TUBEIMOSIDE-1 INHIBITED INVASIVENESS OF PC-3 CELLS THROUGH DEACTIVATE NLRP3 INFLAMMASOME

ZHU KE[1], ZHANG CHAO[2,*]
[1]Urology Surgery department, Chengdu Jin Niu District People's Hospital, Chengdu, Sichuan Province 610036, China - [2]The first people's Hospital of En Yang District, Bazhong City, Sichuan Province, 636600, China

ABSTRACT

Prostate cancer is the most frequently diagnosed urinary tract tumor. Tubeimoside-1(TBMS-1) plays an important role in inducing prostate cancer cells apoptosis and cell cycle arrest. However, the effect of TBMS-1 on the prostate cancer cells invasiveness has never been studied. In our study, PC-3 cells were treated with 0, 0.1, 1.0, and 10.0 μM TBMS-1 for 12, 24, and 48 hours, then Nigericin and LPS, were added to active NLRP3 signaling. The cell viability and invasiveness were detected in PC-3 cells. The expression of inflammasome component proteins (NLRP3, Caspase-1, IL-1β) and MMP-2 were detected by Western blotting. Our study showed that TBMS-1 treatment significantly decreased the of PC-3 cells' viability and invasiveness (P<0.05). The inflammasome component protein (NLRP3, Caspase-1, IL-1β) and MMP-2 were significantly decreased after TBMS-1 exposure (P<0.05), which was nullified by Nigericin (P<0.05). In conclusion, we found that TBMS-1 inhibited the viability and invasiveness of PC-3 cells. We also found that TBMS-1 exposure significantly suppressed the expression of MMP-2 and inflammasome component proteins, which would be abrogated by Nigericin + LPS exposure. All of these proved that deactivation of NLRP3 inflammasome may serve as an important step in TBMS-1 induced PC-3 cell invasiveness reduction.

Keywords: *Tubeimoside-1, prostate cancer, invasiveness, PC-3 cells, Nigericin, NRLP3.*

DOI: 10.19193/0393-6384_2022_3_247

Received *March 15, 2021;* ***Accepted*** *Januaryr 20, 2022*

Introduction

Prostate cancer most frequently diagnosed tumor, which annually resulted in 1-2% of deaths in men[(1)]. Invasion and metastasis are important characteristic of prostate cancer, which is closely associated with the poor prognosis of patients[(2)]. In recent years, more and more attention has been paid to the anticancer drugs of natural compounds, especially the TBMS-1[(3)]. TBMS-1 is a triterpenoid saponin extracted from Koelreuteria paniculate[(4)],which has anti-cancer potential[(5)]. TBMS-1 was shown to suppress the proliferation and invasion of colorectal cancer cells[(6)]. Moreover, it was reported that TBMS-1 could cause the non-small cell lung cancer angiogenesis inhibition[(7)]. It also reported that TBMS-1 plays a key role in inducing prostate cancer cells apoptosis and cell cycle arrest[(8)]. However, no study has yet examined the role of TBMS-1 played in the prostate cancer cells invasiveness. The inflammasome component proteins, which was made up of NLRP3, adaptor protein ASC, and protease caspase-1, which could induce the release of IL-1β and IL-18, thus causing the occurrence of inflammation[(9)]. NLRP3 is an important mediator of tumor cells. Evidence showed that NLRP3 signaling was involved in migration and invasion in colorectal cancer cell[(10)] and lung

cancer cells[11]. It was also reported that decreasing the activity of NLRP3 inflammasomes promotes the metastasis of renal cell cancer[12]. However, the effect of NLRP3 signaling pathway on invasion and metastasis of prostate cancer has not been reported. It remains to be seen if TBMS-1 could regulate the activity of NLRP3 inflammasome.

In our study, we demonstrated that the proliferation and invasiveness of prostate cancer cells (PC-3) decreased after TBMS-1 exposure. In addition, the expression of NLRP3 and MMP-2 was significantly decreased after TBSM-1 stimulation, which could be partly abrogated by Nigericin, an agonist of the NLRP3 receptor. Moreover, we concluded that TBMS-1 inhibited invasiveness of PC-3 cells via NLRP3 deactivation.

