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Study on the influence of metformin on castration-resistant prostate cancer PC-3 cell line biological behavior by its inhibition on PLCε gene-mediated Notch1/Hes and androgen receptor signaling pathway

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Abstract. – OBJECTIVE: To study the regulation of metformin on the biological behaviors of the castration-resistant prostate cancer (CRPC) PC-3 cell such as proliferation, invasion, apoptosis through influencing Notch1/Hes and androgen receptor (AR) signaling pathway activity by its inhibition on the expression of PLCε gene.

MATERIALS AND METHODS: Human prostate cancer-3 (PC-3) cell line was divided into PC-3 cell line (group A), PC-3 cell line + metformin (10 mM) (group B), PC-3 cell line + metformin (20 mM) (group C), PLCε gene knockout cell line (group D), PLCε knockout cell line + metformin (10 mM)_ (group E) and PLCε knockout cell line + metformin (20 mM) (group F), which were respectively tested at 24 h, 48 h and 72 h, and five duplicate wells were set at each time point in each group. Western blot assay and RT-PCR assay were used to test the relative expressions of PLCε, Notch1, Hes, AR protein and mRNA; MTT assay was used to test the cell proliferation. Transwell chamber was used to test the invasion capability. The scratch test was used to test the migration capability and the flow cytometer was used to test cell apoptosis.

RESULTS: The relative expressions of PLCε, Notch1, Hes, AR protein and mRNA in Group A were increased gradually with time, but those values in group B and group C were decreased gradually with time and also significantly lower than those in group A (p <0.05) at each time point. The relative expressions of PLCε, Notch1, Hes, AR protein and mRNA in-group D, group E and group F were not changed at each time point (p>0.05). The proliferation, invasion and migration capabilities of the cells in group A, group D, group E and group F were gradually increased with time, but those in group B and group C were rapidly decreased with time and also significantly lower than those in group A, group D, group E and group F (p<0.05) at each time point. The apoptosis rates of group B and group C were increased gradually with time, and there was no other significant change in each group (p<0.05).

CONCLUSIONS: Metformin can regulate the biological behaviors of CRPC PC-3 cell line such as proliferation, invasion, migration and apoptosis through influencing Notch1/Hes and AR signaling pathway activity by its inhibition on the expression of PLCε gene.

Key Words:
Metformin, PLCε gene, Notch1/Hes, Androgen receptor, Castration-resistant prostate cancer, Proliferation, Invasion, Apoptosis.

Introduction

Prostate cancer has become the most common malignant tumor in urinary system[1]. Patients with localized prostate cancer are mainly treated with surgery or radiation therapy. For those with prostate cancer metastasis or localized prostate cancer without surgical indications, they can receive surgery castration or medical castration, antiandrogen treatment[2]. Although the androgen deprivation therapy (ADT) can be more effective in most patients initially, all patients would be eventually progressed to castration-resistant prostate cancer (CRPC)[3]. Recently, some studies[4] have found that metformin had a prevention and treatment role in breast cancer, colorectal cancer and endometrial cancer. Metformin can significantly reduce the risk of prostate cancer and biochemical recurrence (after two consecutive

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monitoring, the prostate specific antigen (PSA) was elevated by ≥0.2 ng/ml). It may also improve recurrence-free survival of PSA (PSA-RFS), long-term survival without metastasis overall survival (OS) and reduce prostate cancer-specific mortality (PCSM) and the progress of CRPC[5]. In prostate cancer cells, the metformin can up-regulate the insulin growth factor receptor (IGF-IR) by the biological effects such as disrupting the cell membrane androgen receptor (AR) activity and inhibiting the insulin growth factor-I (IGF-I), indicating that the metformin can represent a typical anti-androgen replacement therapy[6]. Also, phospholipase C epsilon (PLCε) gene can play a role in the pathogenesis of prostate cancer by Notch1 signaling pathway and AR signaling pathway[7]. Therefore, we hypothesized that metformin can regulate the castration-resistant prostate cancer (CRPC) PC-3 cell proliferation, invasion, apoptosis and other biological behaviors through influencing Notch1/Hes and androgen receptor (AR) signaling pathway activity by its inhibition on the expression of PLCε gene.

