure 4. Tenacigenin B had effects on relative mRNA expressions as shown by RT-PCR. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus Normal group; # $P<0.05$ versus Drug group; & $P<0.05$ versus Aurora-A group. RT-PCR – real-time polymerase chain reaction.

Table 2. Blood components of difference groups.

	RBC (10^{12}/L)	HGB (g/L)	PLT (10^9/L)	WBC (10^9/L)
Normal	5.04±0.026	141.4±1.054	250.47±0.907	10.33±0.208
Drug	5.11±0.025	139±0.436	250.6±0.917	9.91±0.035
si-Aurora-A	5.04±0.015	139.23±1.986	251.37±0.833	9.59±0.046
si-Aurora-A+Drug	5.17±0.021	140.8±0.4	252.4±0.7	9.43±0.08
Aurora-A	5.24±0.085	141.57±0.85	250.33±1.266	12.23±0.306
Aurora-A+Drug	5.21±0.031	142.42±0.625	252.37±1.258	12.63±0.503

RBC – red blood cell; HGB – hemoglobin; PLT – platelet; WBC – white blood cell.

Tenacigenin B affected relative mRNA expressions and blood components *in vivo*

Compared with the normal tumor tissues, the PTEN, P53, and P21 gene expressions in the Drug group, the si-Aurora-A group, and the si-Aurora-A+Drug group were significantly increased and the PI3K and AKT gene expressions were significantly decreased ($P<0.05$, $P<0.01$, or $P<0.001$, respectively, Figure 4). Meanwhile, the PTEN, P53, and P21 gene expressions in the si-Aurora-A+Drug group were significantly upregulated and the PI3K and AKT gene expression in the si-Aurora-A+Drug group were significantly upregulated compared with the Drug group ($P<0.05$, respectively). The relative data are shown in Figure 4. There were no significantly differences among the groups in red blood cell (RBC), hemoglobin (HGB), platelet (PLT), and white blood cell (WBC) levels in the blood of the nude study mice. The relative blood components data are shown in Table 2.

Tenacigenin B affected relative proteins expression *in vivo*

By immunohistochemistry (IHC) assay, the PTEN, P53, and P21 protein expressions in the Drug, the si-Aurora-A, and the si-Aurora-A+Drug groups were significantly upregulated and the PI3K, AKT, and p-AKT protein expressions in the Drug, the si-Aurora-A,

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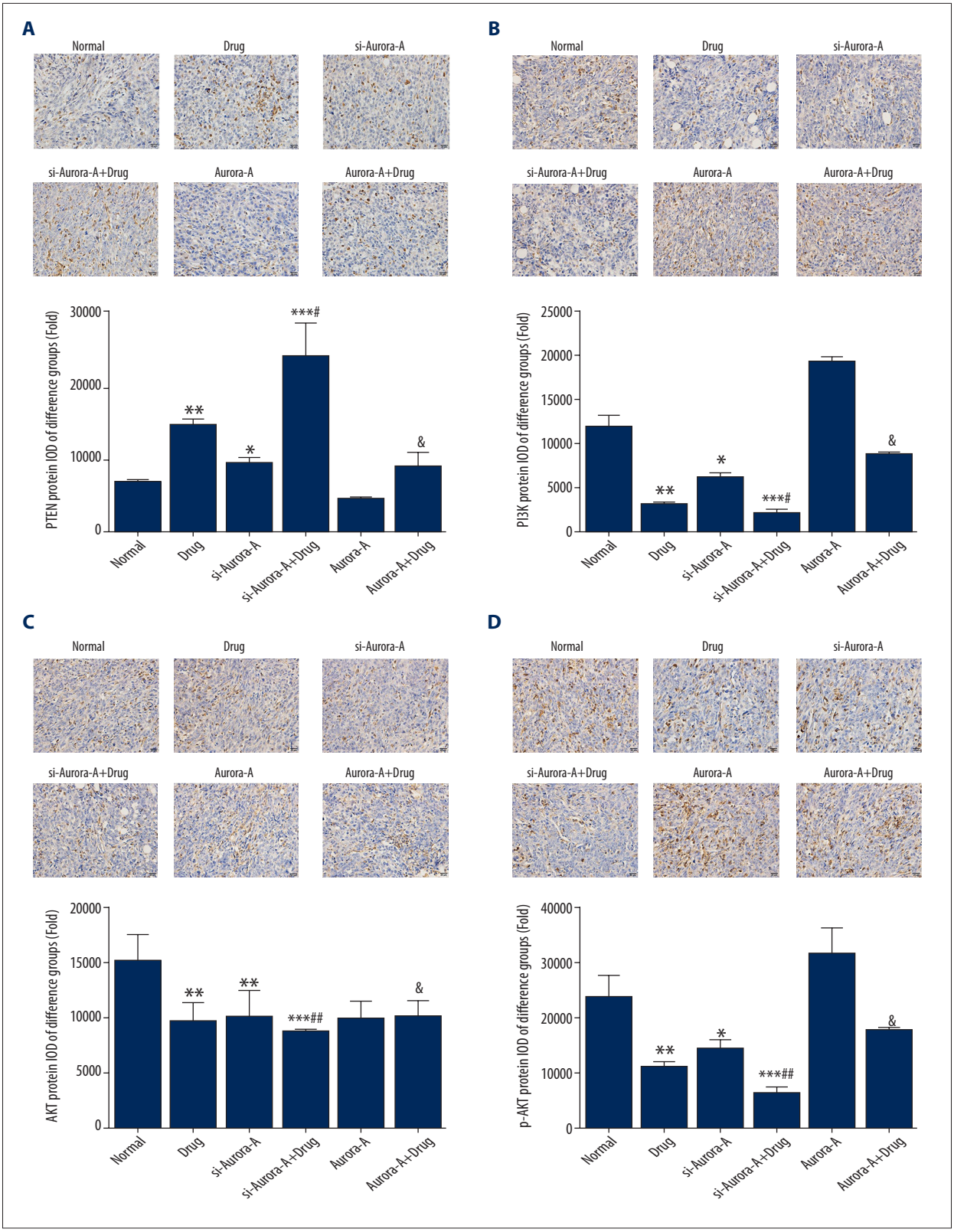

