ORIGINAL ARTICLES

Department of Obstetrics[1]; General Surgery Department[2], Jinan Seventh People's Hospital, Jinan, China

JTC-801 inhibits the proliferation and metastasis of ovarian cancer cell SKOV3 through inhibition of the PI3K - AKT signaling pathway

Jun-Xia Li[1,*], Yan-Ping Bi[1], Jing Wang[1], Xue Yang[2], Yan-Fang Tian[1], Zhong-Fang Sun[1]

Received December 16, 2017, accepted January 19, 2018

**Corresponding author: Jun-Xia Li, Department of Obstetrics, Jinan Seventh People's Hospital, No.17 on XinYuan Road, Jiyang District, Jinan, 251400, Shandong, China*
junxiali12@126.com

Pharmazie 73: 283–287 (2018) *doi: 10.1691/ph.2018.7326*

Abstract. Ovarian cancer (OC) is the commonest cause of gynaecological cancer-associated death because of the wide metastasis and frequent recidivation. JTC-801 is a new synthetic compound with the function of reversing pain and anxiety symptoms as a selective opioid receptor-like1 receptor (belonging to the G-protein-coupled receptor) antagonist. We investigated the role and possible mechanisms of JTC-801 in the cell growth and metastasis of OC. It was observed that JTC-801 inhibited the proliferation, invasion and migration of cancer in SKOV3 cells. The apoptosis rate of SKOV3 cells treated with JTC-801 was significantly increased ($P<0.05$), and the expression results of relevant apoptosis proteins (BCL2, BAX, Active Caspase-3) indicated the JTC-801 could induce the apoptosis of SKOV3. Further, the expression levels of phosphorylated AKT, phosphorylated mTOR, P70 and CyclinD1 in the PI3K/AKT signaling pathway were obviously reduced in the JTC-801 treated SKOV3 group. This suggests that JTC-801 exerts its anticancer effect through the PI3K/AKT signaling pathway. Our data also highlights the possibility of using JTC-801 as a novel therapeutic drug for OC treatment mean while it plays the analgesic effect.

1. Introduction

Ovarian cancer (OC), accounting for an estimated 200,000 new cases and 150,000 deaths worldwide annually, is the seventh most common cancer and the eighth cause of death from cancer in women (Ferlay et al. 2015; Bruggmann et al. 2017). The 5-year relative survival rate is only 29% (Reid et al. 2017), for the disease is apt to widely metastatic and frequently recrudescent (Jayson et al. 2014). Furthermore, the surgical treatment for OC has to be replaced by radiotherapy and chemotherapy on account that it is usually definite diagnosed at late stage. However, the side-effect and intolerance limit the application of radiotherapy and chemotherapy for OC. Therefore, it is highly important for us to find new drugs that suppress proliferation and migration of OC cells.

The G-protein-coupled receptor (GPCR) is the largest family of cell surface receptors involved in signal transduction (Schonenbach et al. 2015). These proteins share a common seven-transmembrane (7TM) topology and mediate cellular responses to a variety of extracellular signals ranging from photons and small molecules to peptides and proteins (Katritch et al. 2013). GPCRs were reported to have a pivotal role in many physiological functions and in multiple diseases, including the development of cancer and cancer metastasis (Lappano and Maggiolini 2011; O'Hayre et al. 2013), for example, GPCRs control the immune cell-mediated functions, proliferation, invasion and survival in the process of tumorigenesis (Bar-Shavit et al. 2016). In addition, overwhelming evidence have implicated GPCRs, G proteins and their downstream signaling targets in cancer initiation and progression, where they can influence aberrant cell growth and survival, largely through activation of AKT/mTOR, MAPKs, and Hippo signaling pathways (O'Hayre et al. 2014). Therefore, the novel GPCR targets may provide promising opportunities for drug discovery in cancer prevention and treatment (Lappano and Maggiolini 2011). JTC-801, high affinity, is a selective opioid receptor-like1 (ORL1) receptor antagonist, binding to ORL1 receptor with a Ki value of 8.2 nM. The ORL1 receptor, also called nociception/orphanin FQ (N/OFQ) peptide (NOP) receptor, belongs to the family of G protein-coupled opioid receptors (covering mu, kappa and delta). JTC-801 is reported to reverse single-prolonged stress-induced anxiety- and pain-like behaviors, and NOP receptor system up-regulation (Zhang et al. 2015b). It also could block cannabinoid-evoked hypothermia in rats (Rawls et al. 2007). Several retrospective studies have suggested that opioid use might promote tumor progression and as a result negatively impact survival in patients with advanced cancer (Afsharimani et al. 2011; Lennnon et al. 2012; Lennon et al. 2014), whether JTC-801 has effects on OC hasn't been reported yet.

