Progesterone and its metabolite allopregnanolone promote invasion of human glioblastoma cells through metalloproteinase-9 and cSrc kinase

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Abstract. Glioblastomas are the most aggressive and common primary brain tumors in adults. Glioblastoma cells have a great capacity to migrate and invade the brain parenchyma, often reaching the contralateral hemisphere. Progesterone (P4) and its metabolite, allopregnanolone (3α-THP), promote the migration and invasion of human glioblastoma-derived cells. P4 induces migration in glioblastoma cells by the activation of the proto-oncogene tyrosine-protein kinase Src (cSrc) and focal adhesion kinase (Fak). In breast cancer cells, cSrc and Fak promote invasion by increasing the expression and activation of extracellular matrix metalloproteinases (MMPs). However, the mechanism of action by which P4 and 3a-THP promote invasion in glioblastoma cells remains unclear. The effects of P4 and 3α-THP on the protein expression levels of MMP-2 and -9 and the participation of cSrc in progestin effects in U251 and U87 human glioblastoma-derived cells were evaluated. It was determined by western blotting that the P4 increased the protein expression level of MMP-9 in U251 and U87 cells, and 3α-THP increased the protein expression level of MMP-9 in U87 cells. None of these progestins modified MMP-2 protein expression levels. The increase in MMP-9 expression was reduced when the intracellular progesterone receptor and cSrc expression were blocked with small interfering RNAs. Cell invasion induced by P4 and 3α-THP was also blocked by inhibiting cSrc activity with PP2 or by cSrc gene silencing. These results suggest that P4 and its metabolite 3α-THP induce the invasion of glioblastoma cells by increasing MMP-9 expression through the cSrc kinase family. The results of this study provide information of interest in the context of targeted therapies against molecular pathways involved in glioblastoma invasion.

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Introduction

Glioblastomas are the primary tumors of the central nervous system that cause the most deaths and therefore have the highest fatality rate. The median survival time of patients with this disease is <15 months, and this statistic has not changed for more than two decades (1). Glioblastoma cells' high migration and invasion capacity into areas of the brain parenchyma adjacent to the tumor, even reaching the contralateral hemisphere, makes it impossible to perform a complete surgical resection, resulting in tumor recurrence (2).

Proto-oncogene tyrosine-protein kinase Src (cSrc) is a non-receptor tyrosine kinase that participates in diverse biological activities, including cell migration. Furthermore, it has been broadly associated with controlling the expression of metalloproteinases (MMPs) and tumor invasiveness (3). cSrc and focal adhesion kinase (Fak) kinase activity inhibition in the breast cancer MCF-7 cell line, was reported to have blocked MMP-9 secretion (4). Gautam *et al* (5) reported that, in triple-negative MDA-MB-231 breast cancer cells, the inhibition of cSrc activity decreased MMP-9 levels and cell invasion. Within the high molecular complexity of the mechanisms that regulate invasion in glioblastomas, MMPs serve a fundamental role in degrading the extracellular matrix (ECM). Upregulation of MMP-2 and -9 in glioblastomas is associated with poor prognosis (6). The involvement of MMP-2 and -9 in the progression of glioblastomas has been studied for >20 years. In 1999, Forsyth *et al* (7) reported that the levels and activity of MMP-2 and -9 were higher in glioblastomas than in normal brain tissue. The expression level of MMP-9 also showed a strong correlation with the tumor grade in gliomas. Later, Kondraganti *et al* (8) reported that impaired expression of MMP-9 reduced glioblastoma cells' invasiveness. More recently, in 2019, Zhou *et al* (6) reported that MMP-2 and -9 expression was higher in recurrent glioma than in primary glioma.

Progesterone (P4) has been associated with increased invasiveness capacity in numerous malignant processes (9-11). In breast cancer cells, P4 increases the formation of the protrusions associated with focal adhesion complex, and migration and invasion processes through cSrc kinase activation (12). Although glioblastoma is not a cancer of the reproductive system, it is sex-dependent since there is a higher prevalence in men than in women, as evidenced by epidemiological data (13). In the context of P4, the results of previous studies indicate that this hormone induces glioblastoma progression when administered at 10-50 nM (14,15). Notably, in terms of glioblastoma, Piña-Medina *et al* (16) reported that P4 increased the number of invasive and migrating cells. More recently, Bello-Alvarez *et al* (15) demonstrated that P4 activates cSrc kinase and Fak, which are fundamental components of focal adhesion complexes. It has also been reported that 3α-tetrahydroprogesterone (3α-THP), an active P4 reduced metabolite, increased cell proliferation of glioblastoma cells. In addition, finasteride, an inhibitor of 5α-reductase, the rate-limiting enzyme for the P4 transformation to 3α-THP, partially inhibited this effect (17). Moreover, 3α-THP regulates migration in glioblastoma cells, and this effect is dependent on the activation of cSrc (18). In rat Schwann cells, the 3α-THP induction of cell migration is regulated by cSrc and Fak (19). However, it is not known whether P4 and 3α-THP regulate MMP-2 and -9 expression through cSrc activity and whether this effect is reflected in the migration and invasiveness of glioblastoma cells.

