European Review for Medical and Pharmacological Sciences 2017; 21: 3185-3192

Suppression of adriamycin resistance in osteosarcoma by blocking Wnt/β-catenin signal pathway

B.-Q. WU, Y. CAO, Z.-G. BI

Department of Orthopedics, First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China

Abstract. – OBJECTIVE: Wnt/β-catenin signal pathway plays a role in regulating cell proliferation and apoptosis, and is correlated with tumor onset, progression and drug resistance. B-cell lymphoma 2 (Bcl-2) is an anti-apoptotic factor inducing tumor cell drug resistance. Wnt/β-catenin signal pathway can modulate Bcl-2 expression. This study established a cell model of drug resistance using adriamycin (ADM) treatment. Wnt/β-catenin signal pathway was intervened to discuss its role in drug resistance of osteosarcoma cells.

MATERIALS AND METHODS: Expr of β-catenin and Bcl-2 was compared b U2OS and hFOB1.19 cells. ADM resista ell line U2OS/ADM was established for comp d β-catenin and Bcl-2 expression. Cell coun kit-8 (CCK-8) assay was used to test cell pro eration, followed by flow cytom r apopto ic rate under ADM concentr OS/ADM cells were further treated w i-β-c in and/ or β-catenin inhibitor X 39. β-c in and Bcl-2 expression were ured. CCK-8 and flow cytom try.

RESULTS: Compa to hF cells, U2OS cells had signific elevate tenin and Bcl-2 expressio OS/ADM cel higher β-catenin and B ssion than U OS, plus lower ADM s tivity uppressed apoptotic rate. Tra fection of s tenin and XAV939 suppress β-catenin an 2 expression, and sig antly enhanced AD sensitivity and ADM- ced a tosis.

C LUSIO Up-regulation of β-catenin play le i tentiati xpression and downstrea ptotic r Bcl-2, and in enhanc- ADM nce steosarcoma U2OS cells.

Key ds:
β- nin, Bcl-2, driamycin, U2OS, Osteosarcoma.

Introduction

sarcoma is one of the primary malignant bone tumors derived from mesenchymal cells commonly ics, with fe s including high ma ncy d disease progression and high mortality[1]. Th a certain number of patie fering from of treatment due to tu astasis or recur nce. Drug resistance is an important reason for treatment failure. iamycin (AD is a common chemotherapy treating va s tumors including osteosarc With its e application, drug resistance for ecome severe[2]. Wnt/β-catenin is a sign athway with high conservation in volution, and is widely involved in regulation of iferation, apoptosis, differentiation and vas Abnormal activation of Wnt/β-catenin signal pathway plays a role in occurrence and progression of multiple tumors such as pulmonary carcinoma[3], breast cancer[4], and liver cancer[5]. Moreover, Wnt/β-catenin signal pathway is also correlated with mediation of osteoblast differentiation and bone formation[6,7]. β-catenin is the core protein in Wnt/β-catenin signal pathway. Enhanced activity of Wnt/β-catenin signal pathway as induced by up-regulation of β-catenin is involved in occurrence of osteosarcoma[8] and tumor progression or metastasis[9]. Previous studies showed that abnormal elevated activity of Wnt/β-catenin signal pathway is correlated with lower chemotherapy sensitivity of multiple tumors including colorectal carcinoma[10], pancreatic cancer[11], oral cavity cancer[12] and glioma[13]. Multiple mechanisms participate in tumor cell drug resistance, including drug intake and metabolism, and excluding cell proliferation and apoptosis. Among those apoptosis escape and suppressed apoptosis are important mechanisms for tumor cell drug resistance. B-cell lymphoma 2 (Bcl-2) is an important anti-apoptotic factor in regulation of mitochondrial dependent apoptotic pathway[14]. Up-regulation of Bcl-2 can induce lower cell apoptosis and is closely correlated with acquire-

Corresponding Author: Yang Cao, MD; e-mail: yuemingsongyyy@163.com

ment of drug resistance of tumor cells[15]. Various studies showed that the activation of Wnt/β-catenin signal pathway plays a role in up-regulating expression of anti-apoptotic factor Bcl-2 and suppressing cell apoptosis[12,16]. This study established ADM resistant cell model by drug treatment, and intervened Wnt/β-catenin signal pathway in those cells to investigate the role of Wnt/β-catenin signal pathway in drug resistance of osteosarcoma cells.