In this present study we elucidated the inhibition effect of JTC-801 on OC cell SKOV3 proliferation and metastasis, and investigated the underling mechanisms of it. We expected that this study could provide a theoretical basis for the effective use of JTC-801 and the treatment of OC.

2. Investigations and results

2.1. *JTC-801 inhibited the proliferation of OC cell SKOV3*

To explore whether JTC-801 affects OC cell SKOV3 proliferation, we performed CCK-8 assay. The results showed that OD values of SKOV3 cells treated with JTC-801 was decreased compared to NC, especially, the OD values of viable SKOV3 cells at 48 h and 72 h in JTC-801 group were significantly reduced than NC group (Figure. 1, $P < 0.05$).

2.2. *JTC-801 inhibited OC cell SKOV3 invasion and migration*

To determine the effect of JTC-801 on OC cell invasion and metastasis, Transwell assay was carried out. In JTC-801 group, the invasive and migratory cell counts were 28±2 and 37±5 respectively. In NC group, the invasive and migratory cell counts were 50±4 and

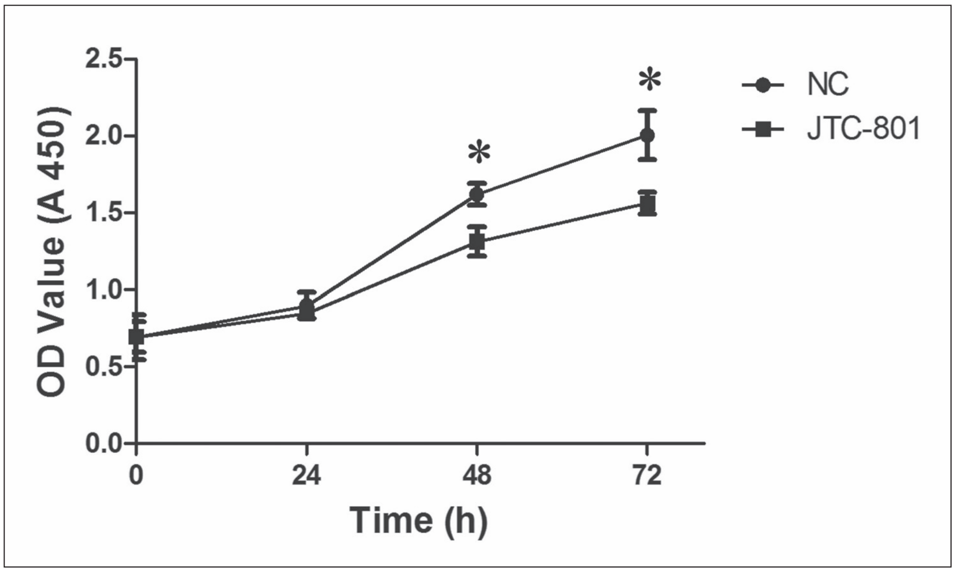

Fig. 1: Proliferation change of SKOV3 cells treated with JTC-801. * P < 0.05 significantly different compared to negative control group. Data were expressed as the mean±SD.

91±8 respectively (Figure. 2A-B). It suggests that JTC-801 treatment could inhibit the invasion and migration of SKOV3 (P<0.05).

2.3. JTC-801 promoted the apoptosis of SKOV3

Then we investigated the effects of JTC-801 on the apoptosis of SKOV3 cells by flow cytometry and western blot assay. After the apoptosis induction of cells with serum-free, compared with the NC group, SKOV3 cell apoptosis rate in the JTC-801 group was significantly increased (23.77%>5.69%, Figure. 3A, P<0.05). The western-blot assay results showed that the expression of anti-apoptotic protein B-cell lymphoma 2 (BCL2) was obviously decreased (P<0.05), and the expression of pro-apoptotic proteins Bcl-associated X (BAX), Active Caspase3 was significantly increased (P<0.05, Figure. 3B-C). These results suggested that JTC-801 could promote the apoptosis of SKOV3.

2.4. JTC-801 inhibited the activation of phosphatidylinositol 3 kinase (PI3K)/serine-threonine kinase (AKT) signal pathway in OC cell SKOV3

PI3K/AKT signal pathway, which is often aberrantly activated in human cancers, including OC, is one of the most classical pro-survival pathways (Ciruelos Gil 2014; Fumarola et al. 2014; Danielsen et al. 2015; Mabuchi et al. 2015). The key proteins called AKT and mechanistic target of rapamycin (mTOR) in the pathway play an important role in the process of tumor cell proliferation and transfer (Edlind and Hsieh 2014). Therefore, we next examined whether the effect of JTC-801 on OC cell SKOV3 was correlated with PI3K/AKT signal pathway. The levels of AKT phosphorylation at Ser473 in SKOV3 were detected by WB method. The WB results showed that the levels of AKT and mTOR phosphorylation were significantly inhibited in JTC-801- treated SKOV3 cells, as compared to the NC group (P<0.05). The downstream protein 70-kDa ribosomal protein kinase I (P70) and CyclinD1 of the PI3K/AKT signaling pathway were obviously reduced in JTC-801 group than that in NC group (P<0.05) (Figure 4). These results indicated that JTC-801 inhibited the activation of PI3K/AKT signal pathway in OC cell SKOV3.