Materials and methods

Cell culture and treatments. U251 (astrocytoma cell line) and U87 (HTB-14; glioblastoma of unknown origin) cells were purchased from the American Type Culture Collection (ATCC) and were previously authenticated by STR profiling using an AmpFISTR® Identifiler™ kit (cat. no. 4322288) and Genetic Analyzer 3130xl (Applied Biosystems; Thermo Fisher Scientific, Inc.). Mycoplasma contamination was routinely monitored using a Universal Mycoplasma Detection Kit (cat. no. 30-1012K; ATCC). Cells were plated in 35 mm culture dishes and maintained in DMEM medium (Vitro SA) supplemented with 10% fetal bovine serum (FBS; Biowest), 1 mM pyruvate, 2 mM glutamine, 0.1 mM MEM Non-Essential Amino Acids Solution (Gibco; Thermo Fisher Scientific) and 1 mM antibiotic (streptomycin 10 g/l; penicillin G 6.028 g/l; and amphotericin B 0.025 g/l, Biowest, cat. no. L0010) at conditions of 37˚C and 5% CO_2. Culture medium was replaced by DMEM phenol red-free (Biowest) and supplemented with 10% charcoal-stripped serum FBS (sFBS; HyClone; Cytiva), to avoid additional hormone supplementation, ~24 h before treatment. On the day of treatment, cells were treated with 50 nM P4 (0.001% DMSO vehicle; cat. no. P-8783; MilliporeSigma) or 100 nM 3α-THP (0.01% ethanol vehicle; cat. no. 195886; MP Biomedicals, LLC) for 3 or 6 h at 37˚C to assess the protein expression levels of MMP-2 and MMP-9.

Cell migration (wound healing assays). For evaluating cell migration, wound healing assays were performed. A total of $3.5x10^5$ U251 or U87 cells were seeded per well in 6-well plates and cultured as aforementioned. After 24 h of culture conditions free of phenol red and 10% sFBS (20), a scratch wound was made in the 90% confluent cells with a fine pipette tip. After washing the detached cells with 1X PBS (137 mM NaCl, 2.7 mM KCl, 1.8 mM KH_2PO_4 and 10 mM $NaHPO_4$), the cell cultures were treated with 10 μM cytosine β-D-arabinofuranoside (Ara-C; Sigma-Aldrich; Merck KGaA) to inhibit cell proliferation 1 h before adding either 50 nM P4 (0.001% DMSO vehicle), 1 μM 1-tert-Butyl-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d] pyrimidin-4-amine (PP2, a Src family kinase inhibitor; 0.001% DMSO vehicle), P4 + PP2 at the aforementioned concentrations, 100 nM 3α-THP (0.01% ethanol vehicle) or 3α-THP + PP2 at the aforementioned concentrations. To determine the percentage of cell migration, images of four fields of view per treatment condition were taken at 0, 6, 12 and 24 h with an Infinity 1-2C camera attached to an Olympus CKX41 inverted microscope. Image processing was performed using the macro-MRI Wound Healing Tool in ImageJ 1.45S software (National Institutes of Health).

Invasion assays. Cells were grown according to the aforementioned method. The invasion assays were performed using Transwell inserts (8.0 μm membrane; Corning, Inc.) and 6-well plates with Matrigel (2 mg/ml; Engelbreth-Holm-Swarn murine sarcoma extract; MilliporeSigma) diluted in DMEM phenol red-free without FBS or antibiotics. This dilution was incubated in the inserts at conditions of 37˚C and 5% CO_2 atmosphere for 2 h. In the upper insert, $3x10^4$ cells suspended in DMEM without phenol red, FBS or antibiotics but with 10 μM Ara-C were seeded. DMEM supplemented with 10% FBS (Biowest) as a chemoattractant was added to the lower wells. Cells in the upper Transwell inserts received the following steroid treatments: 50 nM P4, 100 nM 3α-THP or 1 μM PP2. The incubation conditions were 37˚C and 5% CO_2 atmosphere for 24 h. To evaluate the specific role of cSrc on cell invasion, cSrc expression was first downregulated as described in the small interfering (*siRNA) transfection* subsection of this manuscript, followed by vehicle, P4 or 3α-THP treatments. After incubation, the non-invading cells were washed from the upper surface of the insert. Invasive cells at the membrane were fixed, stained, visualized and quantified as described previously by Piña-Medina *et al* (16).

