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Olanzapine inhibits the proliferation and induces the differentiation of glioma stem-like cells through modulating the Wnt signaling pathway *in vitro*

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Abstract. – OBJECTIVE: Olanzapine, a D2/5-HT2 antagonist, is often used as an atypical antipsychotic drug in clinical. Previous research has found its new pharmacological influence on enhancing the differentiation of neural stem cells (NSCs) to oligodendrocyte-like cells (ODLCs). Glioblastomas are associated with poor prognoses owing to the glioma stem-like cells (GSLCs), which have a great many of similarities with adult NSCs. Hence, in this article, we aim to study the effects and associated mechanisms of olanzapine on GSLCs derived from [illegible] U87MG glioblastoma cell lines.

MATERIALS AND METHODS: The meth[illegible]a-zolyl tetrazolium (MTT) colorimetric assay [illegible] conducted to investigate the effects of olanz[illegible]ine on cell viability of GSLCs. Flow cytomet[illegible] analysis was applied to study [illegible] cycle dy[illegible]namics of GSLCs and Cell C[illegible]8 (CCK-8) was used to further inv[illegible]ate th[illegible]roliferation of GSLCs after treate[illegible]h olanz[illegible]or dimethyl sulfoxide (DMSO[illegible] h. C[illegible]ation assay was carrie[illegible]out[illegible]e differentiation of GSLCs an[illegible]n Ima[illegible] Plus image analysis was use[illegible] measure[illegible]otrusion length of the di[illegible]iated cells. F[illegible]rmore, the confocal [illegible]^{2c} [illegible]urement was conducted to observ[illegible]e influ[illegible]of olanzapine on the opening fu[illegible]tion of Ca^{2+} [illegible]. After the application of [illegible]zapine for 48 [illegible]CR was conducted [illegible]measure mRNA le[illegible]s of calcium-sensi[illegible]ecepto[illegible]aSR) and stromal interaction mol[illegible]e 1 (ST[illegible]), and Western blotting analysis [illegible]rri[illegible]ut to e[illegible]me the expression of myelin [illegible]rotein ([illegible]), glial fibrillary acidic [illegible]ein (C[illegible]CaS[illegible]otein, STIM1 protein and [illegible]in pr[illegible]

R[illegible]LTS: Ou[illegible]ults demonstrated that olanzapi[illegible]nhibited the proliferation of GSLCs by arres[illegible] in G0/G1 phase and facilitated th[illegible]on of such cells to ODLCs. After [illegible]ted with olanzapine for 48 h, cells were very [illegible]itive to 100 mM K^+ stimulation, with inc[illegible]l spontaneous calcium wave. We also foun[illegible]lanzapine increased the protein expression of [illegible] and [illegible]R. In addition, the mRNA transcrip[illegible]on and p[illegible] expression of CaSR and STIM1 were enh[illegible]after treated with ol[illegible] for 48h, while [illegible] protein expression [illegible]atenin was suppressed.

C[illegible]ONCLUSIONS: Our results suggest that [illegible]nzapine mod[illegible]s the Wnt signaling pathway [illegible]ugh activat[illegible] the Ca^{2+} pathway and re-s[illegible]ng the β[illegible]enin pathway, leading to the dif[illegible]GSLCs to ODLCs. It provides excitin[illegible]ects that olanzapine might be a new novel chemotherapeutic modality targeting [illegible]Cs for the treatment of glioblastomas.

Key words:
Olanzapine, Glioma stem-like cell, Oligodendrocyte-like cell, Ca^{2+} channel, Wnt signaling pathway.

Abbreviations

NSCs = neural stem cells; ODLCs = oligodendrocyte-like cells; CaSR = calcium-sensing receptor; CNPase = 2',3'-Cyclic-nucleotide 3'-phosphodiesterase; STIM1 = stromal interaction molecule 1; GSLCs = glioblastoma stem-like cells; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; GFAP = glial fibrillary acidic protein.

Introduction

Gliomas are one of the most invasive and frequent primary malignant neoplasms in human beings, especially in adults, accounting for about 30% of all brain and central nervous system (CNS) neoplasms and 80% of all malignant brain neoplasms[1]. Currently, the treatment for gliomas is commonly using the combined approaches, such as surgery excision, radiation therapy, and chemotherapy, while the mean survival rate after the combination therapy for

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gliomas is often less than one year[2]. According to the phenotype, gliomas are classified into several types: oligodendroglioma, oligoastrocytoma and astrocytoma[3]. Meanwhile, in accordance with their malignancy, they can also be sorted into four grades: from WHO grade I to grade IV[4]. The grade IV astrocytomas, also named glioblastomas or glioblastoma multiforms, are one of the most common primary malignant neoplasms in adult CNS[5]. In spite of multimodal treatment with maximal surgery resection followed by tribuzon and radiation therapy, the prognosis for glioblastomas is still unsatisfactory, of which the 3-year survival rate is with only 10%[6,7]. Previous studies have shown that glioblastomas are associated with poor prognoses on account of the existence of GSLCs, which might be one of the most vital reasons for the recurrence and progression of cancer patients in clinical[8]. Hence, developing new novel therapeutic modalities against GSLCs is a promising field in cancer researches.

Evidence has indicated that GSLCs are defined on account of their consistent properties with adult neural stem cells (NSCs)[9]. NSCs, which plans to become GSLCs, are mito[illegible]lly active and longevial, thereby, accum[illegible] enough oncogenic mutations during their [illegible]le life and undergoing neoplastic transformat[illegible]. Inducing the differentiation of GSLCs to as[illegible]cytes always causes the formation of glial sca[illegible] suppressing the recovery of [illegible]unction. However, inducing GSLC[illegible]ferer[illegible]ed into oligodendrocytes might h[illegible]recover[illegible] neural function, leading to a b[illegible]progn[illegible] tumors. Therefore, in[illegible]cing[illegible]to undergo oriented differentia[illegible]is a ne[illegible]l promising concept for malig[illegible]glioma.