3. Discussion

The transfer and diffusion of OC cells are crucial biological characteristics of malignant tumor, which are also the main causes of death in patients with OC. In this study, we investigated the effect of JTC-801 on the proliferation, invasion, metastasis, and apoptosis in OC cells SKOV3. The results of CCK-8 assay showed that JTC-801 could significantly inhibit the cell proliferation with less OD values of viable SKOV3 cells, as compared to the normal control group. The Transwell assay verified that the JTC-801 could markedly inhibit the migration and invasion of SKOV3 cells (P<0.05). The WB and flow cytometry assays suggested that JTC-801 could promote the apoptosis of SKOV3 through decreasing the expression of anti-apoptotic protein BCL2 and

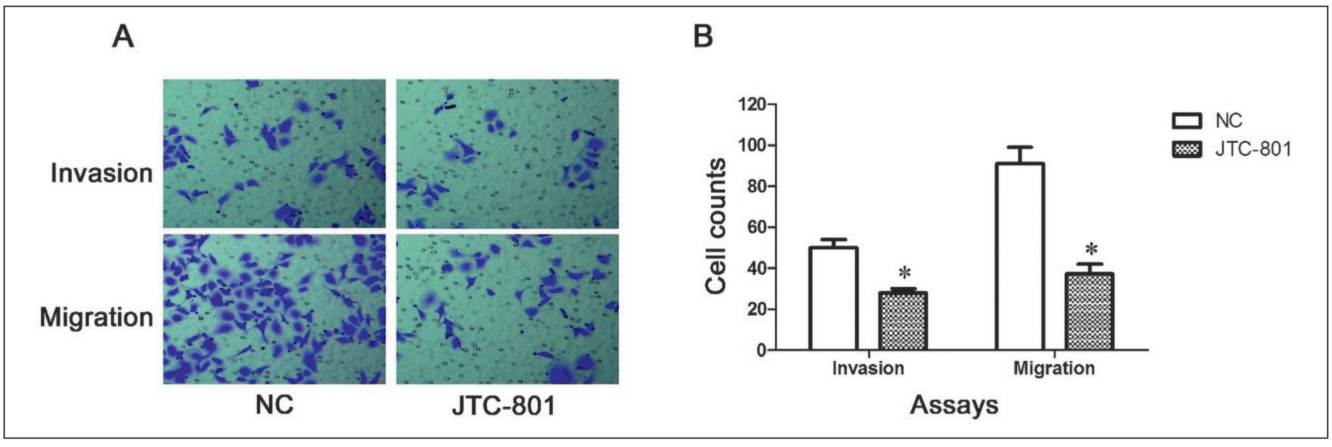

Fig. 2: The effect of JTC-801 on invasion and metastasis abilities in SKOV3 cells. A: Images of SKOV3 stained with 0.1% crystal violet were acquired at 200 magnification under an inverted microscope. B: Statistical significance was analyzed according to the number of invaded and metastasized cells. * P < 0.05 significantly different compared to negative control group. Data were expressed as the mean ± SD.