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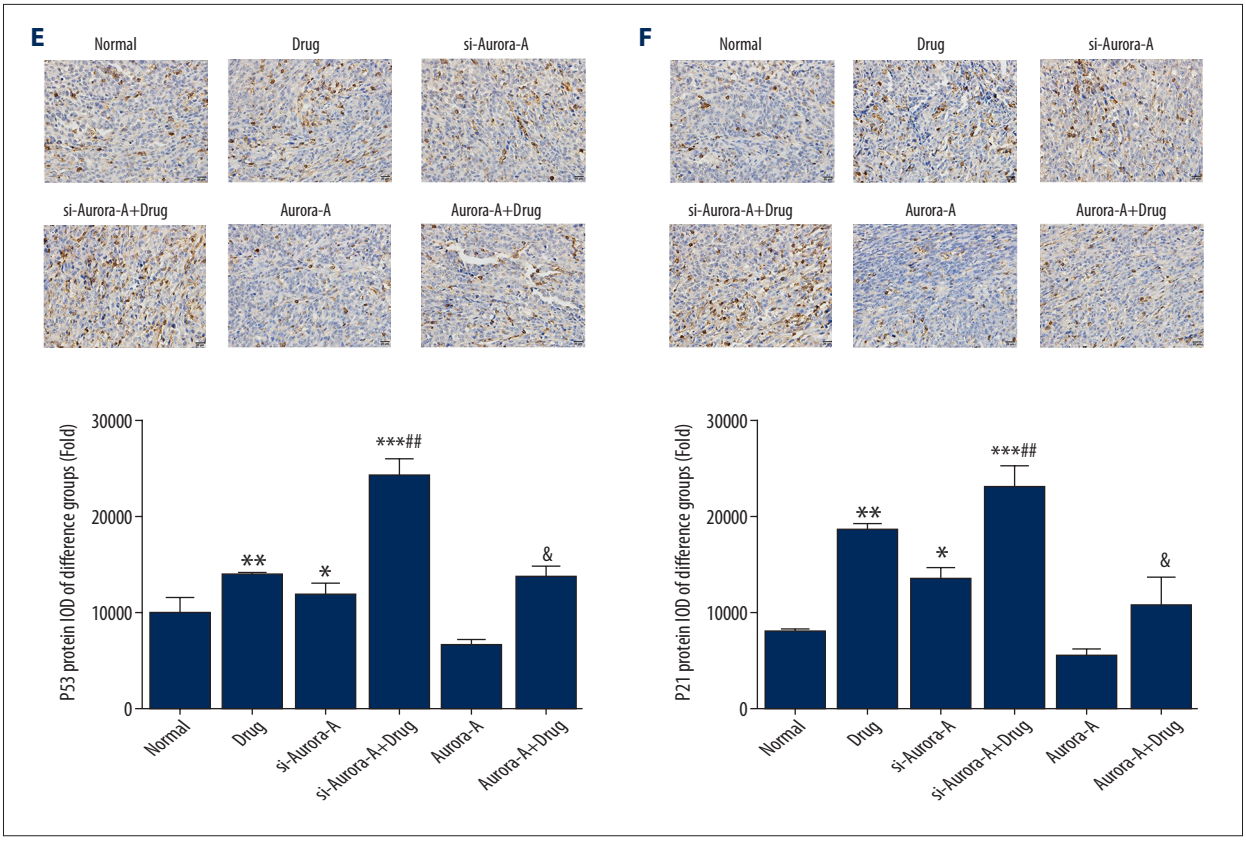