It has been w[illegible]orted that sev[illegible] signaling pathways[illegible]ch a[illegible]h and Wnt, regulate the prolife[illegible]tion of adu[illegible]Cs. As expected, some of t[illegible] signaling path[illegible]ave also been found [illegible] aberrantly activated in human brain tum[illegible]especi[illegible]y the vital role of Wnt in gli[illegible]oma[illegible]ere is [illegible]idence also suggesting that a[illegible]otivati[illegible] Wnt signaling pathway is [illegible]rela[illegible]to the development of [illegible]stoma[illegible]modulating the Wnt signaling [illegible]way wo[illegible]provide a promising strategy to t[illegible] GSLCs, thereby overcoming the proble[illegible]istance and relapse[13].

Previously, several studies have found that the [illegible]all incidence of cancers among schizop[illegible] patients is remarkably lower than that of the g[illegible]eral population[14-16]. These findings suggest that the antipsychotic drugs, such as olanzapine, chlorpromazine, quetiapine a[illegible] done, applied for treating schizor[illegible]a [illegible] have the inhibitory effects on [illegible] cancers[17]. Olanzapine, a D2/5-HT2 ant[illegible]t, is one of several atypical antipsychotics to[illegible]luated in the past decade for use in [illegible]ating sc[illegible]reni[illegible] and bipolar disorder[18,19]. [illegible]cently, rese[illegible] demonstrated that ol[illegible]pine [illegible]ows the pharmacological acti[illegible]s on [illegible]oting the differentiation of NSC[illegible]evi[illegible]studies[illegible]e already found th[illegible]e [illegible] of an[illegible]chotic drugs involve[illegible]atenin[illegible]wi[illegible] reported that β-caten[illegible]y a critical r[illegible] canonical Wnt sign[illegible]way, which i[illegible]verexpressed in huma[illegible]obla[illegible]and inhibiting its expression restrains the pr[illegible]tion and invasion of glio[illegible]a cells[22]. Th[illegible]s likely that modul[illegible] the activity of β-catenin by olanzapine [illegible]nt lead to inh[illegible]iting the growth of glioblas[illegible]as. Another [illegible]ortant signaling pathway in [illegible]inogenesis i[illegible]$^{2+}$ mediated signaling pathw[illegible]hich is[illegible]ely involved in the proliferation[illegible]ation of cancer cells[23]. It is also reported that the involvement of this kind of signaling pathway in the differentiation of cancer [illegible]ugh the extracellular calcium-sensing [illegible]cept[illegible] (CaSR) or the intracellular Ca^{2+} alterations[24]. Studies have demonstrated that, in CNS, CaSR's expression is up-regulated when NSCs are specified to the oligodendrocyte lineage[25]. Ca^{2+} acts as a second messenger regulating diverse processes such as apoptosis, activation of the downstream gene and neural alterations in memorizing and learning[26]. Therefore, in this article, we plan to study the effects of olanzapine on the cell viability, cycle dynamics, proliferation and differentiation of GSLCs derived from human U87MG glioblastoma cell lines, and its relationship with Wnt signaling pathway.

Materials and Methods

Glioma Stem-Like Cell Isolation and Culture

The human glioblastoma U87MG cell lines were acquired from Shandong University and cultured in Dulbecco's Modified Eagle Medium (DMED)/F12 (Boster Biology Co., Wuhan, China) plus 10% fetal bovine serum (FBS, Boster Biology Co., Wuhan, China) with penicillin and streptomycin at 37°C in a 5% CO_2 atmosphere. GSLCs were derived from U87MG cells as previously demonstrated[25]. The combination of

serum-free medium (SFM) with DMEM/F12 was used and supplemented with 2 μg/ml heparin (Zhongshan Co., Beijing, China), 25 ng/ml epidermal growth factors (Zhongshan Co., Beijing, China), B27 reaction solution (Zhongshan Co., Beijing, China) and 25 ng/ml fibroblast growth factors (Zhongshan Co., Beijing, China)[27]. Olanzapine (Zhongshan Co., Beijing, China) stock solution was prepared in dimethyl sulfoxide (DMSO, Zhongshan Co., Beijing, China) and further diluted in complete cell culture medium. The control medium was treated with the equal amount of DMSO alone.

Cell Viability Assay

According to previous studies[28], the influence of olanzapine on GSLCs was determined using the methyl thiazolyl tetrazolium (MTT) colorimetric assay. The GSLCs were cultured at 5000 cells in the 96-well plates, and were hatched at 37°C in a 5% CO_2 atmosphere for 12 h. After that, the cells were treated with caffeine at different doses of concentrations (Figure 1) for 48 h. Cell growth was measured by adding 20 ml of 5 mg/ml MTT (Boster Biology Co., Wuhan, China) to every culture well, and the GSLCs were hatched in a 5% CO_2 atmosphere for 4 h. After that, the supernatant was taken away. Finally, the absorbance at 570 nm was detected through using the multiwell spectrophotometer (Bio-Rad, Hercules, CA, USA). Cell viability (experimental group absorbance value / control group absorbance value) * 100%. Each experiment was done at least quintic.

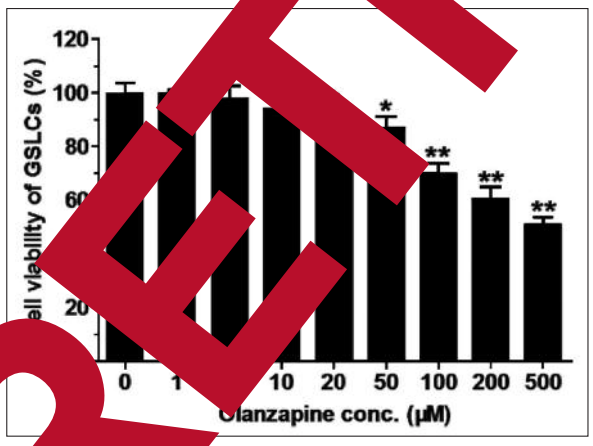


Figure 1. The influence of olanzapine on the cell viability of GSLCs. The cell viability of GSLCs 48 h after the application of different doses olanzapine detected by MTT colorimetric assay. The data express means ± SD. *$p < 0.05$, **p < 0.01 vs. the control.