Materials and Methods

MajorReagent and Materials

Human osteosarcoma cell line U2OS and normal human osteoblast cell line hFOB1.19 were purchased from Yuxin Biotech (Guangzhou, China). RPMI 1640 medium, Dulbecco's Modified Eagle Medium (DMEM)/F12 medium, fetal bovine serum (FBS), streptomycin-penicillin mixture and L-glutamine were purchased from Gibco (Grand Island, NY, USA). G418 was obtained from Amresco Inc. (Solon, OH, USA). Trizol and Lipofectamine 2000 were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). Reverse transcription reagent and SYBR Green dye were purchased from TaKaRa (Dalian, China). PCR primers were synthesized by Sango Co. Ltd. (Tokyo, Japan). Mouse anti-human monoclonal antibody, β-catenin siRNA sequence and negative control (NC) sequence, were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse anti-human β-catenin and β-actin polyclonal antibody were purchased from Active Motif (Waranac Lake, IN, USA). HRP labeled goat anti-rabbit antibody was purchased from Zhongxin Biotechnology (Shanghai, China). ADM was purchased from Hengrui Pharma (Lianyungang, Shanghai, China). Cell counting kit-8 (CCK-8) reagent was obtained from Toyobo Co. Ltd (Osaka, Japan). Annexin V-FITC/PI cell apoptosis analysis kit was purchased from BD Biosciences (San Jose, CA, USA). β-catenin inhibitor XAV939 was obtained from Selleck Chemicals (Shanghai, USA).

Cell Culture

U2OS cells were kept in RPMI 1640 medium containing 10% FBS and 1% streptomycin, in an incubator with 5% CO_2 at 37°C. After paving all dishes, cells were passed at 1:4 ratio. hFOB1.19 cells were kept in DMEM/F12 medium containing 10% FBS, 2.5 mM L-glutamine and 0.3 mg/ml G418, and in an incubator with 5% CO_2 at 34°C. After paving all dishes, cells were passed at 1:4 ratio.

Induction of U2OS/ADM Resistant Cell Line

U2OS cells were treated with 0.2 μg/ml ADM as the starting concentration. 24 h later, ADM was washed out, followed by changing fresh medium. When cells status recovered to normal, ADM drug concentration gradually increased to 2.0 μg/ml. Those cells that can normally grow at 2.0 μg/ml ADM were named as U2OS/ADM.

Assay for Drug Sensitivity

U2OS and U2OS/ADM cells were treated for 48 h with different concentrations of ADM. CCK-8 reagent was then added to observe absorbance values at 450 nm (A45) of each well. Inhibition rate (%) = [1 − A450(drug treatment group)) / A450(control group)] ×100%. IC_{50} value was calculated as the drug concentration required for 50% cell growth. Resistance index (RI) = IC_{50} of drug resistant cells / IC_{50} of home line cells.

Cell Treatment and Grouping

In vitro cultured U2OS/ADM cells were divided into three groups: si-NC transfection group; si-β-catenin group; and si-β-catenin + XAV939 treatment group. 72 hours after transfection, cells were collected for assay. Sequences were: si-β-catenin sense, 5'-CAGGG GGUUG UGGUU AAGCU CUUdTdT-3'; si-β-catenin anti-sense, 5'-AAGAG CUUAA CCACA ACCCC CUGdT-dT-3'; si-NC sense, 5'-UUCUC CGAAC GUGUC ACGUdT dT-3'; si-NC anti-sense, 5'-ACGUG ACACG UUCGG AGAAdT dT-3'.

Quantitative Real-time PCR (RT-PCR) for Gene Expression

PrimeScript RT reagent kit was used to synthesize cDNA by reverse transcription using RNA extracted by Trizol method as the template. Using cDNA as the template, PCR was performed with TaqDNA polymerase. In a 10 μl PCR reaction system, 5.0 μl 2× SYBR Green RT-PCR Master Mix, 0.5 μl of forward/reverse primers, 1 μl Template RNA and ddH_2O, were added. PCR conditions were: 95°C for 5 min pre-denature, followed by 40 cycles each containing 95°C 15 s denature, 58°C 30 s annealing and 72°C 30 s elongation. PCR was performed on ABI ViiA 7 fluores-

cent quantitative PCR cycler. Primer sequences used were: β-cateninP$_F$: 5'-AGGAC CACCG CATCT CTACA T-3'; β-cateninP$_R$: 5'-GCAGT TTTGT CAGTT CAGGG A-3'; Bcl-2P$_F$: 5'-GGTGG GGTCA TGTGT GTGG-3'; Bcl-2P$_R$: 5'-CGGTT CAGGT ACTCA GTCAT CC-3'; β-actinP$_F$: 5'-GAACC CTAAG GCCAA C-3'; β-actinP$_R$: 5'-TGTCA CGCAC GATTT CC-3'.

Western Blot

Cells were collected and extracted for proteins using sodium lauryl sulfate (SDS) lysis buffer. After testing for quality and concentration, 40 μg samples were loaded and separated in 10% sodium lauryl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were then transferred to membrane, which was blocked in 5% defatted milk powder under room temperature incubation. Primary antibody (β-catenin at 1:300, Bcl-2 at 1:200, β-catenin at 1:500) was added for 4°C overnight incubation. After phosphate-buffered saline tween-20 (PBST) rinsing, horse radish peroxidase (HRP) labeled secondary antibody (1:10000 dilution) was added for 1 h incubation. The membrane was rinsed in PBST and developed by enhanced chemiluminescence method. After exposure and fixation, the film was scanned and saved for data.