Materials and Methods

Cell Culture

Human prostate cancer-3 (PC-3) cell line and PLCε gene knockout cell line were respectively cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium containing 1 nm DHT (dihydrotestosterone), 10% fetal calf serum (FCS), l00.000 U/L penicillin and 100 mg/L streptomycin in the incubator demanding 37ºC, 5% CO_2 and saturated humidity for 24 h. CRISPR/Cas9 method was used to construct PLCε-knockout and PC-3 cell line by Beyotime Science and Technology Co., Ltd., (Shanghai, China). The routine cell passage cultivation was adopted. When the cell confluence reached 85%, the cell was trypsinized and re-suspended in phosphate-buffered saline (PBS) to a concentration of 1×10^6/ml and stored at -80ºC.

Experiment Grouping

The experiment included PC-3 cell line (group A), PC-3 cell line + metformin (10 mM) (group B), PC-3 cell line + metformin (20 mM) (group C), PLCε gene knockout cell line (group D), PLCε gene knockout cell line + metformin (10 mM) (group E) and PLCε gene knockout cell line + metformin (20 mM) (group F). All cells were cultured in 96-well plates in 5% CO_2 saturated humidity incubator at 37ºC and tested at 24 h, 48 h and 72 h respectively. Five duplicate wells were set at each time point in each group.

Observation Indicators and Testing Methods

The Relative Protein Expressions of PLCε, Notch1, Hes and AR by Western Blot Assay

The total cellular protein was extracted. The coomassie brilliant blue method was used for rough quantification. Before testing the protein, the dosage standardized testing of the protein amount in each sample was done with β-actin antibody. An equal amount of total protein was taken and placed in 8% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for electrophoretic separation, then transferred from the separation zone to the polyvinylidene fluoride (PVDF) membrane. After the rabbit-anti PLCε, Notch1, Hes and AR monoclonal antibody (1:2000, Sigma-Aldrich, St. Louis, MO, USA) were added, it was stood in the solution for one night, and then incubated with murine-anti PLCε, Notch1, Hes and AR polyclonal antibody (1:500, Sigma-Aldrich, St. Louis, MO, USA) at room temperature for 4 h. The membrane was washed with phosphate buffered saline (PBS) and colored by diaminobenzidine (DAB). The results were saved after scanning. Lab Works 4.5 gel imaging software (Invitrogen, Carlsbad, CA, USA) was used for semi-quantitative analysis. The result was expressed in the integral optical density (IOD). β-actin was taken as the internal reference control. Relative IOD value = IOD value p of 38 MAPK/β-actin.

The Relative mRNA Expression Levels of PLCε, Notch1, Hes and AR with RT-PCR Assay

Conventional Trizol Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. (Beijing, China) method was used for extracting total RNA from the cells. UV spectrophotometer (Applied Biosystems Company, City Foster, CA, USA) was used to determine the concentration and purity. The reverse transcription kit (TaKaRa Company, Otsu, Shiga, Japan) was used to synthesize Cdna. The primer sequence based on Gene Bank sequence was synthesized Shanghai Sangon Co., Ltd., (Shanghai, China), PLCε: (F) 5'-CATGGAAGGATAAGCGTTGGT-3', (R) 5'-CCCAAGTCCCGTGTTAAGA-3'; Notch1: (F) 5'-GGGTCCACCAGTTTGAATGG-3', (R) 5'-GTTTGCTGGCTGCAGGTTCT-3'; Hes: (F) 5'-GCACGAGCAGTTCGAAGCTC-3',

(R) 5'-TTCCATTTGCTTTTATGTCC-3'; AR: (F) 5'-CAGTGAAGCATTGGAAACCCTAT-3', (R) 5'-GCAAAGTCTGAAGGTGCCAT-3' B-actin GAPDH (F): 5'- CGCGAGAAGATGACCCAGAT-3', (R): 5'-GCACTGTGTTGGCGTACAGG-3'. The reaction system was as follows: cDNA 2 μl + forward and reverse primers: 3 μl + Taq respectively, polymerase: 0.5 μl + dNTPs 1 μl + MgCl2 3 μl + 10×Buffer 5 μl + ddH2O2 2.5 μl. The reaction condition was pre-denaturation at 95°C for 5 min; then, 30 cycles containing denaturation 30 s at 95°C, annealing 30 s at 58°C, elongation 60 s at 72°C and extend at 72°C for another 10 min. 2% agarose gel electrophoresis was used to verify polymerase chain reaction (PCR) product. The gel imaging analysis system was used for ultraviolet spectroscopic imaging and digital photography row gray value analysis (Gel-Pro Analyzer4.0 analysis software) was carried out. The results were expressed by $2^{-\Delta\Delta}$Ct method.