Cell Cycle Dynamics Detection

Flow cytometric analysis was used to detect the cell cycle dynamics in different phases of the cell cycle. After the application of olanzapine or DMSO for 48 h, GSLCs were collected and digested with 0.25% trypsin (Sigma Chemical Co., St. Louis, MO, USA) for 15 minutes at 37°C. Following 100-μm mesh sieve separation, the prepared cell suspension was fixed with ethanol at 4°C. The cell suspension was treated with 100 μL RNase (Sigma Chemical Co., St. Louis, MO, USA) at 37°C for 30 minutes, washed with phosphate buffered saline (PBS), stained with 0.5 mg/L propidium iodide (PI) staining solution (Boster Biology Co., Wuhan, China) for 30 minutes at 4°C in the dark. The stained cell suspension was analyzed using FACS flow cell analysis and software (Becton Dickinson, Franklin Lakes, NJ, USA). Each experiment was done at least quintic.

Cell Proliferation Assay

After the application of olanzapine or DMSO for 48 h, GSLCs were hatched at 37°C in a 5% CO_2 condition. Then, the Cell Counting Kit-8 (CCK-8, Zhongshan Co., Beijing, China) was used to detect the cell proliferation. After the cells were inoculated for 0 d, 1d, 2 d and 3 d, CCK-8 was applied to the culture medium and hatched for 1 h, and then the absorbance at 450 nm was measured, respectively. Each experiment was done at least quintic.

Cell Differentiation Assay

In order to investigate the effects of olanzapine on differentiation in GSLCs, the cell spheres were separated by Accutase (Sigma Chemical Co., St. Louis, MO, USA) and attached to Poly-D-lysine-coated cover glasses in DMEM/F12 culture medium plus 10% fetal bovine serum (FBS), with 50 μM olanzapine or DMSO for 48 h. Differentiated cells and its protrusions counting were conducted using the densitometer Image-Pro Plus (Media Cybernetics Co., Silver Spring, MD, USA) image analysis system. Each experiment was done at least quintic.

Confocal $[Ca^{2+}]_c$ Measurement

After olanzapine or DMSO treatment for 48 h, GSLCs were cultured the detection medium under an inverted microscope (Nikon, Tokyo, Japan). GSLCs were treated with 4 μM fluo-3 AM (Sigma Chemical Co., St. Louis, MO, USA) for 25 min and, then, rinsed three to five times

with Krebs-HEPES (Sigma Chemical Co., St. Louis, MO, USA) during 10 min. The 100 mM K^+ pulse was used precisely to modulate the beginning of stimulation. The fluorescence of fluo-3 was detected under the confocal microscope (Bio-Rad, Hercules, CA, USA). Then, cell staining was stimulated by Kr-Ar laser at 488 nm and afterwards the release was determined at 522 nm. Among each cell, control image was subtracted from the succedent images. Data were collected and analyzed by the LaserSharp MRC-1024 software and the NIH Image.

RNA Isolation and Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

After olanolanzapine application, the cell culture mediums of the two groups were collected to detect the mRNA levels of CaSR and stromal interaction molecule 1 (STIM1). The overall RNA was extracted by RNeasy Mini kit (Zhongshan Co., Beijing, China) in accordance with the instructions of the manufacturer. Reverse transcription of the overall RNA was done through the First Strand cDNA Synthesis kit (Zhongshan Co., Beijing, China) in accordance with the ir tions of the manufacturer. The primer se for the detection of CaSR mRNA: fo d, 5 TTCCTGACCGCCTTTGTG3 ; rev 5 CGTGTAGAGCCAGATGATGC3 . The pri sequences for the detection of STIM1 mRN forward, 5 TGTGGAGCTGC TATG3 , reverse, 5 CTTCAGCACA CC TCA3 . The primer sequences for detectio β-actin mRNA: forward, 5 CTG CTCTT3 ; reverse, GC ACCGC1-CATT3 . All the p rs were based from Cellular Institute hanghai Inst for Biological Science ai, China). cycles of polymeras ain were carried out at 94°C for 3 ec, 52°C fo and 72°C for 60 sec. Each eriment was do ast quintic.

Prot Prepa on

h af lanzar application, the extracti eins w one as previously de- ibed[2] ort, Cs were treated on ice e ph uffered saline (PBS) plus 1% idet P- NP40, Boster Biology Co., Wu China), 0.1% Sodium Dodecyl Sulfonate (S hemical Co., St. Louis, MO, X), 20 μl/mL protease inhibitor cocktail ongshan Co., Beijing, China) and 2 mM p ethylsulfonyl fluoride (Zhongshan Co., Beij China). After that, the concentrations of proteins were measured through using the Bradford method[30], and the spare proteins up at –40°C for further use. Each e ent done at least quintic.

SDS-PAGE and Western Blot sis

Equal GSLC lysates we fractio ough SDS-PAGE and then tran d to the ace cellulose sheets electro tically fterward sheets were treated w he TBS uffer (20 mM Tri-HCl, pH 7.3, 15 NaC d 0.1% een-20) supplement wit nfat d milk. Then, GSLCs cultur C 2 h with the appropr rimary anti Zhongshan Co., Beiji that against Pase (1:500), MBP (0), C (1:500), CaSR (1:500), STIM1 (1:500), β-ca (1:500) and GAPDH (1: spectively. A next morning, the s were rinsed with the TBST buffer and cul- d with the s ecific secondary antibodies ongshan Co. ijing, China). The enhanced e chemilum scence kit (Sigma Chemical C Louis , USA) was used to visualize the n bands. The optical density of the bands (normalized with those of GAPDH) was termined by Image Pro Plus image analysis sys- xperiment was done at least quintic.

Statistical Analysis

Data were shown as the mean ± standard deviation (SD). Statistical analysis of variance was used to evaluate the results. SPSS IBM software for Mac was used for statistical analysis of the results (SPSS, Inc., Chicago, IL, USA). Statistical significance was calculated by using the oneway ANOVA with the Bonferroni correction. $p < 0.05$ was regarded as a statistically significant difference.

Results

Olanzapine Decreased the Cell Viability of GSLCs in the Dose-Dependent Way

GSLCs were treated with olanzapine or DMSO for 48 h in culture and the viable cells were detected by MTT colorimetric assay. Our results found that olanzapine reduced the cell viability in the dose-dependent way in GSLCs (Figure 1). Olanzapine at 100 μM reduced the cell viability of GSLCs to less than 70%. Therefore, to avoid any effects on cell viability, the maximal non-cytotoxic concentration of olanzapine on GSLCs was 50 μM and it was thus used in our following investigations.