Materials and methods

Cell culture and regents

PC-3 cell was bought from ATCC company (Manassas, VA, USA). Cells were cultured with DMEM medium supplied with 10% fetal bovine serum in cell incubator (37°C, 5% humidified CO_2). Cells in logarithmic growth phase were used for the experiments and pre-treated with 1 μg/mL of LPS for 4 hours, then incubated with 10 μmol/L of Nigericin to active the NLRP3 inflammasome[13]. The MTS reagent kit was bought from Promega Company (Madison, WI, USA). Primary antibodies for NLRP3, caspase-1, IL-1β, MMP-2 and GAPDH (dilution 1:1,000) were brought from Abcam (Cambridge, MA). TBMS-1, LPS and Nigericin were brought from the Med Chem Express Co (Monmouth Junction, NJ, USA).

Cell viability assay of MTS

PC-3 cells were cultured with medium containing (0, 0.1, 1.0, and 10.0 μM) TBMS-1for 12, 24, 48 h in a 96-well microplate. Then the cells exposed to 10 μL MTS for 2 h. The absorbance values were measured at 490 nm wavelength by a microplate reader (BIO-TEK, Rockville, MA, USA).

Cell invasion assay

Cell invasiveness was detected by trans-well assay. The PC-9 cell suspension (1×10^4 cells) were added into the upper chamber, while DMEM medium with 15% FBS was added into the lower chamber, treated with or without TBMS-1 for 24h.When the cells were attached to a porous polycarbonate membrane with a matrix base membrane as the matrix. The cells that did not adhere to the polycarbonate membrane were removed with a cotton swab. The invaded cells were fixed with 4% paraformaldehyde for 20 min, and then stained with 1% crystal violet at room temperature for 30 min. Three randomly selected visual fields were photographed using an Olympus CX23 light microscope (200×magnification; Olympus Corporation, Tokyo, Japan). The cell number in photos were counted and analyzed.

Western blotting analysis

The PC-3 cells were completely destroyed with the cell lysis buffer on ice for 30 min. A 4°C refrigerated centrifuge was used to centrifuge cell lysis products at 12,000 rpm for 15 min and then supernatants were harvested. Measuring the protein concentration, added into loading buffer, and denature proteins at 100°C for 5 min. Next, 30 μg of protein products were electrophoresed on an 8% SDS-PAGE gel, then transferred onto PVDF membrane. The protein membranes were harvested and blocked with 5% skim milk, and then incubated with primary antibodies at 4°C overnight, washed with Tris-buffered saline supplied with 0.1% Tween20 and incubated with secondary antibody at room temperature for 2 h. The immunoreactivity was visualized by chemiluminescence.

Statistical analysis

All experiments were performed with 3 independent replicates, and data were expressed as mean ±SD. Univariate analysis of variance and Dunnett test were used to analyze the differences between TBMS-1 group and control group, and Tukey's test was used to analyze the differences between the TBMS-1 + Nigericin + LPS group and TBMS-1 group using GraphPad Prism software (version 5.01). When P values were less than 0.05, differences were assumed to be statistically significant.

Results and discussion

TBMS-1 treatment caused the cell viability and invasiveness reduction in PC-3 cells

The cell viability of PC-3 cells was detected after (0, 0.1, 1.0, and 10.0 μM) TBMS-1 exposed for 24 h (Figure 1A). The 10.0 μM, not the 0.1 or 1.0 μM, TBMS-1 significantly inhibited the cell viability of PC-3 cells. Then, we chose the 1.0 μM TBMS-1 treat with PC-3 cells for 12, 24, and 48 h. The result showed that TBMS-1 inducing cell viability

reduction in a time dependent manner, however, a significant difference was observed at 48 h (Figure 1B). In order to eliminate the effect of cell viability on invasion ability, we used 1.0 μM TBMS-1 in the trans-well invasion assay.

The result (Figure 1C and D) revealed that fewer invaded cells were observed in TBMS-1 group when compared to control cultures (P<0.05).