Cell Proliferation, Invasion and Migration Capabilities

MTT (3-(4,5)-dimethylthiazolyl-3, 5-diphenyltetrazoliumbromide) assay was used to test the cell proliferation. Transwell chamber was used to test the invasion capability, and the scratch experiment was made to test the migration capability. MTT method: 1 ml cell was taken and transferred to 96-well plates. 40 μl MTT solution were added to each well and cultured in 5% CO_2 saturated humidity incubator at 37ºC for 4 h. The supernatant was discarded, 150 μl dimethyl sulfoxide (DMSO) were added and the plate was shaken for 10 min. Then the solution was placed on an enzyme mark instrument to measure the 490 nm wave length absorbance (OD). 630 nm was taken as the reference wavelength. The above procedure was repeated for 3 times, then the average was taken. Transwell chamber: Matrigel was placed in refrigerator at 4ºC for 24 h for chamber preparation. 50 μl Matrigel and 400 μl Opti-ME-MI medium (1:8) was added into 1.5 ml Eppendorf (EP) tube and mixed evenly. All the procedure was operated on ice. The tube was placed into the small chamber and cultured in 5% CO_2 saturated humidity incubator at 37ºC for 1 h. 50-μl cell suspension was added into the chamber and cultured continuously for 48 h. Then the chamber was taken out and washed with phosphate buffered saline (PBS). Then the cell was fixed with ethanol for 5 min. 95% crystal violet staining solution was added for staining for 7 min. Then the chamber was washed with phosphate-buffered saline (PBS) and air-dried at the room temperature. The chamber film was removed and placed on the glass slide. The slide was sealed with the neutral gum. Five horizons were randomly selected under an optical microscope (200×) for cell counting. Graph Pad Prism 5 software was used for analysis. Scratch experiment: marker pen was used to draw line on the back of the well plate and then the cells were added into the plate. The sterile pipette tip (200 μL) scratched a line in one direction along the bottom of the culture hole. Then it was placed in the Dulbecco's Modified Eagle Medium (DMEM) culture medium for culturing, and finally the cell migration situation in the scratching zone was observed.

Testing on cell Apoptosis by Flow Cytometer

The cells were collected and centrifuged at 2000 g for 10 min and washed twice with buffer. The cell concentration was adjusted to 1×10^6/ml. It was fixed with 70% ethanol. 1×Buffer A phosphate-buffered saline (PBS) was added for washing and then cells were centrifuged. 500 μl 1×Buffer A phosphate buffered saline (PBS) and then 10 mg/L RNase A was added. The final concentration was 0.25 mg/ml. Then the solution was placed in 5% CO_2 saturated humidity incubator at 37ºC for 30 min. Then, 5 μl 50 mg/L PI solution was added for staining. After 30 min's incubation in the dark at room temperature, it was observed on the machine (FACS Caliber type flow cytometer, BD Biosciences, Franklin Lakes, NJ, USA).

Statistical Analysis

SPSS software (SPSS Inc., Chicago, IL, USA) was used for statistics analysis. The measurement data was expressed in mean ± standard deviation. The comparison between groups was analyzed by one-way ANOVA. The pairwise comparison was tested with LSD-t assay. The comparison in groups was analyzed with the variance of repeated measurement data. $p<0.05$ indicated that the difference was statistically significant.

Results

Analysis of Testing Results by Western Blot Assay

In-group A, the relative protein expressions levels of PLCε, Notch1, Hes and AR were increased gradually with the time. In group B and group C, those values were decreased gradually with time and also significantly lower than in-group A ($p<0.05$) at each time point. The relative protein expression levels of Notch1, Hes and AR in-group D, E and F were not changed ($p>0.05$) (Table I).

Table I. Analysis of testing results by Western blot assay.