CCK-8 Assay for Cell Proliferation Activity

All cells were collected, digested and seeded into 96-well plate, which was incubated for 24, 48 or 72 h. 10 μl CCK-8 was added for continuous incubation. Absorbance values at 450 nm (A450) were measured. Relative proliferation activity = (A450 of treated cells – A450 of blank group) / (A450 of control group – A450 of blank group) * 100%.

Cell Apoptosis Assay

Cells were digested in trypsin and were collected. After re-suspension in Binding buffer, 5 μl Annexin V-FITC and 5 μl were sequentially added. Beckman Coulter Cytomics flow cytometer was used to test cell apoptosis.

Statistical Analysis

SPSS 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data were presented as mean ± standard deviation (SD). Comparison of measurement data between groups was performed by student t-test. Statistical significance was denoted when $p < 0.05$.

Results

Elevated β-catenin and Bcl-2 Expression Potent Proliferation Ability in U2OS Cells

qRT-PCR results showed that, compared to hFOB1.19 cells, U2OS cells had significantly elevated β-catenin and Bcl-2 mRNA levels (Figure 1A). Western blot results also showed significantly higher β-catenin and Bcl-2 protein levels in U2OS cells compared to hFOB1.19 cells (Figure 1B). CCK-8 assay showed significantly potentiated proliferation potency of U2OS cells than hFOB1.19 cells (Figure 1C). These results showed possible correlation between β-catenin up-regulation and osteosarcoma occurrence.

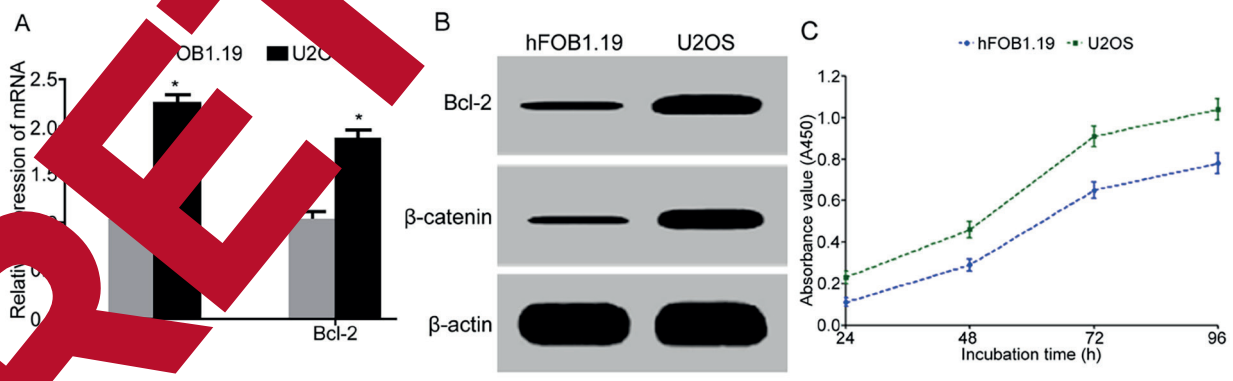

Figure 1. Elevated β-catenin and Bcl-2 expression with potent proliferation ability in U2OS cells. ***(A)*** qRT-PCR for gene expression; ***(B)*** Western Blot for protein expression; (C) CCK-8 assay for cell proliferation activity. *, $p < 0.05$ comparing between U2OS cells and hFOB1.19 cells. Bcl-2: B-cell lymphoma 2; CCK-8: cell counting kit-8; qRT-PCR : quantitative Real-time PCR.

Correlation between β-catenin and Bcl-2 up-regulation with U2OS Cell Drug Resistance

CCK-8 assay showed significantly lower proliferation activity of U2OS cells under various concentrations of ADM compared to U2OS/ADM cells (Figure 2A). IC50 of U2OS cells was 0.157 mg/l, whilst U2OS/ADM cells had IC_{50} value at 4.282 mg/l. U2OS/ADM cells had RI of 27.27 compared to parental U2OS cells. Under treatment by 0.157 mg/l ADM, U2OS cells had significantly higher apoptotic rate than U2OS/ADM cells (Figure 2B). qRT-PCR results showed significantly elevated expression of β-catenin and Bcl-2 mRNA in U2OS/ADM cells compared to U2OS cells (Figure 2C). Western blot results showed significantly higher β-catenin and Bcl-2 protein expression in U2OS/ADM cells compared to U2OS cells (Figure 2D).