Senescence assays. Based on preliminary results, growth pattern and cell size, a total of $5x10^4$ U87 cells or $3x10^4$ U251 cells were plated per well in 6-well plates with phenol red-free DMEM and 10% sFBS for 24 h at 37˚C. After 24 h, the following treatments were added: 50 nM P4, 1 μM PP2, 50 nM P4 + 1 μM PP2, 100 nM 3α-THP, 100 nM 3α-THP + 1 μM PP2 or vehicle (0.01% DMSO for PP2 and P4 or 0.01% ethanol for 3α-THP). After a further 24 h, cells were washed with PBS and senescence was evaluated using the Senescent Cells Staining Kit (cat. no. CS0030-1KT; MilliporeSigma), according to the manufacturer's instructions, to determine the expression of β-galactosidase in senescent cells. Incubation with β-galactosidase staining solution was performed overnight at 37˚C. A total of three independent experiments were performed for each cell line. After staining, images of four fields of view per treatment condition were taken with an Infinity 1-2C camera attached to an Olympus CKX41 inverted

microscope. ImageJ 1.45S software (National Institutes of Health) was used to perform the image processing and counting of β-galactosidase positive cells to determine the proportion of senescent cells.

siRNA transfection. A total of $2.5x10^5$ U251 cells were plated in 35-mm culture dishes with DMEM and 10% FBS for 24 h. After incubation, the medium was replaced with DMEM without phenol red, FBS, or antibiotics. Transfection was performed with 100 nM commercial cSrc siRNA (cat. no. 4392420; ID, s13412; Thermo Fisher Scientific, Inc.) or with 100 nM control siRNA (Silencer Select Negative Control #1; cat. no. 4390844; Thermo Fisher Scientific, Inc.), an aleatory sequence that did not recognize a specific target in the cell, using Lipofectamine RNAiMAX (Thermo Fisher Scientific, Inc.) for 48 h at 37˚C. This commercial cSrc siRNA was also used to evaluate the effect of P4 on MMP-2 and MMP-9 expression promoted by cSrc. After transfection with the cSrc siRNA or control siRNA, the medium was refreshed for 12 h. Cells were harvested for protein extraction to evaluate transfection efficiency 48 h after the addition of siRNAs, and the wound healing assays were performed. Commercial siRNA against progesterone receptor (PR) (Ambion, PGR Silencer Select, Pre-designed; cat. no. 4392420) and control siRNA (Silencer Select Negative Control #1; cat. no. 4390844) (both Thermo Fisher Scientific, Inc.) was used to evaluate the effect of PR on MMP-2 and -9 expression 48 h after the addition of siRNAs. The transfection protocol was performed as previously described (15,18).

Protein extraction and western blotting. The protein expression levels of MMP-2, MMP-9, cSrc and PR were determined by western blotting. After any hormone treatment or transfection procedure described above was performed, cells were homogenized in RIPA buffer with protease inhibitors (cat. no. P8340; Sigma-Aldrich; Merck KGaA). Proteins were obtained as described by Bello-Alvarez *et al* (15). For protein electrophoresis, 40 μg of total extracted proteins were loaded on an 8.5% SDS-PAGE gel. Proteins were transferred to nitrocellulose membranes in semi-dry conditions (Bio-Rad Laboratories, Inc.) at 25 V for 1 h (MMP-2, MMP-9 and cSrc) or 2 h (PR). Membranes were blocked with 5% bovine serum albumin (cat. no. A-420-100; Gold Biotechnology) in Tris-buffered saline with 0.1% Tween, at 37˚C for 2 h. Membranes were then incubated with primary antibodies against MMP-2 (cat. no. 4042; Cell Signaling Technology, Inc.), MMP-9 (cat. no. 3852; Cell Signaling Technology, Inc.), cSrc (cat. no. 2108; Cell Signaling Technology, Inc.), PR (cat. no. B-30 sc-811; Santa Cruz Biotechnology, Inc.) or the α-tubulin loading control (cat. no. sc-398103; Santa Cruz Biotechnology, Inc.). The dilution of all primary antibodies was 1:1,000 and antibodies were incubated for 24 h at 4˚C. Secondary antibodies were against rabbit (cat. no. 1858415; Thermo Fisher Scientific, Inc.) or mouse (cat. no. sc-516102; Santa Cruz Biotechnology, Inc.), and were incubated at a dilution of 1:10,000 at room temperature for 45 min. Finally the signal was detected with Super Signal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, Inc.) (15). Densitometric analysis was performed with ImageJ 1.45S software (National Institutes of Health). To determine changes in the protein expression levels of MMP-2 and MMP-9, the same membrane was re-probed three times, alongside the α-tubulin loading control.