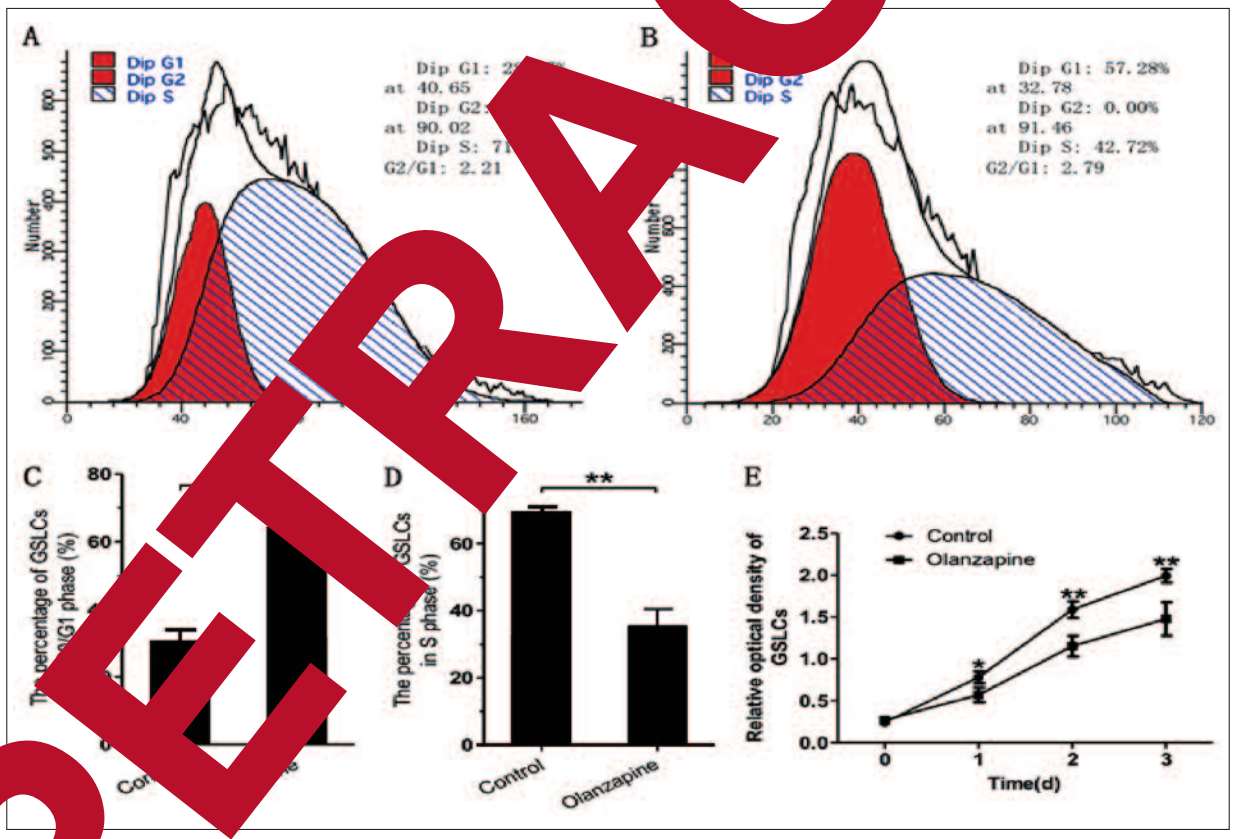
Olanzapine Inhibited the Proliferation of GSLCs by Arresting Cell Cycle in G0/G1 Phase

To study the influence of olanzapine on the cell cycle dynamics and proliferation of GSLCs, flow cytometric analysis and CCK-8 assay were conducted. The results showed that treatment for 48 h with olanzapine significantly arrested the cell cycle of GSLCs in G0/G1 phase, when comparing to the normal control ($p < 0.01$, Figure 2 C). However, the percentage of GSLCs in S phase was lower than that of the normal control ($p < 0.01$, Figure 2 D). In addition, as shown in Figure 2 E, after the cells were inoculated for 0d, 1 d, 2 d and 3 d, the number of GSLCs significantly decreased in olanzapine treatment group, when comparing to that of the normal control (day 1, day 2, day 3: $p < 0.05$, $p < 0.01$, $p < 0.01$, Figure 2 E). These results confirmed that the proliferation of GSLCs was remarkably suppressed through changing the cell cycle dynamics after treated with olanzapine.

Olanzapine Facilitated the Differentiation of GSLCs to ODI

Since olanzapine inhibited the p[illegible]ration GSLCs, we planed to investigate [illegible]ether it can activate the differentiation proc[illegible]GSLCs. After being cultured with 50 μM [illegible]ine plus 10% FBS for 48 h, the p[illegible]entage [illegible]LCs with long protrusions [illegible]ficantly in[illegible] compared with that of [illegible]norma[illegible]ontrol [illegible] was treated with 10[illegible]BS fo[illegible]h alone ($p <$ 0.01, Figure 3 A a[illegible] F[illegible]rmore, [illegible]per-centage of cell[illegible]it[illegible] man[illegible] small branches, whi[illegible]sembled [illegible]der[illegible]yte cells in microsco[illegible]henotype, inc[illegible]when comparing to [illegible]e normal co[illegible]l ($p < 0.01$, Figure 3[illegible]nd D,

To further confirm[illegible]es of the differentiated [illegible]e Western b[illegible]g analysis was carri[illegible]t to detect the relative expression of MBP GFAP, which are the markers of oligodendrocytes and as[illegible]ytes, respectively. As shown



Fig[illegible] [illegible]ence of olanzapine on the cell cycle dynamics and proliferation of GSLCs. ***A, B,*** Flow cytometric cell cy-[illegible]files of GSLC suspension in the normal control group and the olanzapine treated group. ***C,*** The percentage of GSLCs in [illegible]G1 phase is depicted in the bar graphs, respectively. The data express means ± SD. *$p < 0.05$, **$p < 0.01$ vs. control. ***D,*** [illegible]rcentage of GSLCs in S phase is depicted in the bar graphs, respectively. The data express means ± SD. *$p < 0.05$, **$p <$ 0.[illegible]ntrol. ***E,*** The relative optical density of GSLCs is depicted in the broken line graph. The data express means ± SD. *$p <$ [illegible] **$p < 0.01$ vs. control.