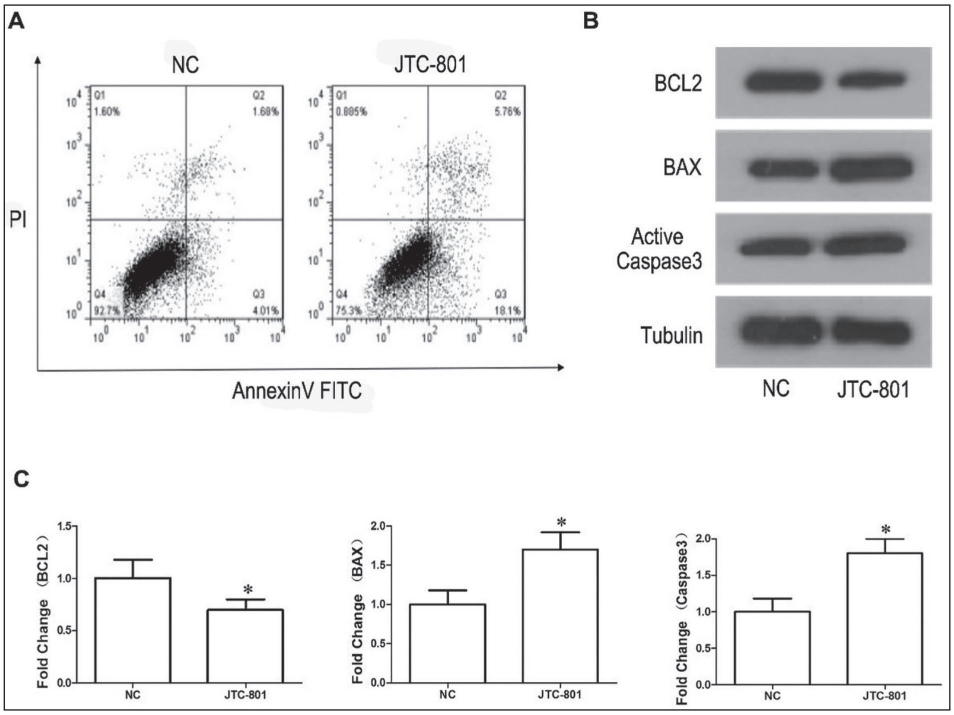

Fig. 3: Effect of JTC-801 on apoptosis of SKOV3 human ovarian cancer cells. A: Apoptosis was detected using flow cytometry. FITC, fluorescein isothiocyanate; PI, propidium iodide. B: Expression levels of proteins (BCL2, BAX and Active Caspase3) involved in the apoptotic pathway in the different SKOV3 groups, as detected by western blotting. Lane 1, protein expression in the SKOV3 normal control group; Lane 2, protein expression in the SKOV3 JTC-801-treated group. C: Statistical significance was analyzed according to the expression levels of proteins. * $P < 0.05$ significantly different compared to negative control group. Data were expressed as the mean ± SD.

increasing the expression of pro-apoptotic proteins BAX, Active Caspase3. These findings suggested that JTC-801 may function as a tumor suppressor in SKOV3 cells. Furthermore, the possible function mechanism of JTC-801 on OC was explored by detecting the relevant proteins expression levels in the signal pathways based on the WB assays. As a result, the levels of phosphorylated AKT and mTOR, the downstream protein P70 and CyclinD1 of the PI3K/AKT signaling pathway were obviously reduced, indicating that JTC-801 inhibited the activation of PI3K/AKT signal pathway in OC cell SKOV3.

JTC-801 is a new synthetic selective ORL1 receptor antagonist. The ORL1 receptor belongs to the family of GPCRs which are involved in many pathophysiological processes including pathways. In the previous studies, JTC-801 was reported to reverse single-prolonged stress-induced mechanical allodynia, thermal hyperalgesia, anxiety-like behaviour and hypocortisolism in rats not only by intravenous injection but also oral administration. It also reversed NOP receptor protein and mRNA up-regulation in amygdala and periaqueductal grey (Yamada et al. 2002; Zhang et al. 2015b). JTC-801 was used to examine the involvement of endogenous N/OFQ in pathophysiological conditions, and the result was that increases in nitric oxide (NO) synthase (NOS) activity and NO production in neuropathic mice were blocked by pretreatment of oral JTC-801, which suggested that N/OFQ is involved in the maintenance of neuropathic pain and that the analgesic effect of JTC-801 on neuropathic pain is mediated by inhibition of NO production by neuronal NOS (Mabuchi et al. 2003). Besides, Koyama et al examined the JTC-801 on N(2)O-induced analgesia in 129Sv mice by the writhing test and tail flick test, and demonstrated that the analgesic effect of N(2)O was suppressed by the intraperitoneal administration of JTC-801 (Koyama and Fukuda 2009). In addition, Rawls et al firstly verified