Group		Group A	Group B	Group C	Group D	Group E	Group F
PLCε	24 h	0.46±0.12	0.42±0.13	0.41±0.15	-	-	-
	48 h	0.55±0.16	0.38±0.14	0.36±0.12	-	-	-
	72 h	0.72±0.18	0.35±0.12	0.33±0.11	-	-	-
Notch1	24 h	0.53±0.16	0.46±0.13	0.44±0.16	0.16±0.08	0.15±0.06	0.14±0.07
	48 h	0.62±0.18	0.42±0.15	0.40±0.13	0.15±0.09	0.16±0.05	0.17±0.08
	72 h	0.68±0.21	0.38±0.14	0.39±0.12	0.17±0.07	0.16±0.06	0.15±0.05
Hes	24 h	0.55±0.19	0.52±0.17	0.53±0.16	0.19±0.06	0.18±0.07	0.20±0.05
	48 h	0.64±0.23	0.46±0.15	0.47±0.15	0.20±0.09	0.19±0.08	0.21±0.09
	72 h	0.73±0.25	0.43±0.14	0.44±0.13	0.18±0.08	0.17±0.06	0.19±0.08
AR	24 h	0.62±0.18	0.53±0.15	0.52±0.18	0.13±0.05	0.14±0.05	0.13±0.03
	48 h	0.75±0.22	0.46±0.17	0.47±0.16	0.14±0.03	0.13±0.04	0.13±0.04
	72 h	0.83±0.24	0.42±0.14	0.41±0.17	0.12±0.04	0.15±0.06	0.14±0.05

Note: group A: PC-3 cell line; B group: PC-3 cell line + metformin 10 mM; group C: PC-3 cell line + metformin 20 mM; group D: PLCε gene knockout cell line; Group E: PLCε gene knock cell lines + metformin 10 mM; F group: PLCε gene knock cell line + metformin 20 mM.

Analysis of Testing Results by PCR-RT Assay

The relative mRNA expressions levels of PLCε, Notch1, Hes and AR mRNA in group A were increased gradually with time, but those values in group B and group C were decreased gradually with time and also significantly lower than those in group A ($p<0.05$) at each time point. The relative mRNA expression levels of Notch1, Hes and AR mRNA were not changed in group E and group F group ($p>0.05$) (Table II).

Comparison of cell Proliferation, Invasion and migration Capabilities

The proliferation, invasion and migration capabilities of the cell were increased gradually with time in-group A, group D, group E and group F. Those values were decreased gradually with time in-group B and group C, and also significantly lower than those in-group A, group D, group E and group F ($p<0.05$) at each time point (Table III).

Comparison of Cell Apoptosis Rate

The apoptosis rates of cell in group B and group C were increased gradually with time, and there was no significant change in other groups ($p<0.05$) (Table IV).

Discussion

There are two RA domains (ra1 and ra2) bonded with RAS at C end of phosphatidylinositol

Table II. Analysis of testing results by PCR-RT assay.

Group		Group A	Group B	Group C	Group D	Group E	Group F
PLCε	24 h	0.5326±0.1254	0.4754±0.1326	0.4659±0.1421	-	-	-
	48 h	0.5748±0.1326	0.4213±0.1241	0.4321±0.1325	-	-	-
	72 h	0.6324±0.1527	0.3965±0.1162	0.3865±0.1174	-	-	-
Notch1	24 h	0.6125±0.1529	0.5216±0.1462	0.5326±0.1624	0.1256±0.0629	0.1325±0.0528	0.1268±0.0342
	48 h	0.6459±0.1648	0.4856±0.1325	0.4795±0.1432	0.1326±0.0754	0.1254±0.0469	0.1277±0.0528
	72 h	0.6857±0.1857	0.4421±0.1247	0.4521±0.1274	0.1352±0.0632	0.1296±0.0538	0.1321±0.0169
Hes	24 h	0.5496±0.1326	0.5126±0.1462	0.5021±0.1342	0.1629±0.0567	0.1754±0.0628	0.1845±0.0865
	48 h	0.5895±0.1254	0.4658±0.1385	0.4765±0.1524	0.1754±0.0628	0.1792±0.0527	0.1762±0.0764
	72 h	0.6352±0.1754	0.4321±0.1295	0.4215±0.1262	0.1823±0.0727	0.1826±0.0429	0.1932±0.0932
AR	24 h	0.4958±0.1325	0.4526±0.1258	0.4621±0.1328	0.2154±0.0867	0.2215±0.0216	0.2054±0.0628
	48 h	0.5216±0.1417	0.4147±0.1414	0.4215±0.1452	0.1865±0.0923	0.2146±0.0347	0.2153±0.0732
	72 h	0.5548±0.1629	0.3856±0.1029	0.3865±0.1285	0.1923±0.0821	0.2035±0.0428	0.1896±0.0918

Note: group A: PC-3 cell line; B group: PC-3 cell line + metformin 10 mM; group C: PC-3 cell line + metformin 20 mM; group D: PLCε gene knockout cell line; Group E: PLCε gene knock cell lines + metformin 10 mM; F group: PLCε gene knock cell line + metformin 20 mM.