Figure 5. Tenacigenin B had effects on relative protein expressions as shown by IHC (200×). (**A**) The PTEN protein expression of difference groups as shown by IHC assay. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus Normal group; # $P<0.05$ versus Drug group; & $P<0.05$ versus Aurora-A group. (**B**) The PI3K protein expression of difference groups as shown by IHC assay. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus Normal group; # $P<0.05$ versus Drug group; & $P<0.05$ versus Aurora-A group. (**C**) The AKT protein expression of difference groups as shown by IHC assay. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus Normal group; # $P<0.05$ versus Drug group; & $P<0.05$ versus Aurora-A group. (**D**) The p-AKT protein expression of difference groups as shown by IHC assay. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus Normal group; # $P<0.05$ versus Drug group; & $P<0.05$ versus Aurora-A group. (**E**) The P53 protein expression of difference groups as shown by IHC assay. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus Normal group; # $P<0.05$ versus Drug group; & $P<0.05$ versus Aurora-A group. (**F**) The P21 protein expression of difference groups as shown by IHC assay. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus Normal group; # $P<0.05$ versus Drug group; & $P<0.05$ versus Aurora-A group. IHC – immunohistochemistry.

and the si-Aurora-A+Drug groups were significantly downregulated compared with the Normal group ($P<0.05$, $P<0.01$, or $P<0.001$, respectively). Meanwhile, there were significant differences between the si-Aurora-A+Drug group and the Drug group in PTEN, PI3K, AKT, p-AKT, P53, and P21 protein expressions ($P<0.05$, respectively). The relative data are shown in Figure 5.

Discussion

Aurora kinase is a centrosome-related kinase existed in eukaryotic cells, playing important roles in centrosome replication, bipolar spindle formation, chromosome rearrangement, and chromosome checkpoint detection during critical mitosis process [7]. Abnormal expression of Aurora kinase may interfere with functions of checkpoints in mitosis, resulting in genetic instability and induced tumor growth [8,9]. The majority of current studies pay attention to Aurora B; the mechanism of Aurora-A in anti-tumor drugs remains unclear. Compared with Aurora B, Aurora A is over-expressed in various tumors such as ovarian cancer, colon cancer, gastric cancer, and breast cancer, which has certain guiding significance for the treatment of lymphoma [10]. The function of Aurora A is correlated with the separation and maturation of the centrosome and spindle assembly, which may result in the termination of mitosis and the formation of aneuploidy by activating the checkpoints of the spindle [11]. Over-expression of Aurora A disrupts the dynamic balance of centrosome and microtubules, alters genomic stability, and leads to the occurrence of tumors [11]. The mRNA and protein expression of Aurora A are extremely low in the G1 and S phases, and they are then increased at the peak of the G2 and M phases. The expression of Aurora A

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may decrease rapidly when entering the next G1 phase following the completion of the former cell cycle [12].

In this study, we found that tenacigenin B could effectively inhibit the proliferation of Raji cells *in vivo* and *in vitro*, and promote the apoptosis of Raji cells by inducing a large number of cells to arrest in the G1 phase. However, as shown in Figure 1B and 1C, the inhibitory effects of tenacigenin B on cell apoptosis as well as the cell cycle did not disappear, but were reduced in the Raji cells. These results indicated that Aurora A may play a crucial role in interfering in the anti-tumor effect of tenacigenin B. In addition, there was no significant difference in blood composition among the nude mice groups, suggesting that tenacigenin B had no significant toxicity to nude mice.

Phosphatidylinositol-3 kinase (PI3K)/AKT functions significantly in tumor cell proliferation, angiogenesis, radiotherapy, and chemotherapy, which is important in maintaining the biological characteristics of hemangioma [13]. With external stimulation, the body may produce a variety of cytokines, induce activation of PI3K phosphorylation, and promote the production of the second messenger phosphatidylinositol 3,4,5-triphosphate. Furthermore, the second messenger can bind to proteins carrying PH domains such as PDK1 and Akt to promote Akt phosphorylation, activate or inhibit target gene expression in downstream pathways, and regulate cell proliferation and apoptosis [14,15]. Significantly, Aurora-A can regulate the expression of PI3K/AKT signaling pathway effectively [16–18]. Meanwhile, the PI3K/AKT signaling pathway can exert negative regulatory effects on P53 and P21 [19,20]. As a tumor suppressor gene, P53 can directly regulate cell cycle and apoptosis, and can be activated by oxidative stress and DNA damage, leading to upregulation of its protein expression. As one of the target genes of P53, P21 is a negative regulator of cell cycle that can be upregulated by activating P53 to induce G1 phase arrest of cells [21,22]. In this study, the results showed that expressions of P53 and P21 were increased with the inhibition of the PI3K/AKT signaling pathway, resulting in a great quantity of Raji cells arrested in the G1 phase.

Conclusions

Tenacigenin B can effectively inhibit the proliferation of lymphoma cells and increase the apoptosis of lymphoma cells by arresting a large number of cells in the G1 phase. *In vivo* and *in vitro* experiments suggest that the anti-tumor mechanism of tenacigenin B may be achieved through the suppression of Aurora-A expression, thereby inhibiting the activity of the PI3K/AKT signaling pathway, and increasing the activity of P53 and P21, resulting in decreased proliferation of Raji cells *in vivo* and *in vitro*.

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