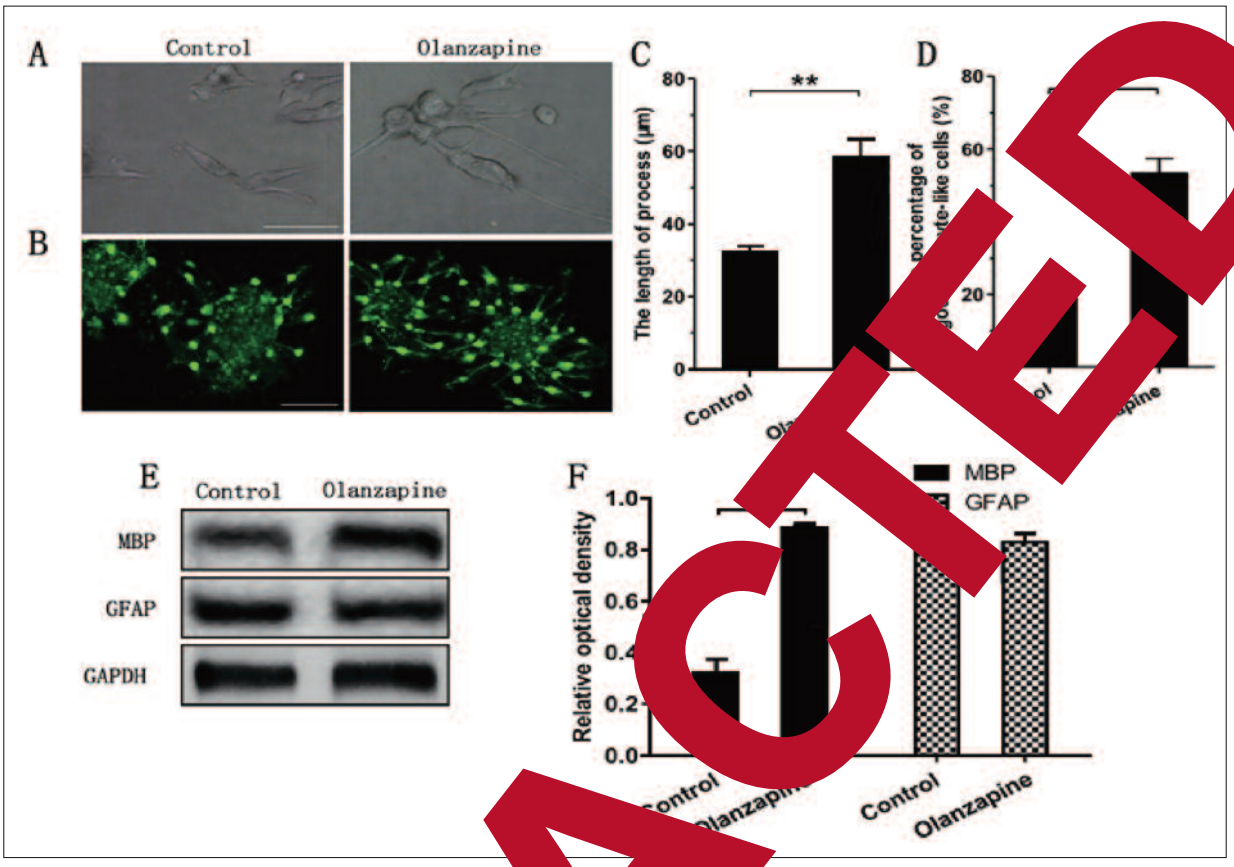


Figure 3. The influence of olanzapine on differentiation of GSLCs. ***A,*** The images of differentiated GSLCs in 48 h after olanzapine or DMSO treatment under the contrast phase ordinary microscope. Bar = 50 μm. ***B,*** The images of differentiated GSLCs in 48 h after olanzapine or DMSO application under the fluorescence microscope. Bar = 50 μm. ***C,*** The length of process of differentiated GSLCs in 48 h after olanzapine or DMSO treatment is depicted in the bar graphs. The data express means ± SD. *$p < 0.05$, **$p < 0.01$ vs. control. ***D,*** The percentage of oligodendrocyte-like cells in GSLCs after treated with olanzapine or DMSO is depicted in the bar graphs. The data express means ± SD. *$p < 0.05$, **$p < 0.01$ vs. control. ***E,*** The protein expression of MBP and GFAP in the normal control and the olanzapine treated groups was detected by Western blot analysis. ***F,*** Relative quantification of Western blot analysis is depicted in the bar graphs. The data express means ± SD. *$p < 0.05$, **$p < 0.01$ vs. control.

in Figure 3 E and F, we observed that olanzapine remarkably increased the expression of MBP in the differentiated cells ($p < 0.05$, Figure 3 E and F). Nevertheless, the expression of GFAP remained unchanged in the olanzapine treated group when comparing to the normal control ($p > 0.05$, Figure 3 E and F). Our results suggested that olanzapine promoted the differentiation of GSLCs to oligodendrocyte-like cells.

Olanzapine Promoted the Oligodendrogenesis of GSLCs Through Opening the Ca^{2+} Channel, Activating CaSR and Inhibiting Wnt/β-catenin Signaling Pathway

To further study the associated mechanisms of olanzapine on promoting the differentiation of GSLCs to oligodendrocyte-like lineage, the confocal $[Ca^{2+}]_c$ measurement was used to examine the function of calcium channel, the RT-PCR was carried out to measure relative mRNA levels of CaSR and STIM1, and the Western blotting analysis was conducted to detect the relative expression of CaSR, STIM1 and β-catenin. The confocal $[Ca^{2+}]_c$ measurement demonstrated that after treated with olanzapine for 48 h, the cells were very sensitive to 100 mM K^+ stimulation, with the increased spontaneous calcium wave, when comparing to the normal control ($p < 0.01$, Figure 4 A and B). The RT-PCR detection showed that the mRNA levels of CaSR and STIM1 in olanzapine treatment group were higher than those in normal control group ($p < 0.01$, $p < 0.01$, Figure 4 C and D). As for the results of Western blot analysis, we found that significant increases in the