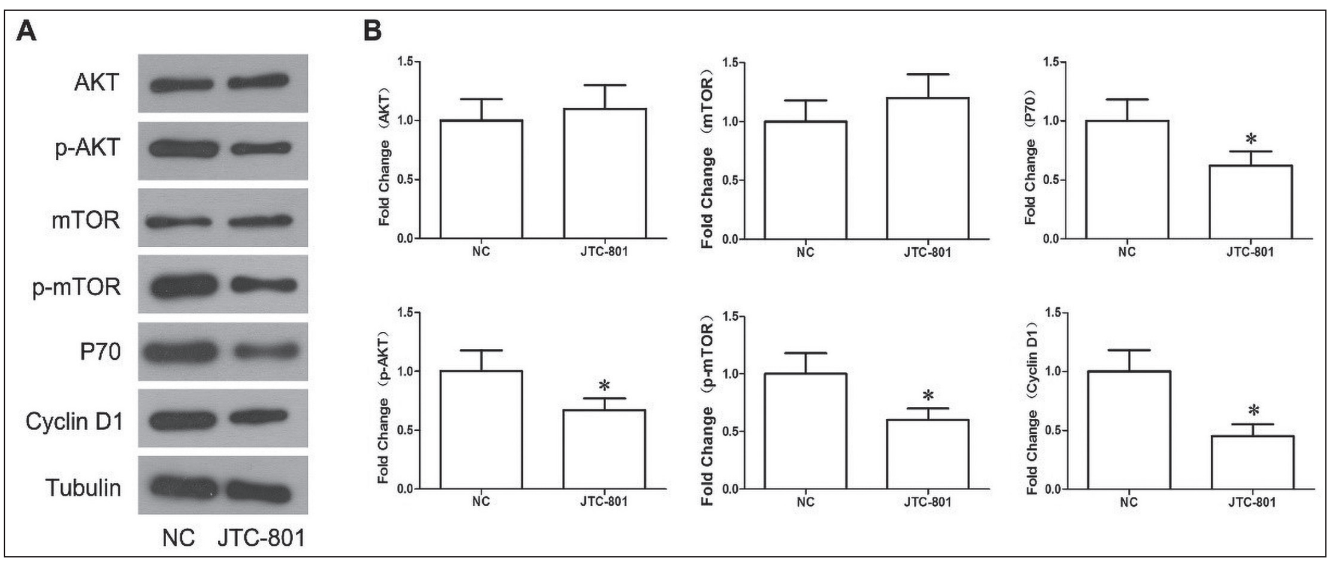

Fig. 4: JTC-801 inhibited the expression of PI3K/AKT signal pathway-related proteins in SKOV3. A: Expression levels of proteins in PI3K/AKT signal pathway in the different SKOV3 groups, as detected by western blotting. Lane 1, protein expression in the SKOV3 normal control group; Lane 2, protein expression in the SKOV3 JTC-801-treated group. B: Statistical significance was analyzed according to the expression levels of proteins. * $P < 0.05$ significantly different compared to negative control group. Data were expressed as the mean ± SD.

that JTC-801 could block cannabinoid-evoked hypothermia in rats (Rawls et al. 2007). In our present study, for the first time, we found the JTC-801 exerted suppressive effect on the tumorigenesis and development of OC. The proliferation, invasion and migration of OC cells SKOV3 were significantly inhibited by JTC-801, and the apoptotic SKOV3 cells were obviously increased in JTC-801-treated group, than that in NC group. Thus, it was certainly excellent for JTC-801 to be used to remit the pain caused by the tumor and to treat the tumor at the same time, which indicted that JTC-801 may be widely applied in clinical practice in the future.

The genesis, development and maintenance of pain usually comes from the interconnected network systems consisting of a variety of factors, and so the radical way to solve the clinical problem is to explore the pathogenesis of the molecular biology about pain. Cell signaling pathway is an important way that the cells participate in the biological process, including receiving and transmission of the information inside and outside environment, making a reply after the integration of complex signaling network, regulating the expression of related genes, and affecting cell proliferation, differentiation, migration, and apoptosis. The existing researches have showed that the PI3K/AKT signal pathway may be an important way in the network of pain. PI3Kγ/AKT has been reported to mediate the function of ephrinBs/EphBs in the central sensitization under bone cancer pain (BCP) condition, and inhibition of spinal PI3Kγ suppressed BCP-associated behaviors (Jin et al. 2015). Moreover, Xu et al have found that inhibition of spinal PI3K signaling pathway prevented pain behaviors (dose-dependent) and spinal Fos protein expression caused by plantar incision (Xu et al. 2014). Therefore, JTC-801 reduced pain sensation through the inhibition of PI3K/Akt pathway. PI3K/AKT signaling pathway, as one of the important signal transduction pathways in cells, plays a key role on cell proliferation and migration through affecting the activation state of a variety of downstream responsive molecules, and is closely related to the genesis and development of many human tumors. For example, inhibition of PI3K/AKT signaling by knockdown of Hexokinase-2 has been found to suppress aerobic glycolysis, increase cell apoptosis and reduce ability of colony formation (Zhuo et al. 2015). Liu et al have recently shown that blocking the PI3K/AKT signaling in human hepatocellular carcinoma cells largely abolished the inhibitory effect of Glutaminase 2 (a direct target gene of tumor suppressor p53) on the anchorage-independent cell growth and xenograft tumor growth (Liu et al. 2014). Further, signalling from the PI3K/AKT pathway has been found to activate numerous survival, growth, metabolic and metastatic functions characteristic of aggressive cancer (Toren and Zoubeidi 2014). Therefore, to excavate the possible mechanism behind these effects of JTC-801 on OC, we detected the relevant proteins in the PI3K/AKT pathway. As a result, we found that the levels of phosphorylated AKT and mTOR, the downstream protein P70 and CyclinD1 were obviously reduced in the SKOV3 group treated with JTC-801. Furthermore, we also found JTC-801 could promote SKOV3 cells apoptosis, and promoting apoptosis was the main way to exert effect for anti-tumor drugs, even more important, studies have reported that the occurrence of apoptosis was also related to the inhibition of PI3K/Akt pathway (Shrivastava et al. 2015; Zhang et al. 2015a). Therefore, PI3K/AKT pathway inhibition have the potential to significantly improve the future care of cancer patients in the current era of personalized cancer medicine (Polivka and Janku 2014), and this point out a new direction for OC treatment and OC pain remission.