Table III. Comparison of cell proliferation, invasion and migration capabilities.

Group		Group A	Group B	Group C	Group D	Group E	Group F
Proliferation rate	24 h	0.8±0.2	0.6±0.2	0.5±0.2	0.7±0.3	0.6±0.2	0.7±0.3
	48 h	1.2±0.3	0.4±0.1	0.4±0.2	1.3±0.5	1.2±0.4	1.4±0.5
	72 h	1.4±0.3	0.3±0.1	0.3±0.1	1.5±0.6	1.6±0.5	1.7±0.8
Invasion ability (cell count/horizon)	24 h	130±26	120±22	115±24	120±24	125±23	135±30
	48 h	150±34	100±23	105±18	145±36	140±37	145±35
	72 h	160±51	90±16	85±13	165±45	170±42	160±46
Migration ability (mm)	24 h	12.5±2.6	10.5±2.3	10.6±2.4	13.6±3.2	13.5±3.4	13.8±3.5
	48 h	16.3±2.7	9.2±2.2	9.4±2.3	17.2±3.3	17.5±3.6	17.3±3.4
	72 h	17.8±2.8	8.4±1.8	8.3±1.6	18.4±3.4	18.6±3.3	18.2±3.6

specific phospholipase C epsilon (PLCε). cdc25 domain in the N end (also known as the guanylic acid conversion binding domain) can activate Ras. Therefore, PLCε is an important dual intermediate effect molecule[8] in Ras signaling transduction pathway. The study[9] found that the expression of PLCε in bladder cancer was significantly higher than that in normal bladder tissues, which was correlated with clinical stages. If PLCε was used to interfere with plasmid and adenovirus, after making its expression in bladder cancer silent, the bladder cancer cell malignant biological behaviors including proliferation, invasion, metastasis can be inhibited[10]. Androgen receptor (AR) is a member of the nuclear receptor super family, which plays an important role in the generation of normal prostate and prostate cancer. The overexpressed androgen with its translocation capability to the nucleus remaining, it will continuously activate the downstream target gene expression, thereby causing androgen resistance[11]. Notch gene plays an important role in the differentiation and growth of cells. It can express four transmembrane protein receptors, including Notch1 to 4. The expressions of Notch1 protein and its ligand Jagged 1 protein were increased in prostate cancer, which was positively correlated with the cancer staging[12-14]. The results of this study showed that the relative protein and mRNA expressions levels of PLCε, Notch1, Hes, AR in group A were gradually increased with time, but those values in group B and group C were decreased gradually with time, and also significantly lower than those in group A at each time point. Those in group D, group E and group F were not changed at all time points, suggesting that PLCε gene can mediate Notch1/Hes and AR signaling pathway in CRPC PC-3 cell line. Metformin can act on PLCε specifically despite the concentration, thereby affecting the degree of activation of Notch1/Hes and AR signaling pathways. The proliferation, invasion and migration capabilities of cells in group A, group D, group E and group F were increased gradually with time, but those values in group B and group C were decreased gradually with time, and also significantly lower than those in group A, group D, group E and group F at each time point. The apoptosis rates of group B and group C were increased gradually with time, but there was no significant change in other groups, suggesting the proliferation, invasion, migration, apoptosis and other biological behaviors of CRPC PC-3 cell line were closely related with the protein and mRNA expressions levels of PLCε, Notch1, Hes, AR. PLCε can play a anti-oncogene role, affecting the malignant tumor behavior of PC-3 cell line. But we also found simultaneously that the proliferation, invasion and migration capabilities of cells in group D, group E and group F were increased gradually with time and there was no change in apoptosis, suggesting that PLCε gene knockout cell line could also play

Table IV. Comparison of cell apoptosis rate (%).

Group	Group A	Group B	Group C	Group D	Group E	Group F
24 h	6.8±2.3	25.6±6.9	27.8±7.5	7.8±2.1	8.3±2.2	9.2±2.6
48 h	7.2±2.6	43.2±12.4	38.9±13.5	6.9±2.3	8.9±2.5	9.3±2.5
72 h	7.5±2.8	54.7±21.5	53.5±24.1	8.2±2.5	7.8±2.4	10.5±2.8

the malignant tumor behavior by other regulatory pathways and PLCε gene may only is one of the action targets.