Suppression of β-catenin Expression Enhanced ADM Sensitivity of U2OS/ADM Cells

Setting ADM concentration at 2.0 mg/l, we observed change of ADM resistance of U2OS/ADM cells after inhibition of β-catenin expression. Results showed that after silencing β-catenin expression, U2OS/ADM cells had increased Bcl-2 expression (Figure 3A). Under 2.0 mg/l ADM, cells had weaker proliferation

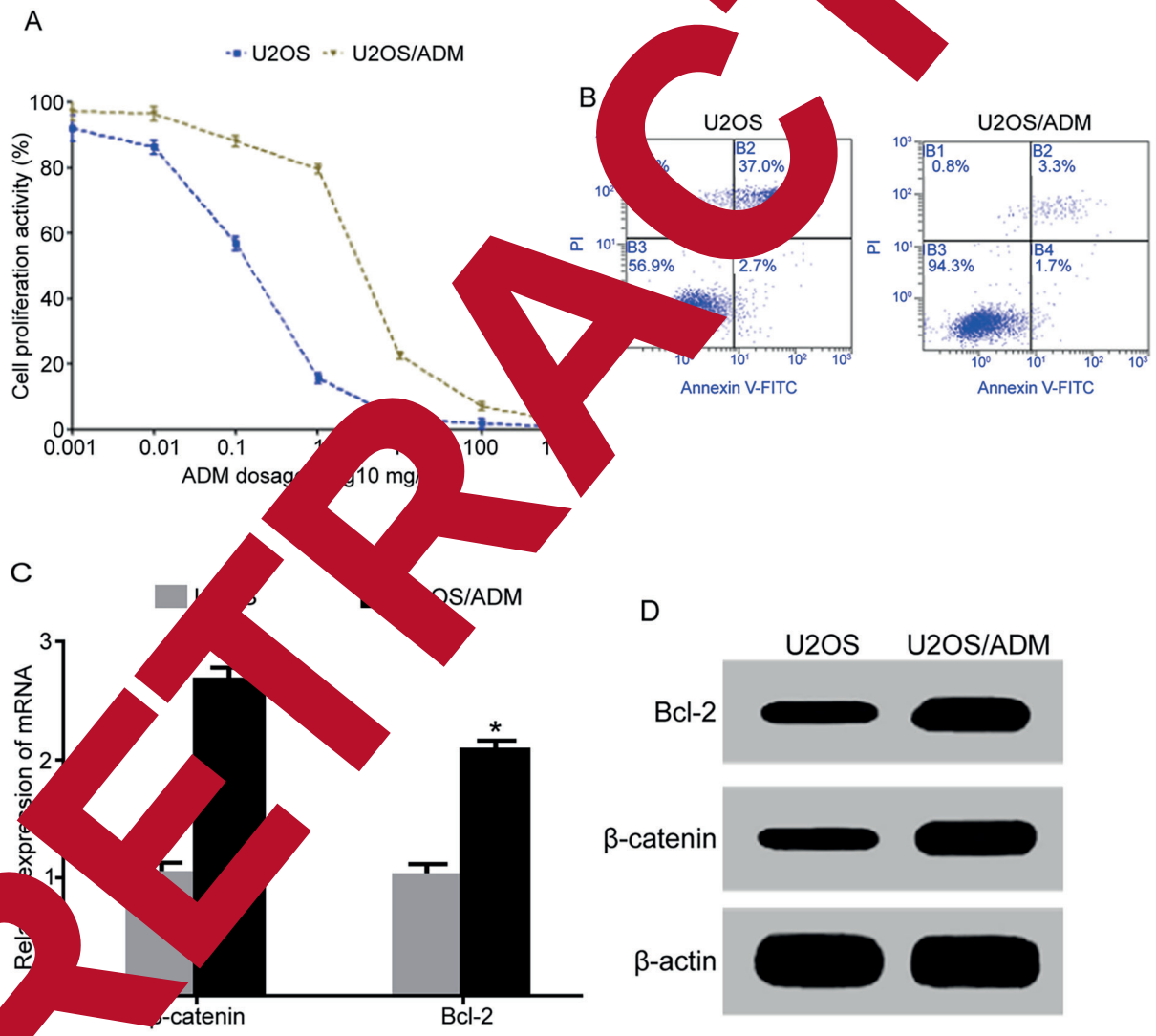

Figure 2. Correlation between β-catenin and Bcl-2 up-regulation with U2OS cell drug resistance. ***(A)*** CCK-8 assay for cell proliferation activity; ***(B)*** Flow cytometry for cell apoptosis; ***(C)*** qRT-PCR for gene expression; ***(D)*** Western Blot for protein expression. *, $p < 0.05$ comparing between U2OS cells and hFOB1.19 cells. Bcl-2: B-cell lymphoma 2; CCK-8: cell counting kit-8; qRT-PCR: quantitative Real-time PCR.