Statistical analysis. All statistical analyses were performed using Graph Pad Prism 5 software (GraphPad Software; Dotmatics). A one-way ANOVA and Bonferroni post hoc test, or unpaired Student's t-test were used to analyze differences between comparable groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

P4 and 3α-THP enhance cell migration through activation of the Src family of kinases. The impact of the Src kinase family on the actions of P4 and 3α-THP in cell migration using the kinase inhibitor, PP2, which has a high affinity for many members of the Src kinase family, was evaluated. Both progestins (P4 and 3α-THP) induced cell migration (Figs. 1 and 2). The effect of P4 in the U251 and U87 cell lines was significative compared to the vehicle following 24 h of treatment (Figs. 1A-C and S1). 3α-THP also promoted cell migration but significance compared with vehicle was only demonstrated at 12 and 24 h of treatment in both cell lines (Figs. 2A-C and S2). The inhibitor PP2 completely blocked the effect of P4 in U251 cells and significantly blocked the effect of P4 in U87 cells (Fig. 1A-C). PP2 significantly, partially blocked the effect of 3α-THP at 12 and 24 h of treatment in U251 cells (Fig. 2A and B); whereas in U87 cells, PP2 completely blocked its effect (Fig. 2C). No marked changes in cell migration were observed for any treatments at 6 h (Figs. 1, 2, S1 and S2).

Furthermore, the potential of any of the treatment conditions to induce senescence was evaluated in both cell lines using a β-galactosidase staining assay (β-galactosidase catalyzes the hydrolysis of β-galactosidase only in senescent cells). In U251 cells, the proportion of β-galactosidase positive cells was 0% for any treatment (Figs. S3 and S4). In U87 cells, the proportion of β-galactosidase positive cells in each treatment was 0.88-1.5%. However, no significant differences between treatments were demonstrated (Figs. S5 and S6).

cSrc activity participates in the invasion induced by P4 and 3α-THP in human glioblastoma-derived cells. Considering that the addition of P4 increased cell migration, the effect of cSrc on the invasiveness of P4-stimulated human glioblastoma-derived cells was evaluated. A Transwell invasion assay was performed to determine whether inhibiting Src kinase family activity with PP2 altered the invasion triggered by P4 in U251 and U87 cells. In P4-treated cells, a significant enhancement of invasion compared with the vehicle group was observed at 24 h in both U251 (Fig. 3A and B) and U87 cells (Fig. 3C and D). This enhancement was significantly suppressed by PP2 in U87 cells (Fig. 3D). In U251 cells, the effect of PP2 on invasiveness was not significant; however, there was a marked trend towards a decrease in invasion (Fig. 3B). This result demonstrated that the Src kinase family modulates the invasion of glioblastoma cells triggered by P4.

The effect of cSrc silencing on the invasiveness of U251 cells after treatment with P4 or 3α-THP was also assessed. Silencing in U87 was not performed due to limited siRNA availability). As with the PP2 experiments, P4 significantly induced U251