In conclusion, our study demonstrates that JTC-801 leads to subdued OC cell SKOV3 growth and invasion through inhibiting the PI3K/AKT pathway. Therefore, JTC-801 may be a promising compound against malignant progression of OC. Of course, our work has some limitations, and it would be better for JTC-801 to be found the similar antagonistic effects on growth and metastasis of OC in vivo experimental systems.

4. Experimental

4.1. Cell culture

The JTC-801were provided from MCE Company (NJ, USA). OC cells (SKOV3) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). RPMI-1640 culture medium (Gibco Invitrogen Corporation) supplemented with 10% FBS, 0.1 mg/mL of streptomycin, and 100 U/mL of penicillin were used to culture the cells in 5 % CO_2 cell incubator at 37 °C and changed the fluid every 48 h and then transfected the cells when the cell fusion was up to 70-80 %. The SKOV3 cells were subjected to growth arrest for 24 h before different treatments were added to the indicated groups described below. The groups were JTC-801group (with 20 μM JTC-801) and negative control (NC, with the same volume of DMSO diluted by 1:1000).

4.2. Western Blot (WB)

The methodology has been described in previous report (Wu et al. 2016). In brief, the cells which grew well in the two groups were split by using pre-cooling cell lysis solution and extracted total protein. Bicinchoninic acid (BCA) protein assay was used to test protein concentration. The samples containing 20 μg of protein were electrophoresed on polyacrylamide SDS gels and then transferred to polyvinylidene diFuoride (PVDF) membranes. The membranes were blocked for 1 h, with 5% skimmed milk powder. Tris-buffered saline containing 0.2-0.4 % Tween-20 (TBST) was used to wash it every 5 min and added coating antibody and incubated the cells at 4 °C overnight. After washing (3 × 5 min TBST) the membrane, HRP labeled anti-rabbit IgG detection antibody (1:5000, PTG, Shanghai, China) was added and incubated the cells for 1 h at room temperature and washed (3×10 min TBST) the membrane. Equivalent amount of detection reagents 1 and 2 of an ECL Western Blotting Analysis System was added and mixed and put onto the membrane for 1 min. The extra fluid was removed from the edge, and plastic wrap was used to cover it and the images were exposed, developed, and fixed. Western blot result image was fed into the computer, and QUANTITY ONE Biomedicine Image Analysis System was used to test integrated grey level of stripes. Tubulin (1:5000, PTG, Shanghai, China) was taken as internal reference for correction, and semi-quantitative ratio of optical density (OD) of stripes to OD of Tubulin protein was used for indication.

4.3. CCK-8 cell proliferation assay

The Cell Counting Kit-8 (CCK-8; Sollebao, Beijing) cell proliferation assay was conducted following the manufacturer's instructions (Xing et al. 2014). SKOV3 cells were plated at a density of 3-5 × 10^3 cells/well in a 96-well plate for 24 h. The medium was removed, and cells were treated with either DMSO as a control or 20 μM JTC801 in a total volume of 100 μl. The cells were regularly incubated in 5 % CO_2 cell incubator, and the cell vitality were tested every 24 h. Before testing, CCK-8 solution (10 μl) was added to each well and the cultures were incubated at 37°C for 90 min. Absorbance at 450 nm was measured using an immune reader to obtain growth curve of OC cells.

4.4. In vitro invasion and migration assays

The cell invasion assay was performed according to the manufacturer's instructions (Ahn et al. 2015). The chamber of a non-type I -coated 24-well culture insert (Millipore, Billerica, MA, USA) was used. Each well was coated with fresh Matrigel (BD Biosciences, 100ul; diluted at 1:6 with the serum) before the invasion assay. Briefly, 10,000 cells incubated for 24 h in NC and JTC-801groups were plated on the Matrigel coated membrane in the top chamber (24-well insert; pore size, 8mm; Corning Costar). After a 24-h incubation period at 37°C, the non-invading cells were mechanically removed with a cotton swab, and then the filters were individually fixed with 4 % paraformaldehyde. Cells that had crossed through the Matrigel assay were stained with 0.1% crystal violet. The cell numbers were counted in five random fields of each chamber under the microscope. The procedure of cell migration assay was similar to the invasion assay. The only difference was that the Transwell did not need to be coated with Matrigel, and the number of cells plated were 5000.