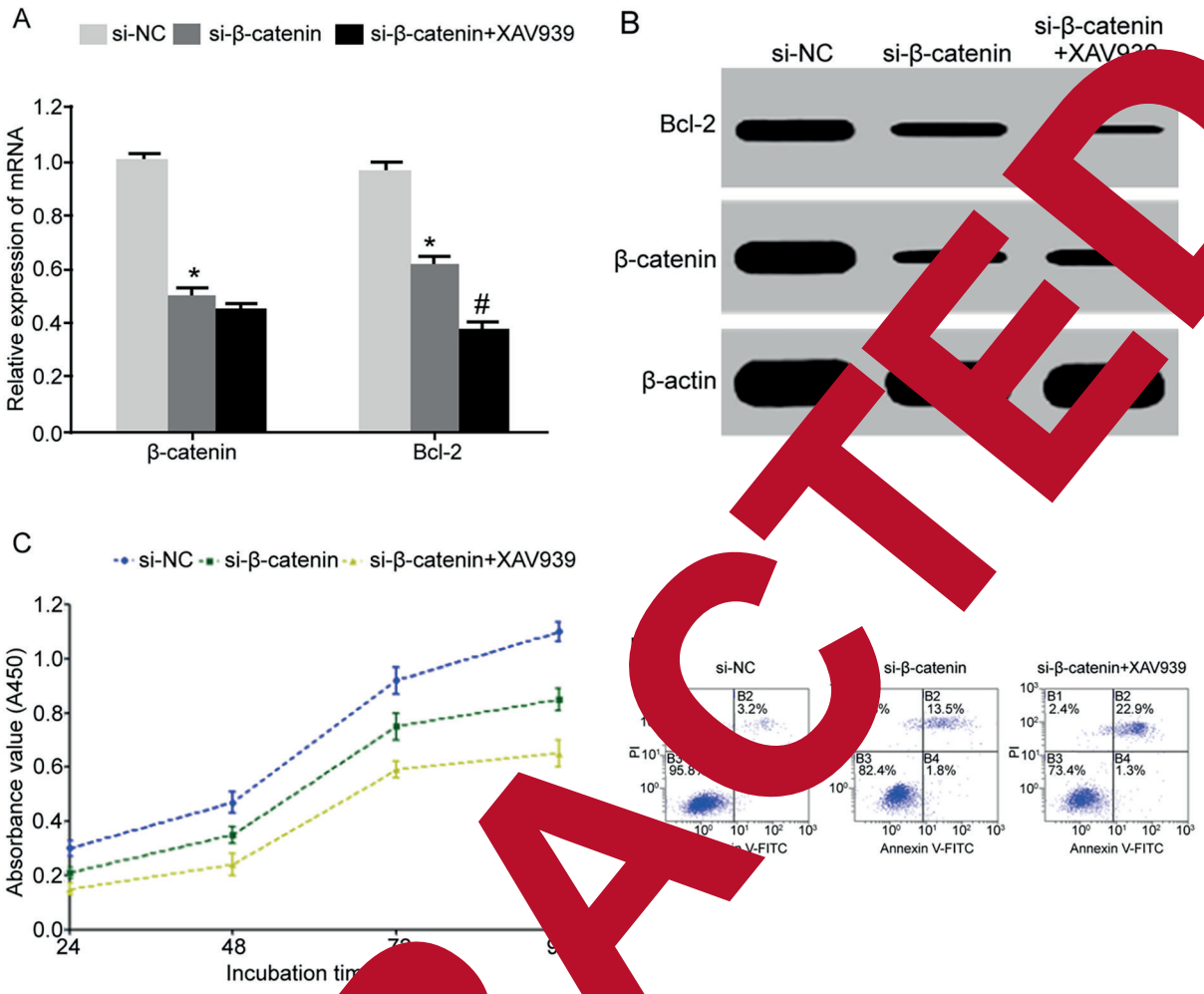

Figure 3. Suppression of β-cat express enhanced A M sensitivity of U2OS/ADM cells. ***(A)*** qRT-PCR for gene expression; ***(B)*** Western Blot otein ex CCK-8 assay for cell proliferation activity; ***(D)*** Flow cytometry for cell apoptosis. *, $p < 0.05$ c g b in and si-NC group; #, $p < 0.05$ comparing between si-β-catenin + XAV939 and si-β-caten roup cell lymphoma 2; CCK-8: cell counting kit-8; ADM: adriamycin; qRT-PCR: quantitative Real-time P

potency (Figu significantly nhanced cell apoptosis igure fter silencing β-catenin expre n, β-catenin tor XAV939 was further d to suppress Bc ctivity (Figure 3A an). Tho ells had more apoptosis after AD ductio nd more potent inhibitory effects ol tion (F e 3C).

scussion

athway is a highly conserved l pathw in evolution, and consists of canical and non-canonical signal pathway. Canal Wnt signal pathway is closely correlated wi mor onset, progression and metastasis, and is the most widely studied signal pathway so far[17]. β-catenin protein is firstly identified as cell adhesion protein molecule. Expression level of β-catenin largely determines Wnt/β-catenin signal activity, and is the core regulatory factor in canonical Wnt signal pathway[18]. Without signal stimuli, Wnt/β-catenin signal pathway is at test phase, and β-catenin can be degraded to keep relatively lower cytoplasmic level[19]. When Wnt signal pathway is activated, Wnt protein can pass the signal to degradation complex via a series of downstream proteins, impeding degradation complex function and phosphorylated degradation of β-catenin, therefore elevating its cytoplasmic expression. β-catenin further enters into cell nucleus, where it can form transcription complex with T-cell factor/lymphoid enhancing factor (TCF/LEF) to initiate transcription and