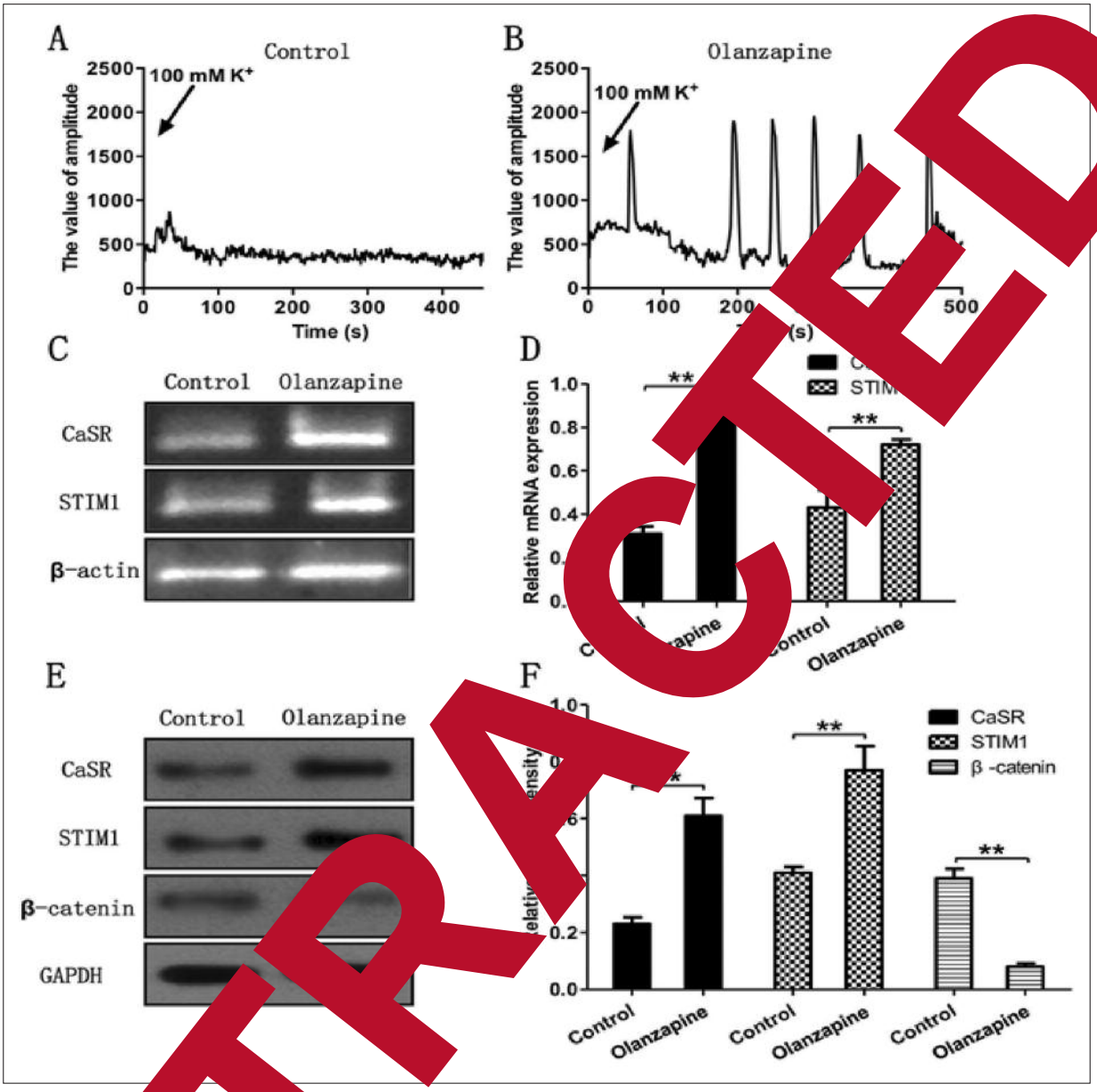


Figure 4. The influence of olanzapine on the function of calcium channel, the transcription of CaSR and STIM1, and the protein expression of CaSR, STIM1 and β-catenin in GSLCs. ***A, B,*** The spontaneous calcium waves in GSLCs after treated with olanzapine or DMSO for 48 h detected by the confocal $[Ca+]_c$ measurement. ***C,*** The mRNA levels of CaSR and STIM1 in GSLCs after treated with olanzapine or DMSO detected by RT-PCR. ***D,*** Relative quantification of RT-PCR analysis is depicted in the bar graphs. The data express means ± SD. $*p < 0.05$, $**p < 0.01$ vs. control. ***E,*** The protein expression of CaSR, STIM1 and β-catenin in GSLCs after treated with olanzapine or DMSO for 48 h detected by Western blot analysis. ***F,*** Relative quantification of Western blot analysis is depicted in the bar graphs. The data express means ± SD. $*p < 0.05$, $**p < 0.01$ vs. control

protein expression of CaSR and STIM1 were obviously increased in olanzapine treatment group, compared with normal control group ($p < 0.01$, $p <$ Figure 4 E and F). However, the expression of β-catenin remarkably reduced in olanzapine treatment group, when comparing to that of the normal control ($p < 0.01$, Fig. 4 E and F). The above results indicated that olanzapine promoted the differentiation of GSLCs to ODLCs by activating the Ca^{2+} channel, up-regulating CaSR signaling pathway as well as down-regulating β-catenin signaling pathway.

Discussion

Gliomas are one of the most common primary malignant neoplasms in the brains of human beings all over the world. As is know to every researcher, the treatment for gliomas, especially the glioblastomas, is one of the most difficult challenges because of its infiltrative and aggressive nature, so that it cannot be fully defeated by surgical interference followed by chemotherapy, making it the main death of brain tumors[31]. Recently, many studies have found that the schizophrenic patients were less easily to suffer from cancers than the normal people[32]. The point of view that the application of antipsychotics may lower the risk of cancers in schizophrenic patients was first reported in two separated articles, which showed the anti-cancer effects of reserpine and chlorpromazine[33,34]. Even though the cytotoxic influence of antipsychotic drugs on the cells were weak, they carry out the effects against the proliferation on the separating cells, with the cancer stem cells or cancer cells being selectively targeted[35,36]. Research has suggested that chlorpromazine combined with statins, which coul... through the blood-brain barrier, has sho... positive and powerful therapeutic effects ... cancers in CNS[37]. These findings will provid... tremely vital strengths for studying the che... prevention property of antipsychoti... medicatio... against tumors in clinical trials.