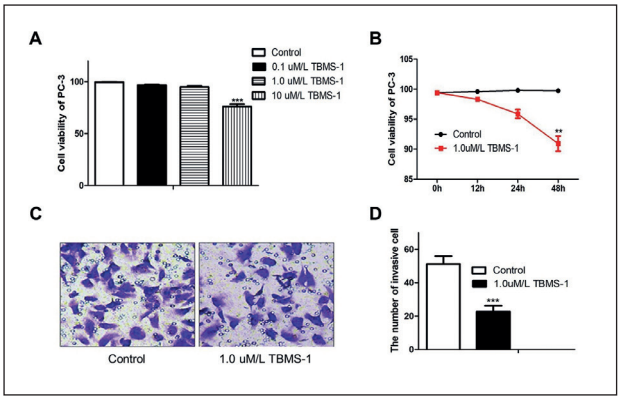

Figure 1: TBMS-1 showed anticancer effects in PC-3 cells. A) PC-3 cells were cultured with 0.0, 0.1, 1.0 and 10.0 μM TBMS-1 for 12 h, and then the MTS kit was used to detect the cell viability. B). PC-3 cells were cultured with 1.0 μM of TBMS-1 for 12, 24, and 48 h, then cell viability was tested. C and D) PC-3 cells were cultured with TBMS-1 1.0 μM for 12 h, then the invasiveness of the cells were detected by trans-well assay.
$^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ represent significance when compared to control.

The protein level of NLRP3, caspase-1, IL-1β and MMP-2 were decreased after TBMS-1 exposure in PC-3 cells

To explore the role of TBMS-1 in mediating the expression of inflammasome component proteins (NLRP3, Caspase-1, IL-1β) and MMP-2 in PC-3 cells, 10 μM TBSM-1 exposed to PC-3 cells for 12 h. TBMS-1 exposure significantly decreased the expression of NLRP3, caspase-1, IL-1β and MMP-2 when compared with the control group (Figure 2 A- E).

TBMS-1 abrogated LPS + Nigericin induced NLRP3, caspase-1, IL-1β and MMP-2 expression and cell invasiveness increasement

The PC-3 cells were treated with TBMS-1 and Nigericin (an NLRP3 receptor agonist) to explore the involvement of NLRP3 inflammasomes and MMP-2 following TBMS-1 exposure. We used 1 μg/mL of LPS for 4 hours, followed by treatment with 10 μmol/L of Nigericin to active the NLRP3 receptor[14]. LPS + Nigericin significantly increased inflammasome component proteins (NLRP3, cleaved caspase-1, IL-1β) and MMP-2 protein level, which was abrogated by TBMS-1 treatment (Figure 3 A-E). In the trans-well invasion assay experiment, we observed that LPS + Nigericin increased the PC-3 cells invasiveness, which was reversed by TBMS-1 treatment (Figure 3 F and G).

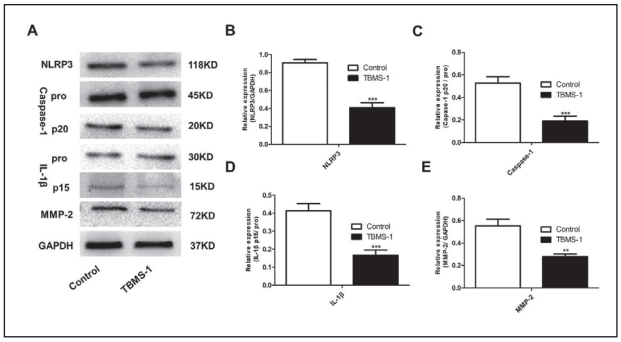

Figure 2: Protein level of NLRP3, caspase-1, IL-1β and MMP-2 in TBMS-1 treated or untreated PC-3 cells. A-E) The expression of NLRP3, caspase-1, IL-1β and MMP-2 were decreased after 1.0 μM TBMS-1 treatment for 12 h, which was visualized by western blotting. The results were analyzed by Prism, version 5.01 software. The graph represents densitometry of the results of three independent experiments (mean±SD).
Statistical significance compared with the control is indicated ($^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$).