expression of multiple downstream target genes, thus modulating cell growth, proliferation, apoptosis, differentiation and invasion[20]. Bcl-2 protein family can be classified into anti-apoptotic factors and pro-apoptotic factors based on differential functions. B-cell lymphoma 2 (Bcl-2) is the most important and widely studied in Bcl-2 family, and can inhibit mitochondrial dependent cell apoptosis pathway[14]. Previous studies have shown important regulatory functions of Bcl-2 induced cell apoptosis in acquirement of drug resistance in cervical cancer[21], gastric carcinoma[22] and pulmonary cancer[23]. Multiple researches showed the role of Wnt/β-catenin signal pathway activation in up-regulating anti-apoptotic factor Bcl-2 expression and decreasing cell apoptosis[12,16]. This study generated an ADM-resistant cell model by drug treatment, and intervened Wnt/β-catenin signal pathway activity, to investigate its role in drug resistance of osteosarcoma cells. Results of this study showed that compared to normal human osteoblast cells hFOB1.19, osteosarcoma cells U2OS showed significantly elevated β-catenin mRNA and protein expression. Bao et al[24] showed significantly elevated β-catenin e[illegible]sion in tumor tissues of osteosarcoma [illegible] and its close correlation with distal met[illegible]is and histo-pathology grading. β-catenin ex[illegible] sion level can also work as a predictive index [illegible] patient's survival and prognosis. [illegible]et al[25] al[illegible] showed abnormally elevated β[illegible]pression in osteosarcoma tissues, an[illegible] close[illegible]relation with distal metastasis, cli[illegible] stage, a[illegible]tient's survival or prognosis. In t[illegible]per cells had abnormally [illegible]vate[illegible]nin expression, indicating th[illegible]e of β-ca[illegible]up-regulation in pathogene[illegible]chanism of [illegible]rcoma, as similar with [illegible] and Lu et al[illegible] who revealed the co[illegible]ation [illegible]n β-catenin up-regulation an[illegible]teosarcoma [illegible]ence. Moreover, this stu[illegible]so showed signifi[illegible]y higher Bcl-2 expre[illegible] in ost[illegible]arcoma U2OS cells than that in h[illegible]1.19 [illegible] as consistent with β-catenin expr[illegible]rn. Th[illegible]results indicated the role of p[illegible]n up-[illegible]ation in enhancing Bcl-[illegible]ressio[illegible]et revealed the role of β-catenin up-regu[illegible]n increasing anti-apoptotic fact[illegible]Bcl-2 expr[illegible]ssion and inhibiting osteosarco[illegible] apoptosis, as consistent with our [illegible]rvation showing elevation of both β-catenin [illegible] Bcl-2 in osteosarcoma cells. In a comparison [illegible] between parental cells and drug resistant cel[illegible]e found significantly higher β-catenin and Bcl-2 expression in U2OS/ADM cells compared to their parent drug sensitive cells U2OS. Under IC50 concentration of U2OS [illegible] of ADM, apoptotic rate of U2OS was [illegible] but with minor effects on apopt[illegible] of U2O[illegible] ADM cells. Results showed the[illegible]relation between β-catenin and Bcl-2 up-re[illegible]n with not only osteosarcoma pathogenesis, [illegible] drug resistance of osteosarcom[illegible]ls. Mar[illegible]ve et al[27] showed that chem[illegible]rapy drug tre[illegible] significantly enhance[illegible]ansduc[illegible] activity of Wnt/β-catenin signa[illegible]hway [illegible]de oste[illegible]sarcoma cells MNNG-[illegible]O[illegible]egulate[illegible]xpression of drug res[illegible]t prot[illegible]BCG, [illegible]making chemotherap[illegible]ure, and e[illegible]xpression of stem tra[illegible]n factor insi[illegible]ls to endow their fea[illegible] o[illegible] stem cells. In this study, chemotherapy reage[illegible]tment further elevated β-ca[illegible]xpression [illegible]osarcoma cells, as co[illegible] with Martins-[illegible]ves et al[27]. Further [illegible]ts showed that, after suppression of β-cat[illegible]n expression [illegible]siRNA, drug resistant cells [illegible]S/ADM sh[illegible]d significantly lower Bcl-2 e[illegible]ion and [illegible]akened proliferation ability, plus [illegible]tivity for ADM-induced cell apoptosis. [illegible] combination of β-catenin inhibitor XAV939 treatment further suppressed Bcl-2 expression, weakened proliferation ability of U2OS/ADM cells, and enhanced apoptosis. These results showed that long-term of ADM treatment up-regulated expression of β-catenin and downstream anti-apoptotic factor Bcl-2 in osteosarcoma cells, thus enhancing ADM resistance of cells. On the other hand, inhibition of β-catenin expression or activity can somehow weaken ADM resistance of osteosarcoma cells. Zhang et al[28] showed that inhibition of β-catenin expression significantly weakened malignant biological features of sarcoma MG63 cells including migration and motility. Ma et al[29] showed the correlation between β-catenin up-regulation plus enhanced activity of Wnt/β-catenin signal pathway and potentiated proliferation and invasion potency of osteosarcoma cells. The inhibition of Wnt/β-catenin signal pathway activity or β-catenin expression could weaken proliferation or invasion potency of osteosarcoma cells. Liu et al[30] found that interference of β-catenin expression by siRNA significantly suppressed proliferation or migration of osteosarcoma U2OS and MG63 cells. Xu et al[26] found that after inhibition of β-catenin expression or activity using Polydatin, osteosarcoma MG63 cells showed significantly increased apoptosis. Li et al[31] showed that over-expression of metalloprotease TIKI2 for suppressing Wnt/β-catenin