Atypical, also known as ...ond-...eration, antipsychotics have repla... ypical, ...named first generation, antipsy... in c... count of their relative... bet... roperties[38]. Olanzapine, one of ...seconda... eration antipsychotic drug... commonly ... for its ability to imp... positive an... egative symptoms in ...zop... patients[39]. Evidence has shown ...at the major ...acological influence of o... apine is blocka... e serotonin 5-HT2 r... tor as well as the dopamine D2[18,19]. Rece... studie... ve showed the effects of several ...psych... medic...ons on β-catenin and calciu... stasis i... NS[40]. Consistent with ...vious ... in ... present article, we found ...lanzap... ...ited the proliferation of GS... and indu... the differentiation of GSLCs to O... Cs by activating Ca^{2+} channel and down-re... enin signaling pathway. Furthermore, evidence has also found that, in tumors, ...R shows the vital effects on controlling the d... tiation and proliferation balance, respon... g to the alterations of the calcium concentrations in extracellular matrix[41]. It has been reported that the pernicious tumors ... related to the loss of normal home... me... nisms, in spite of the cell effects ...CaSR moti... vation[42]. Interrupting this kind ...eptor might cause abnormal differentiation a... alignant progression. In addition, ...$^{2+}$ may ...te it... chemopreventive activity ...ough restra... activation of β-catenin ...enhan...g the ex... sion of E-cadherin[43]. ...Anothe... portant calcium protein is STIM... let... f STIM... ans-forms the wea... m... c B16... mouse melanoma cel... o more ...siv... s, accelerates cell m... ty *in vitro* a... ses experimental m... a mouse mo...[45,46]. Similar with ab... indin... results also demonstrated that the activatio... CaSR and STIM1 increa... tracellular c... m concentrations, w... might cause the degradation of β-catenin.

...nclusions

O... ggest that olanzapine modulates the Wnt pathway through activating the Ca^{2+} ...thway and restraining the β-catenin pathway, ... the differentiation of GSLCs to ... It provides exciting prospects that olanzapine might be a new novel chemotherapeutic modality targeting GSLCs for the treatment of malignant gliomas.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Goodenberger ML, Jenkins RB. Genetics of adult glioma. Cancer Genet 2012; 205: 613-621.
2) Gritsenko PG, Ilina O, Friedl P. Interstitial guidance of cancer invasion. J Pathol 2012; 226: 185-199.
3) Kalinina J, Peng J, Ritchie JC, Van Meir EG. Proteomics of gliomas: initial biomarker discovery and evolution of technology. Neuro Oncol 2011; 13: 926-942.
4) Rousseau A, Mokhtari K, Duyckaerts C. The 2007 WHO classification of tumors of the central nervous system - what has changed? Curr Opin Neurol 2008; 21: 720-727.
5) Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 2007; 114: 97-109.

6) Zhou Q. WHO classification of tumors of central nervous system (2007): an introduction. Zhonghua Bing Li Xue Za Zhi 2008; 37: 5-7.

7) Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG, Mirimanoff RO; European Organisation for Research and Treatment of Cancer Brain Tumour and Radiation Oncology Groups; National Cancer Institute of Canada Clinical Trials Group. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009; 10: 459-466.

8) Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB. Identification of a cancer stem cell in human brain tumors. Cancer Res 2003; 63: 5821-5828.

9) Zhang Y, Wang SX, Ma JW, Li HY, Ye JC, Xie SM, Du B, Zhong XY. EGCG inhibits properties of glioma stem-like cells and synergizes with temozolomide through downregulation of P-glycoprotein inhibition. J Neurooncol 2015; 121: 41-52.

10) Jackson EL, Garcia-Verdugo JM, Gil-Perotin S, Roy M, Quinones-Hinojosa A, VandenBerg S, Alvarez-Buylla A. PDGFR alpha-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling. Neuron 2006; 51: 187-199.

11) Roth W, Wild-Bode C, Platten M, Grimmel [illegible] Melkonyan HS, Dichgans J, Weller M. Secreted Frizzled-related proteins inhibit motility and promote growth of human malignant glioma cells. Oncogene 2000; 19: 4210-4220.

12) Sareddy GR, Panigrahi M[illegible]halla S, [illegible] Babu PP. Activation of Wnt/beta-catenin signaling pathway in human astrocytoma. Neurochem Int 2009; 55: 307-311.

13) Liu S, Dontu G[illegible]ha MS. Mammary stem cells, self-renewal pathways, and carcinogenesis. Breast Cancer Res 2006; 8: 86-95.

14) Gulbinat W, Dupont A[illegible]nsky A, Jensen OM, Marsel[illegible], Nakane Y, Sa[illegible]N. Cancer incidence in schizophrenic patients. Results of record linkage studies in three countries. Br J Psychiatry [illegible]. 1992; [illegible]5-83.

15) [illegible]nsen[illegible] The incidence of cancer in schizophrenic patients. J [illegible]emiol Community Health 1989; [illegible]47.

16) [illegible] Sund[illegible]ng Y, Kendler KS, Sundquist J, [illegible]n X. Incidence of cancer in patients with schizophrenia and their first-degree relatives: a population study in Sweden. Schizophr Bull 2013; [illegible].

17) Ackenheil M, Weber K. Developments in antipsychotic therapy with regard to hypotheses for schizophrenia. Dialogues Clin Neurosci 2002; 4: 431.

18) Littlewood RA, Claus ED, Arenella P, Bogenschutz M, Karoly H, Ewing SW, Bryan AD, H[illegible] Dose specific effects of olanzapine treatment of alcohol dependence. Psychopharmacology 2015; 232: 1261-1268.

19) Zarghami M, Sheikhmoonesi F, [illegible]zdani J, Farnia S. A comparative study of beneficial effects of Olanzapine and sodium valproate on impulsive behavior of patients with [illegible] on methadone maintenance therapy: a randomized triple blind trial. Eur Rev Med Pharmacol [illegible]2013;17:1079-1081.

20) Kurosawa S, Hashim[illegible]W, Toki[illegible]aito S, Saito T. Olanzapine protects neuronal survival and neural stem cell differentiation against regulation of endoplasmic reticulum stress-response proteins. J Neur[illegible]2007; 114: 112[illegible]28.