4.5. Flow cytometry (Meusch et al. 2013; Wu et al. 2016)

Pancreatin/EDTA (0.05 %) was used to digest the cells of two groups, and PBS was used to wash the cells twice and 75 % ethanol was used to fix them (-20 °C). Four hours later, 400 μL of binding buffer was used to resuspend cells and 5 μL of AnnexinV-FITC (4A Biotech Co., Ltd, Beijing, China) was added and blended for reaction at room temperature for 5 min. 10 μL 20 μg/mL of PI dye liquor was added for incubation away from light at 4 °C for 30 min, and flow cytometry (GUAVA Technologies, Inc., Billerica, MA, USA) was used to test for analysis. A total of 10,000 fluorescence signals of each sample were collected, and the results were analyzed using FlowJo software (Tree Star, Inc, Ashland,OR, USA).

4.6. Statistical analysis

The results were dealt with statistics process by Statistical Product and Service Solutions (SPSS, Chicago, IL, USA). The data were expressed as (mean ± standard deviation). We implemented student's t TEST to compare the differences between groups, in addition, $P < 0.05$ was considered to be statistically significant difference.

Conflict of Interest: None declared

Acknowledgments: None.

References

Afsharimani B, Cabot P, Parat MO (2011) Morphine and tumor growth and metastasis. Cancer Metast Rev 30: 225-238.

Ahn JH, Choi YS, Choi JH (2015) Leptin promotes human endometriotic cell migration and invasion by up-regulating MMP-2 through the JAK2/STAT3 signaling pathway. Mol Human Reprod 21: 792-802.