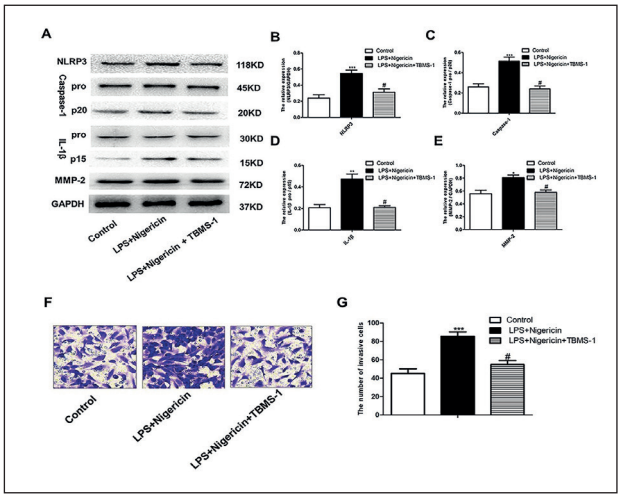

Figure 3: TBMS-1 abrogated LPS + Nigericin induced NLRP3, caspase-1, IL-1β and MMP-2 expression and cell invasiveness increasement. A-E) PC-3 cells were exposed to 1.0 μM TBMS-1 with or without 1 μg/mL of LPS for 4 hours, followed by incubation with 10 μmol/L of Nigericin for 24 h. The protein level of NLRP3, caspase-1, IL-1β and MMP-2 were visualized by western blotting and then analyzed. F and G) The invasiveness of PC-3 cells was detected by trans-well assay so that the invaded cells could be counted and analyzed. The graph represents densitometry of the results of three independent experiments (mean ± SD),
$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$and #P<0.05 represent significance comparing TBMS-1+LPS+Nigericin versus LPS+ Nigericin alone.

Discussion

In this study, we found that TBMS-1 inhibited the cell viability and invasiveness of PC-3 cells, as well as caused MMP-2 expression reduction and NLRP3 inflammasome deactivation, which was partly reversed by LPS+ Nigericin treatment.

TBMS-1 is a kind of triterpenoid saponin with antitumor properties, which is extracted from natural herbs[4]. TBMS played a role in inhibiting cell growth, inducing cell differentiation, apoptosis, autophagy and suppressing angiogenesis, invasion and metastasis[3]. Exposure to TBMS-1 has been associated with inhibiting cervical cancer cell growth by triggering cell cycle arrest and apoptosis[15]. Yan et al.[16] demonstrated that TBMS inhibit colorectal cancer cell viability and trigger apoptosis. Wang et al.[17] reported TBMS-1 could inhibit the proliferation of hepatoma (HepG2) cells. Huang et al.[18] found that TBMS-1 exerted strong proliferation inhibitory effects on human choriocarcinoma cell. Our study observed that TBMS-1 decreased the cell viability of PC-3 cells in concentration and time dependent manner (Figure 1 A and B). It has been reported TBMS-1 inducing the inhibition of cell proliferation and metastasis in glioblastoma[19]. Our study showed that TBMS-1 inhibited the PC-3 cells invasiveness (Figure 1 C and D).

Prostate cancer caused the major deaths among men. It was reported that 366,000 men die of prostate cancer among 1.6 million diagnosed patients for each year[20]. Most deaths from cancer occurs as a result of metastasis[21]. Matrix metalloproteinases play a key role in tumor invasion and metastasis by degrading almost all the protein components of ECM and destroying the histological barrier of tumor cell invasion[3]. MMP-2 can degrade structural components of the extracellular matrix and promote tumor invasion. It reported that MMP-2 is an early response protein in metastasis of ovarian cancer[22]. Increased MMP-2 expression by malignant prostatic epithelia is an independent predictor of decreased prostate cancer disease-free survival[23]. MMP-2 may function as a mediator for migration and invasion of PC-3 cells[24]. We found that TBMS-1 exposure inhibited the protein level of MMP-2 in PC-3 cells (Figure 2 E). Therefore, TBMS-1 may exert the inhibitory effect on the invasiveness of PC-3 cells through suppression of MMP-2 expression.

NLRP3 inflammasomes are an activating platform of caspase, consisting of NLRP3, ASC, and caspase-1. IL-1β is one of the critical cytokines to promote tumor growth and metastasis, which produced by activated NLRP3 inflammasomes[25]. It was reported that inhibition of the NLRP3 inflammasome suppressed the metastatic potential of tumor cells[26]. Activation of NLRP3 inflammasome promoted the cell growth and migration of lung cancer cells[11].The NLRP3 inflammasome activates caspase-1 to regulates the secretion of mature IL-1β[27]. Our study observed that TBMS-1 decreased the expression of NLRP3 and cleaved caspase-1 and IL-1β (Figure 2 A-D), which indicated that TBMS-1 could deactivate the NLRP3 inflammasome.