21) Frey[illegible]Z, Fe[illegible]SJ, Javitch JA. Roles of the Akt/GSK-3 and Wnt signaling pathways in schizophrenia and antipsychotic drug action. Am J Psychiatry 2010; 167: 388-[illegible].

22) [illegible]ue X, Lan F, Yang W, Yang Y, Han L, Zhang A, Liu J, Zeng H, Ji[illegible]T, Pu P et al. Interruption of beta-catenin suppresses the EGFR pathway by blocking multiple oncogenic targets in human glioma cells. Brain R[illegible]010; 1366: 27-37.

23) [illegible]nlocking the secrets of cell signaling. [illegible]ev Physiol 2005; 67: 1-21.

24) Bikle DD, Oda Y, Xie Z. Calcium and 1,25(OH)2D: interacting drivers of epidermal differentiation. J [illegible]d Biochem Mol Biol 2004; 89-90: 355-360.

25) Chattopadhyay N, Espinosa-Jeffrey A, Tfelt-Hansen J, Yano S, Bandyopadhyay S, Brown EM, de Vellis J. Calcium receptor expression and function in oligodendrocyte commitment and lineage progression: potential impact on reduced myelin basic protein in CaR-null mice. J Neurosci Res 2008; 86: 2159-2167.

26) Qiang L, Yang Y, Ma YJ, Chen FH, Zhang LB, Liu W, Qi Q, Lu N, Tao L, Wang XT, You QD, Guo QL. Isolation and characterization of cancer stem like cells in human glioblastoma cell lines. Cancer Lett 2009; 279: 13-21.

27) Huang Q, Zhang QB, Dong J, Wu YY, Shen YT, Zhao YD, Zhu YD, Diao Y, Wang AD, Lan Q. Glioma stem cells are more aggressive in recurrent tumors with malignant progression than in the primary tumor, and both can be maintained long-term *in vitro*. BMC Cancer 2008; 8: 304.

28) Ku BM, Lee YK, Jeong JY, Ryu J, Choi J, Kim JS, Cho YW, Roh GS, Kim HJ, Cho GJ, Choi WS, Kang SS. Caffeine inhibits cell proliferation and regulates PKA/GSK3beta pathways in U87MG human glioma cells. Mol Cells 2011; 31: 275-279.

29) Kamsteeg M, Rutherford T, Sapi E, Hanczaruk B, Shahabi S, Flick M, Brown D, Mor G. Phenoxodiol – an isoflavone analog – induces apoptosis in chemoresistant ovarian cancer cells. Oncogene 2003; 22: 2611-2620.

30) Kruger NJ. The Bradford method for protein quantitation. Methods Mol Biol 1994; 32: 9-15.

31) Ostrom QT, Gittleman H, Liao P, Rouse C, Chen Y, Dowling J, Wolinsky Y, Kruchko C, Barnholtz-Sloan J. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2007-2011. Neuro Oncol 2014; 16: Suppl 4: iv1-63.

32) Chou FH, Tsai KY, Su CY, Lee CC. The incidence and relative risk factors for developing cancer among patients with schizophrenia: a nine-year follow-up study. Schizophr Res 2011; 129: 97-103.

33) Belkin M, Hardy WG. Effect of reserpine and chlorpromazine on sarcoma 37. Science 1957; 125: 233-234.

34) Csatary LK. Chlorpromazines and cancer. Lancet 1972; 2: 338-339.

35) Sachlos E, Risueno RM, Laronde S, Shapovalova Z, Lee JH, Russell J, Malig M, McNicol JD, Fiebig-Comyn A, Graham M et al. Identification of drugs including a dopamine receptor antagonist that selectively target cancer stem cells. Cell 2012; 149: 1284-1297.

36) Wiklund ED, Catts VS, Catts SV, Ng TF, Whitaker NJ, Brown AJ, Lutze-Mann LH. Cytotoxic effects of antipsychotic drugs implicate cholesterol homeostasis as a novel chemotherapeutic target. Int J Cancer 2010; 126: 28-40.

37) Argyriou AA, Antonacopoulou A, Iconom... G, Kalofonos HP. Treatment options for m... gliomas, emphasizing towards new mo... targeted therapies. Crit Rev Oncol Hemato... 9; 69:199-210.

38) Choure BK, Gosavi D, Nanotkar S. Compara... cardiovascular safety of risperid... and olanza... ine, based on electrocardio... rameters and blood pressure: A pros... ve ... label observational study. Indian ... harmac... 014; 46: 493-497.

39) Lou HG, Ruan ZR, Jiang B, Chen JL. Simultaneous quantification of olanzapine and its m... desmethylolanzapine in human pl... chromatography tandem mass ... rometry ... therapeutic drug monitoring. Bi... Chromatogr... 2015; 29: 671-678.

40) Wiklund ED, Catts VS, Catts SV, ... Whitaker NJ, Brown AJ, Lutze-Man... H. Cytot... cts o... antipsychotic drugs in... te choleste... ostasis as a novel ch... therapeutic targe... Cancer 2010; 126: ... .

41) Singh N, Liu G, ... barty ... lation a... d char-acterization of calc... recepto... cells: a highly ma... t a... resista... enotype of colon c... . Int J C... 201... 32: 1996-2005.

42) Rodla... role of the ca... m-sensing recept... canc... Calcium 2004; 35: 291-295.

43) Chakrabarty S, Ra... ne V, Appelman H, Varani ... cellular calci... calcium sensing re... function in huma... colon carcinomas: promotion of E-cadherin expression and suppression of beta-catenin/TCF activation. Cancer Res 2003; 63: 67-71.

... Whitfield JF. ... cium, calcium-sensing receptor ... colon c... . Cancer Lett 2009; 275: 9-16.

45) ... NLM. Identification of metastasis-relate... genes in a mouse model using a library of randomized ribozymes. J Biol Chem 2004; 279: ...083-38086.

... -Massler G, Kast RE, Westhoff MA, Dwucet A, Welscher N, Nonnenmacher L, Hlavac M, Siegelin MD, Wirtz CR, Debatin KM, Halatsch ME. Olanzapine inhibits proliferation, migration and anchorage-independent growth in human glioblastoma cell lines and enhances temozolomide's antiproliferative effect. J Neurooncol. 2015;122:21-33.