Conclusions

The metformin can inhibit the expression of PLCε gene and influence Notch1/Hes and AR signaling pathway activity, so as to regulate the biological behaviors of CRPC PC-3 cell line such as proliferation, invasion, migration and apoptosis.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Yung KW, Yung TT, Chung CY, Tong GT, Liu Y, Henderson J, Welbeck D, Oseni S. Principles of cancer staging. Asian Pac J Surg Oncol 2015; 1: 1-16.
2) Antonarakis ESC, Eisenberger MA. Novel targeted therapeutics for metastatic castration-resistant prostate cancer. Cancer Lett 2010; 291: 1-13.
3) Schweizer MT, Antonarakis ES, Wang H, Ajiboye AS, Spitz A, Cao H, Luo J, Haffner MC, Yegnasubramanian S, Carducci MA, Eisenberger MA, Isaacs JT, Denmeade SR. Effect of bipolar androgen therapy for asymptomatic men with castration-resistant prostate cancer: Results from a pilot clinical study. Sci Transl Med 2015; 7: 269ra2.
4) Cazzaniga M, Bonanni B. Breast cancer metabolism and mitochondrial activity: the possibility of chemoprevention with metformin. Biomed Res Int 2015; 2015: 972193.
5) Hall C, Stone RL, Gehlot A, Zorn KK, Burnett AF. Use of metformin in obese women with type i endometrial cancer is associated with a decreased incidence of cancer recurrence. Int J Gynecol Cancer 2016; 26: 313-317.
6) Kim J, Lee J, Kim C, Choi J, Kim A. Anti-cancer effect of metformin by suppressing signaling pathway of HER2 and HER3 in tamoxifen-resistant breast cancer cells. Tumour Biol 2016; 37: 5811-5819.
7) Sehdev A, Shih YC, Vekhter B, Bissonnette MB, Olopade OI, Polite BN. Metformin for primary colorectal cancer prevention in patients with diabetes: a case-control study in a US population. Cancer 2015; 121: 1071-1078.
8) Yu H, Yin L, Jiang X, Sun X, Wu J, Tian H, Gao X, He X. Effect of metformin on cancer risk and treatment outcome of prostate cancer: a meta-analysis of epidemiological observational studies. PLoS One 2014; 9: e116327.
9) Spratt DE, Zhang C, Zumsteg ZS, Pei X, Zhang Z, Zelefsky MJ. Metformin and prostate cancer: benefit for development of castration-resistant disease and prostate cancer mortality. Eur Urol 2013; 63: 709-716.
10) Malaguarnera R, Sacco A, Morcavallo A, Squatrito S, Migliaccio A, Morrione A, Maggiolini M, Belfiore A. Metformin inhibits androgen-induced IGF-IR up-regulation in prostate cancer cells by disrupting membrane-initiated androgen signaling. Endocrinology 2014; 155: 1207-1221.
11) Wang Y, Wu X, Ou L, Yang X, Wang X, Tang M, Chen E, Luo C. PLCε knockdown inhibits prostate cancer cell proliferation via suppression of notch signalling and nuclear translocation of the androgen receptor. Cancer Letters 2015; 362: 61-69.
12) Soylu H, Acar N, Ozbey O, Unal B, Koksal IT, Bassorgun I, Ciftcioglu A, Ustunel I. Characterization of notch signalling pathway members in normal prostate, prostatic intraepithelial neoplasia (PIN) and prostatic adenocarcinoma. Pathol Oncol Res 2016; 22: 87-94.
13) Zhang QD, Xu MY, Cai XB, Qu Y, Li ZH, Lu LG. Myofibroblastic transformation of rat hepatic stellate cells: the role of notch signaling and epithelial-mesenchymal transition regulation. Eur Rev Med Pharmacol Sci 2015; 19: 4130-4138.
14) Zhang J, Yin XJ, Xu CJ, Ning YX, Chen M, Zhang H, Chen SF, Yao LQ. The histone deacetylase SIRT6 inhibits ovarian cancer cell proliferation via down-regulation of notch 3 expression. Eur Rev Med Pharmacol Sci 2015; 19: 818-824.