Nigericin is an NLRP3 receptor kinase agonist that induces the activation of the NLRP3 inflammasome[28]. It has been reported that Nigericin is often used in combination with LPS to activate NLRP3 inflammasome[13]. In our study, we observed that the expression of NLRP3, caspase-1 and IL-1β were significantly increased by LPS+Nigericin exposure (Figure 3 A, B, C, D), which indicated that NLRP3 inflammasomes were activated by LPS+Nigericin. Studies of NLRP3 inflammasome promote the metastasis of cancer cells have been reported. NLRP3 promoted metastasis in human oral squamous cell carcinoma[29]. Activation of NLRP3 inflammasome promoted the migration of esophageal squamous cell carcinoma[30]. In our study, we found that LPS+Nigericin significantly increased the expression of MMP-2 and the number of invasive PC-3 cells (Figure 3 E, F, G), which could be partly reversed by TBMS-1 exposure (Figure 3).

Conclusion

In summary, our research indicated that TBMS-1 may inhibit the invasiveness of prostate cancer PC-3 cells through deactivation of NLRP3 inflammasomes to suppress the MMP-2 production. TBMS-1 may be a potential therapeutic agent in prostate cancer treatment.

References

1) Attard G, Parker C, Eeles RA, Schroder F, Tomlins SA, Tannock I, et al. Prostate cancer. Lancet. 2016; 387(10013): 70-82.

2) Wang W, Yang X, Dai J, Lu Y, Zhang J, Keller ET. Prostate cancer promotes a vicious cycle of bone metastasis progression through inducing osteocytes to secrete GDF15 that stimulates prostate cancer growth and invasion. Oncogene. 2019; 38(23): 4540-59.