Bar-Shavit R, Maoz M, Kancharla A, Nag JK, Agranovich D, Grisaru-Granovsky S, Uziely B (2016) G Protein-coupled receptors in cancer. Int J Mol Sci 17: pii: E1320.
Bruggmann D, Pulch K, Klingelhofer D, Pearce CL, Groneberg DA (2017) Ovarian cancer: density equalizing mapping of the global research architecture. Int J Health Geographics 16: 3.
Ciruelos Gil EM (2014) Targeting the PI3K/AKT/mTOR pathway in estrogen receptor-positive breast cancer. Cancer Treatm Rev 40: 862-871.
Danielsen SA, Eide PW, Nesbakken A, Guren T, Leithe E, Lothe RA (2015) Portrait of the PI3K/AKT pathway in colorectal cancer. Biochim Biophys Acta 1855: 104-121.
Edlind MP, Hsieh AC (2014) PI3K-AKT-mTOR signaling in prostate cancer progression and androgen deprivation therapy resistance. Asian J Androl 16: 378-386.
Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136: E359-386.
Fumarola C, Bonelli MA, Petronini PG, Alfieri RR (2014) Targeting PI3K/AKT/mTOR pathway in non small cell lung cancer. Biochem Pharmacol 90: 197-207.
Jayson GC, Kohn EC, Kitchener HC, Ledermann JA (2014) Ovarian cancer. Lancet 384: 1376-1388.
Jin D, Yang JP, Hu JH, Wang LN, Zuo JL (2015) MCP-1 stimulates spinal microglia via PI3K/Akt pathway in bone cancer pain. Brain Res 1599: 158-167.
Katritch V, Cherezov V, Stevens RC (2013) Structure-function of the G protein-coupled receptor superfamily. Ann Rev Pharmacol Toxicol 53: 531-556.
Koyama T, Fukuda K (2009) Nociceptin receptor antagonist JTC-801 inhibits nitrous oxide-induced analgesia in mice. J Anesthes 23: 301-303.
Lappano R, Maggiolini M (2011) G protein-coupled receptors: novel targets for drug discovery in cancer. Nature Rev Drug Discov 10: 47-60.
Lennnon FE, Mirzapoiazova T, Mambetsariev B, Salgia R, Moss J, Singleton PA. (2012) Abstract 2232: Overexpression of the mu opioid receptor in human non-small cell lung cancer promotes Akt and mTOR activation, tumor growth and metastasis. Cancer Res 72: 2232-2232.
Lennon FE, Mirzapoiazova T, Mambetsariev B, Poroyko VA, Salgia R, Moss J, Singleton PA (2014) The Mu opioid receptor promotes opioid and growth factor-induced proliferation, migration and Epithelial Mesenchymal Transition (EMT) in human lung cancer. PloS one 9: e91577.
Liu J, Zhang C, Lin M, Zhu W, Liang Y, Hong X, Zhao Y, Young KH, Hu W, Feng Z (2014) Glutaminase 2 negatively regulates the PI3K/AKT signaling and shows tumor suppression activity in human hepatocellular carcinoma. Oncotarget 5: 2635-2647.
Mabuchi S, Kuroda H, Takahashi R, Sasano T (2015) The PI3K/AKT/mTOR pathway as a therapeutic target in ovarian cancer. Gynecol Oncol 137: 173-179.
Mabuchi T, Matsumura S, Okuda-Ashitaka E, Kitano T, Kojima H, Nagano T, Minami T, Ito S (2003) Attenuation of neuropathic pain by the nociceptin/orphanin FQ antagonist JTC-801 is mediated by inhibition of nitric oxide production. Eur J Neurosci 17: 1384-1392.
Meusch U, Klingner M, Baerwald C, Rossol M, Wagner U (2013) Deficient spontaneous in vitro apoptosis and increased tmTNF reverse signaling-induced apoptosis of monocytes predict suboptimal therapeutic response of rheumatoid arthritis to TNF inhibition. Arthritis Res Ther 15: R219.
O'Hayre M, Degese MS, Gutkind JS (2014) Novel insights into G protein and G protein-coupled receptor signaling in cancer. Current Opin Cell Biol 27: 126-135.
O'Hayre M, Vazquez-Prado J, Kufareva I, Stawiski EW, Handel TM, Seshagiri S, Gutkind JS (2013) The emerging mutational landscape of G proteins and G-protein-coupled receptors in cancer. Nature Rev Cancer 13: 412-424.
Polivka J, Jr., Janku F (2014) Molecular targets for cancer therapy in the PI3K/AKT/mTOR pathway. Pharmacol Ther 142: 164-175.
Rawls SM, Schroeder JA, Ding Z, Rodriguez T, Zaveri N (2007) NOP receptor antagonist, JTC-801, blocks cannabinoid-evoked hypothermia in rats. Neuropeptides 41: 239-247.
Reid BM, Permuth JB, Sellers TA (2017) Epidemiology of ovarian cancer: a review. Cancer Biol Med 14: 9-32.
Schonenbach NS, Hussain S, O'Malley MA (2015) Structure and function of G protein-coupled receptor oligomers: implications for drug discovery. Wiley interdisciplinary reviews Nanomed Nanobiotechnol 7: 408-427.
Shrivastava S, Jeengar MK, Reddy VS, Reddy GB, Naidu VG (2015) Anticancer effect of celastrol on human triple negative breast cancer: possible involvement of oxidative stress, mitochondrial dysfunction, apoptosis and PI3K/Akt pathways. Exp Mol Pathol 98: 313-327.
Toren P, Zoubeidi A (2014) Targeting the PI3K/Akt pathway in prostate cancer: challenges and opportunities (review). Int J Oncol 45: 1793-1801.
Wu YR, Qi HJ, Deng DF, Luo YY, Yang SL (2016) MicroRNA-21 promotes cell proliferation, migration, and resistance to apoptosis through PTEN/PI3K/AKT signaling pathway in esophageal cancer. Tumour Biol 37: 12061-12070.
Xing X, Zhang L, Wen X, Wang X, Cheng X, Du H, Hu Y, Li L, Dong B, Li Z, Ji J (2014) PP242 suppresses cell proliferation, metastasis, and angiogenesis of gastric cancer through inhibition of the PI3K/AKT/mTOR pathway. Anti-cancer Drugs 25: 1129-1140.
Xu B, Guan XH, Yu JX, Lv J, Zhang HX, Fu QC, Xiang HB, Bu HL, Shi D, Shu B, Qin LS, Manyande A, Tian YK (2014) Activation of spinal phosphatidylinositol 3-kinase/protein kinase B mediates pain behavior induced by plantar incision in mice. Exp Neurol 255: 71-82.
Yamada H, Nakamoto H, Suzuki Y, Ito T, Aisaka K (2002) Pharmacological profiles of a novel opioid receptor-like1 (ORL(1)) receptor antagonist, JTC-801. Br J Pharmacol 135: 323-332.
Zhang XD, Yang YN, Wang SJ, Zhu DL, Wang LW, Sheng JJ, Song SS (2015a) [Effect of puerarin on PI3K/AKT pathway-mediated apoptosis of PASMCs]. Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China journal of Chinese materia medica 40: 3041-3046.
Zhang Y, Simpson-Durand CD, Standifer KM (2015b) Nociceptin/orphanin FQ peptide receptor antagonist JTC-801 reverses pain and anxiety symptoms in a rat model of post-traumatic stress disorder. Br J Pharmacol 172: 571-582.
Zhuo B, Li Y, Li Z, Qin H, Sun Q, Zhang F, Shen Y, Shi Y, Wang R (2015) PI3K/Akt signaling mediated Hexokinase-2 expression inhibits cell apoptosis and promotes tumor growth in pediatric osteosarcoma. Biochem Biophys Res Commun 464: 401-406.