activity played an important role in inhibiting osteosarcoma cell proliferation, clonal formation or migration. Martins-Neves et al[27] found that the inhibition of β-catenin expression or activity significantly weakened acquirement of stem property of MNNG-HOS cells after chemotherapy, or their transformation towards tumor stem cells with lower drug sensitivity. This study supported Martins-Neves et al[27] results from another perspective. Li et al[12] also showed the role of β-catenin over-expression in enhancing Bcl-2 expression and enhancing chemotherapy drug resistance of tumor cells. This study revealed the role of β-catenin in down-regulating Bcl-2 expression and weakening chemotherapy resistance of tumor cells, further supporting Li et al[12] study.

Conclusions

Up-regulation of β-catenin plays a role in enhancing expression of downstream anti-apoptotic factor Bcl-2, potentiating ADM resistance of osteosarcoma U2OS cells.

Acknowledgements

This work was supported by Medicine and health technology innovation project of Harbin City (...QYJ083).

Conflict of Interest

The Authors declare that they ...

...rences

1) Sarman H, ...tram R, ...SB. Anticancer drugs with ch...therapeutic i...ions with thymoguinon... osteosarcoma cel... Rev Med Pharma... Sci 201...; 20: 1263-127...

2) ..., Zhao ...u CY, Sha H, Bi GB, Chen L, Ye ...ng ... TH. P...ntiation of the antitumor a...driamyc... against osteosarcoma by car... WIN... 12-2. Oncol Lett 2015; 10: 415-2...

3) ... K, Gu ..., Zeng G, Peng L, Huang H, Ji... M, Yang ..., M, Yang Y, Wang Y, Peng Q, Zhu ... K. Inhibition of disheveled-2 resensitiz... resistant lung cancer cells through down-regulating Wnt/beta-catenin signaling. Exp Cell Res 2016; 347: 105-113.

... C, Wu CH, Hu HZ. LncRNA UCA1 pro...es epithelial-mesenchymal transition (EMT) of breast cancer cells via enhancing Wnt/beta-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2016; 20: 2819-2824.

5) Shen YN, He HG, Shi Y, Cao J, Yuan ..., Shi CF, Zhu N, Wei YP, Liu F, Huang ...ng GS, JH. Kruppel-like factor 8 promo...cancer stem cell-like traits in hepatocellular...oma through Wnt/beta-catenin signaling. Mo...og 2017; 56: 751-760.

6) Wu Y, Xu SY, Liu SY, Xu ...g SY, He ..., Liu YH, Ni GX. Upregu...n seurm scleros... in the T2DM patien...th femu...cture in... the expression of ...e forma...remodeling-associated biomarke...nt...lizing W...gnaling. Eur Rev ... F... Sci 20...: 470-478.

7) Glass DA, ...ek P, Ahn JD, ..., Patel MS, Clevers ... MM, Long F, ...ahon AP, Lang RA, ...nty ...onical Wnt signaling in differentiat... osteobl... ntrols osteoclast differentiation. Dev Cell 20... 751-764.

8) ... RC, Deyrup A, Is...wa A, Heck R, Jiang W, Zhou L, Feng T, King D, Cheng H, Breyer B, Peabody T, Simon MA, M...tag AG, He TC. Cytoplasmic and/or nuclear ac...ulation of the beta-catenin protein is a frequ...event in human osteosarcoma. ...J Cancer ...2; 102: 338-342.

9) ...an BT, Qi W, Li HL, Shen XZ, Zhao G, ..., Liu YJ. MicroRNA-26a induces osteosarcoma cell growth and metastasis via the Wnt/beta-catenin pathway. Oncol Lett 2016; 11: 1592-...

10) Wu KF, Liang WC, Feng L, Pang JX, Waye MM, Zhang JF, Fu WM. H19 mediates methotrexate resistance in colorectal cancer through activating Wnt/beta-catenin pathway. Exp Cell Res 2017; 350: 312-317.

11) Cui J, Jiang W, Wang S, Wang L, Xie K. Role of Wnt/beta-catenin signaling in drug resistance of pancreatic cancer. Curr Pharm Des 2012; 18: 2464-2471.

12) Li L, Liu HC, Wang C, Liu X, Hu FC, Xie N, Lu L, Chen X, Huang HZ. Overexpression of beta-catenin induces cisplatin resistance in oral squamous cell carcinoma. Biomed Res Int 2016; 2016: 5378567.