3) Islam MS, Wang C, Zheng J, Paudyal N, Zhu Y, Sun H. The potential role of tubeimosides in cancer prevention and treatment. Eur J Med Chem. 2019; 162: 109-21.
4) Yin Y, Chen W, Tang C, Ding H, Jang J, Weng M, et al. NF-kappaB, JNK and p53 pathways are involved in tubeimoside-1-induced apoptosis in HepG2 cells with oxidative stress and G(2)/M cell cycle arrest. Food Chem Toxicol. 2011; 49(12): 3046-54.
5) Ma R, Song G, You W, Yu L, Su W, Liao M, et al. Anti-microtubule activity of tubeimoside I and its colchicine binding site of tubulin. Cancer Chemother Pharmacol. 2008; 62(4): 559-68.
6) Bian Q, Liu P, Gu J, Song B. Tubeimoside-1 inhibits the growth and invasion of colorectal cancer cells through the Wnt/beta-catenin signaling pathway. Int J Clin Exp Pathol. 2015; 8(10): 12517-24.
7) Gu Y, Korbel C, Scheuer C, Nenicu A, Menger MD, Laschke MW. Tubeimoside-1 suppresses tumor angiogenesis by stimulation of proteasomal VEGFR2 and Tie2 degradation in a non-small cell lung cancer xenograft model. Oncotarget. 2016; 7(5): 5258-72.
8) Yang JB, Khan M, He YY, Yao M, Li YM, Gao HW, et al. Tubeimoside-1 induces oxidative stress-mediated apoptosis and G0/G1 phase arrest in human prostate carcinoma cells in vitro. Acta Pharmacol Sin. 2016; 37(7): 950-62.
9) Shao BZ, Wang SL, Pan P, Yao J, Wu K, Li ZS, et al. Targeting NLRP3 Inflammasome in Inflammatory Bowel Disease: Putting out the Fire of Inflammation. Inflammation. 2019; 42(4): 1147-59.
10) Deng Q, Geng Y, Zhao L, Li R, Zhang Z, Li K, et al. NLRP3 inflammasomes in macrophages drive colorectal cancer metastasis to the liver. Cancer Lett. 2019; 442: 21-30.
11) Wang Y, Kong H, Zeng X, Liu W, Wang Z, Yan X, et al. Activation of NLRP3 inflammasome enhances the proliferation and migration of A549 lung cancer cells. Oncol Rep. 2016; 35(4): 2053-64.
12) Wang K, Xu T, Ruan H, Xiao H, Liu J, Song Z, et al. LXRalpha promotes cell metastasis by regulating the NLRP3 inflammasome in renal cell carcinoma. Cell Death Dis. 2019; 10(3): 159.
13) Han C, Yang Y, Guan Q, Zhang X, Shen H, Sheng Y, et al. New mechanism of nerve injury in Alzheimer's disease: beta-amyloid-induced neuronal pyroptosis. J Cell Mol Med. 2020; 24(14): 8078-90.
14) Huang CF, Chen L, Li YC, Wu L, Yu GT, Zhang WF, et al. NLRP3 inflammasome activation promotes inflammation-induced carcinogenesis in head and neck squamous cell carcinoma. J Exp Clin Canc Res. 2017; 36.
15) Feng X, Zhou J, Li J, Hou X, Li L, Chen Y, et al. Tubeimoside I induces accumulation of impaired autophagolysosome against cervical cancer cells by both initiating autophagy and inhibiting lysosomal function. Cell Death Dis. 2018; 9(11): 1117.
16) Yan J, Dou X, Zhou J, Xiong Y, Mo L, Li L, et al. Tubeimoside-I sensitizes colorectal cancer cells to chemotherapy by inducing ROS-mediated impaired autophagolysosomes accumulation. J Exp Clin Cancer Res. 2019; 38(1): 353.
17) Wang Y, Deng L, Zhong H, Wang Y, Jiang X, Chen J. Natural plant extract tubeimoside I promotes apoptosis-mediated cell death in cultured human hepatoma (HepG2) cells. Biol Pharm Bull. 2011; 34(6): 831-8.
18) Li W, Zhang HX, Nie MX, Tian YL, Chen X, Chen CS, et al. Ursolic acid derivative FZU-03,010 inhibits STAT3 and induces cell cycle arrest and apoptosis in renal and breast cancer cells. Acta Bioch Bioph Sin. 2017; 49(4): 367-73.
19) Cao J, Zhao E, Zhu Q, Ji J, Wei Z, Xu B, et al. Tubeimoside-1 Inhibits Glioblastoma Growth, Migration, and Invasion via Inducing Ubiquitylation of MET. Cells. 2019; 8(8).
20) Pernar CH, Ebot EM, Wilson KM, Mucci LA. The Epidemiology of Prostate Cancer. Cold Spring Harb Perspect Med. 2018; 8(12).
21) Brower V. Researchers tackle metastasis, cancer's last frontier. J Natl Cancer Inst. 2007; 99(2): 109-11.
22) Kenny HA, Lengyel E. MMP-2 functions as an early response protein in ovarian cancer metastasis. Cell Cycle. 2009; 8(5): 683-8.
23) Trudel D, Fradet Y, Meyer F, Harel F, Tetu B. Significance of MMP-2 expression in prostate cancer: an immunohistochemical study. Cancer Res. 2003; 63(23): 8511-5.
24) Liu H, Chen A, Guo F, Yuan L. A short-hairpin RNA targeting osteopontin downregulates MMP-2 and MMP-9 expressions in prostate cancer PC-3 cells. Cancer Lett. 2010; 295(1): 27-37.
25) Carmi Y, Voronov E, Dotan S, Lahat N, Rahat MA, Fogel M, et al. The role of macrophage-derived IL-1 in induction and maintenance of angiogenesis. J Immunol. 2009; 183(7): 4705-14.
26) Lee HE, Lee JY, Yang G, Kang HC, Cho YY, Lee HS, et al. Inhibition of NLRP3 inflammasome in tumor microenvironment leads to suppression of metastatic potential of cancer cells. Sci Rep-Uk. 2019; 9.
27) Davis BK, Wen HT, Ting JPY. The Inflammasome NLRs in Immunity, Inflammation, and Associated Diseases. Annu Rev Immunol. 2011; 29: 707-35.
28) Zhang K, Shi Z, Zhang M, Dong X, Zheng L, Li G, et al. Silencing lncRNA Lfar1 alleviates the classical activation and pyroptosis of macrophage in hepatic fibrosis. Cell Death Dis. 2020; 11(2): 132.
29) Wang H, Luo Q, Feng X, Zhang R, Li J, Chen F. NLRP3 promotes tumor growth and metastasis in human oral squamous cell carcinoma. BMC Cancer. 2018; 18(1): 500.
30) Yu S, Yin JJ, Miao XJ, Li SG, Huang CZ, Huang N, et al. Activation of NLRP3 inflammasome promotes the proliferation and migration of esophageal squamous cell carcinoma. Oncol Rep. 2020; 43(4): 1113-24.

Corresponding Author:
Zhang Chao
The first people's Hospital of Enyang District, Bazhong City, China
Email: zhukecd1234@163.com
(China)