13) Yu G, Wu F, Wang E. KLF8 promotes temozolomide resistance in glioma cells via beta-catenin activation. Cell Physiol Biochem 2016; 38: 1596-1604.

14) Gahl RF, Dwivedi P, Tjandra N. Bcl-2 proteins bid and bax form a network to permeabilize the mitochondria at the onset of apoptosis. Cell Death Dis 2016; 7: e2424.

15) Han J, Chen Q. MiR-16 modulate temozolomide resistance by regulating BCL-2 in human glioma cells. Int J Clin Exp Pathol 2015; 8: 12698-12707.

16) He X, Mo Y, Geng W, Shi Y, Zhuang X, Han K, Dai Q, Jin S, Wang J. Role of Wnt/beta-catenin in the tolerance to focal cerebral ischemia induced by electroacupuncture pretreatment. Neurochem Int 2016; 97: 124-132.

17) Peng Y, Zhang X, Feng X, Fan X, Jin Z. The crosstalk between microRNAs and the Wnt/beta-catenin signaling pathway in cancer. Oncotarget 2017; 8: 14089-14106.

18) Deitrick J, Pruitt WM. Wnt/beta catenin-mediated signaling commonly altered in colorectal cancer. Prog Mol Biol Transl Sci 2016; 144: 49-68.

19) Wang Z, Li B, Zhou L, Yu S, Su Z, Song J, Sun Q, Sha O, Wang X, Jiang W, Willert K, Wei L, Carson DA, Lu D. Prodigiosin inhibits Wnt/beta-catenin signaling and exerts anticancer activity in breast cancer cells. Proc Natl Acad Sci U S A 2016; 113: 13150-13155.

20) Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. Cell 2012; 149: 1192-1205.

21) Leisching G, Loos B, Botha M, Engelbrecht AM. Bcl-2 confers survival in cisplatin treated cervical cancer cells: circumventing cisplatin dose-dependent toxicity and resistance. J Transl Med 2015; 13: 328.

22) Han Z, Hong L, Han Y, Wu K, Han S, Shen H, Li C, Yao L, Qiao T, Fan D. Phospho Akt mediates multidrug resistance of gastric cancer cells through regulation of P-gp, Bcl-2 and Bax. J Exp Clin Cancer Res 2007; 26: 261-268.

23) Yang TM, Barbone D, Fennell DA, Broaddus VC. Bcl-2 family proteins contribute to apoptotic resistance in lung cancer multicellular spheroids. Respir Cell Mol Biol 2009; 41: 14-23.

24) Bao ZQ, Zhang CC, Xiao YZ, Zhou JS, Tao Y[illegible]ai DM. Over-expression of Sox4 and beta-ca[illegible] is associated with a less favorable prognosi[illegible] osteosarcoma. J Huazhong Univ Sci Technol Med Sci 2016; 36: 193-199.

25) Lu Y, Guan GF, Chen J, Hu B, Sun C, Ma Q, Wen YH, Qiu XC, Zhou Y. Aberrant CXCR4 and beta-catenin expression in osteosa[illegible] relates with patient survival. Oncol[illegible] 2123-2129.

26) Xu G, Kuang G, Jiang W, Jiang R[illegible]D. Polydatin promotes apoptosis through up[illegible]ion the ratio of Bax/Bcl-2 and inhibits prolife[illegible]y attenuating the beta-catenin s[illegible]ing in hu[illegible]teo sarcoma cells. Am J T[illegible]Res 2016; 8:

27) Martins-Neves SR, P[illegible]Oliveira[illegible]Wijers-N[illegible] PM, Abrunhosa AJ[illegible]tes-Ribe[illegible], Bovee JV, Cleton-Jansen AM, G[illegible]M, C[illegible]notherap[illegible]nduces stemness i[illegible]oste[illegible] cells th[illegible]h activation of W[illegible]a-cate[illegible]naling[illegible]ncer Lett 2016; 370[illegible]-295.

28) Zhang F[illegible] Chen J, Yu T, [illegible]. SiRNA-mediate[illegible]nc[illegible]beta-catenin suppresses invasion [illegible] chemo[illegible]ivity to doxorubicin in MG-63 osteosarcoma c[illegible]sian Pac J Cancer Prev [illegible]2: 239-245.

2[illegible]a Y, Zhu B, Liu X, Yu H, Yong L, Liu X, Shao J, Liu Z. Inhibition of [illegible]eandrin on the proliferation show and invasion[illegible]osteosarcoma cells in vitro by suppressing [illegible]beta-catenin signaling pathway. [illegible]Exp Clin C[illegible]r Res 2015; 34: 115.

30) [illegible] Chen D, Shen L, Xie Z. Apigenin inhi[illegible]oliferation and invasion of osteosarcoma cells by suppressing the Wnt/beta-catenin signaling pathway. Oncol Rep 2015; 34: 1035-

[illegible] Liu J, Wu H, Liu L, Wang L, Zhang S. TIKI2 suppresses growth of osteosarcoma by targeting Wnt/beta-catenin pathway. Mol Cell Biochem 2014; 392: 109-116.