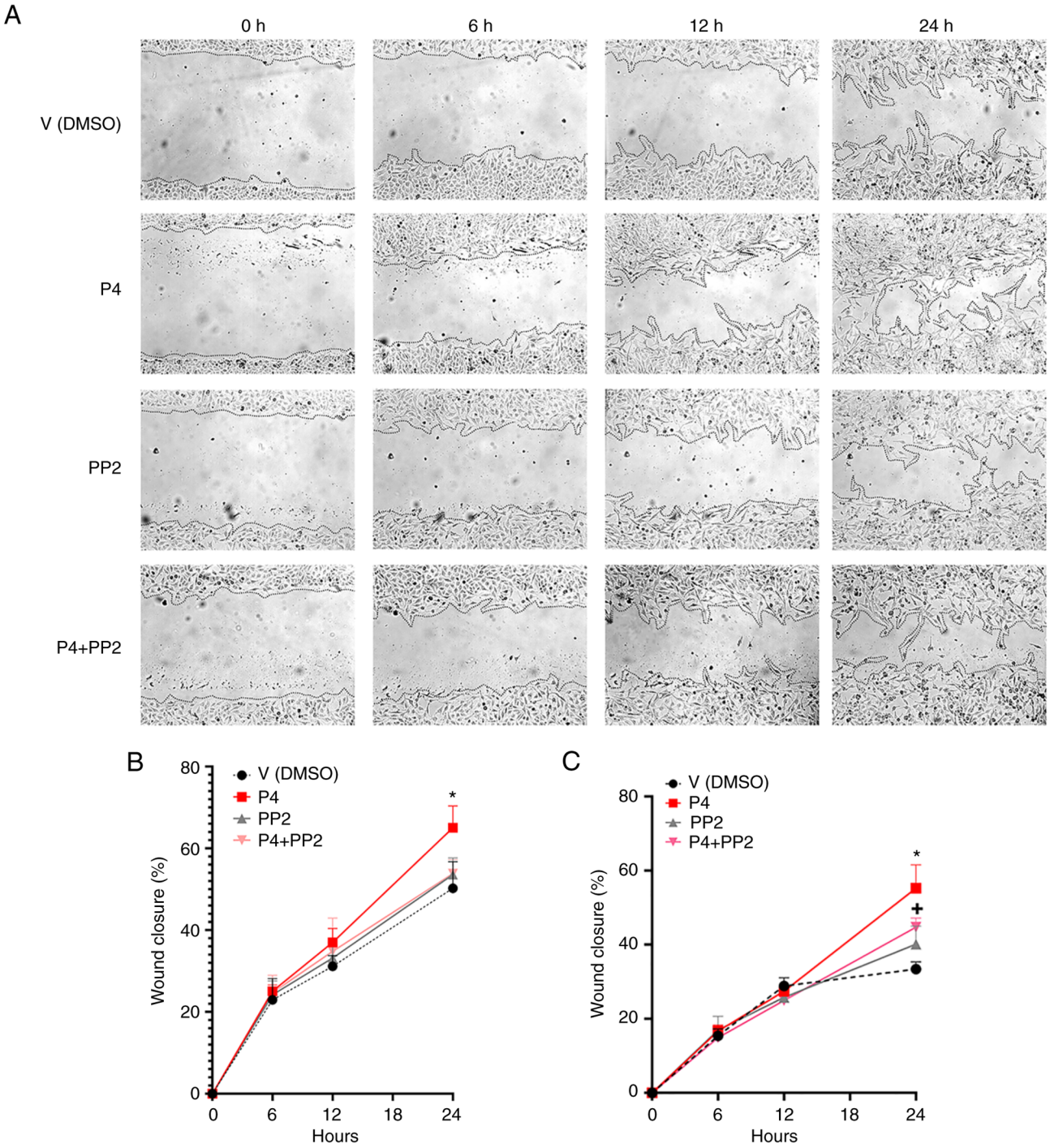

Figure 1. The pharmacological inhibition of cSrc interferes with the effect of P4 on glioblastoma cell migration. (A) Representative images of U251 cells treated with P4 (50 nM), PP2 (1 μM) and the P4 + PP2 conjunct treatment at 0, 6, 12 and 24 h. Percentage cell migration graphs of (B) U251 (*P<0.05, P4 vs. V at 24 h) and (C) U87 cells (*P<0.05, P4 vs. all other treatments at 24 h; $^{+}$P<0.05, P4 + PP2 vs. V at 24 h). Each point represents the mean ± SEM, n=4. All images were taken using a 10X magnification lens. P4, progesterone; V, vehicle; PP2, 1-tert-Butyl-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d] pyrimidin-4-amine.

cell invasion compared with the untreated group and this effect was significantly reduced by cSrc silencing (Fig. 4A and B) compared with the siRNA control. 3α-THP also significantly promoted cell invasion compared with the untreated group and this effect decreased significantly when cSrc was silenced compared with the siRNA control (Fig. 4C and D). Notably, marked inhibition of cell invasion by cSrc silencing was evident in vehicle-treated cells (Fig. 4D). When treatment with 3α-THP was administered to the cells, invasion returned to basal levels.

P4 and 3α-THP increase the protein expression level of MMP-9 in human glioblastoma-derived cells. MMPs perform a vital role in ECM degradation to promote cell invasion and are frequently upregulated in malignant tumors (21). MMP-2 and MMP-9 are upregulated in glioblastomas and are associated with poor prognosis (6). Therefore, the effect of P4 and 3α-THP on MMP-2 and MMP-9 protein expression levels in human glioblastoma-derived cell lines was evaluated. U87 and U251 cells were treated with 50 nM P4 and 100 nM 3α-THP

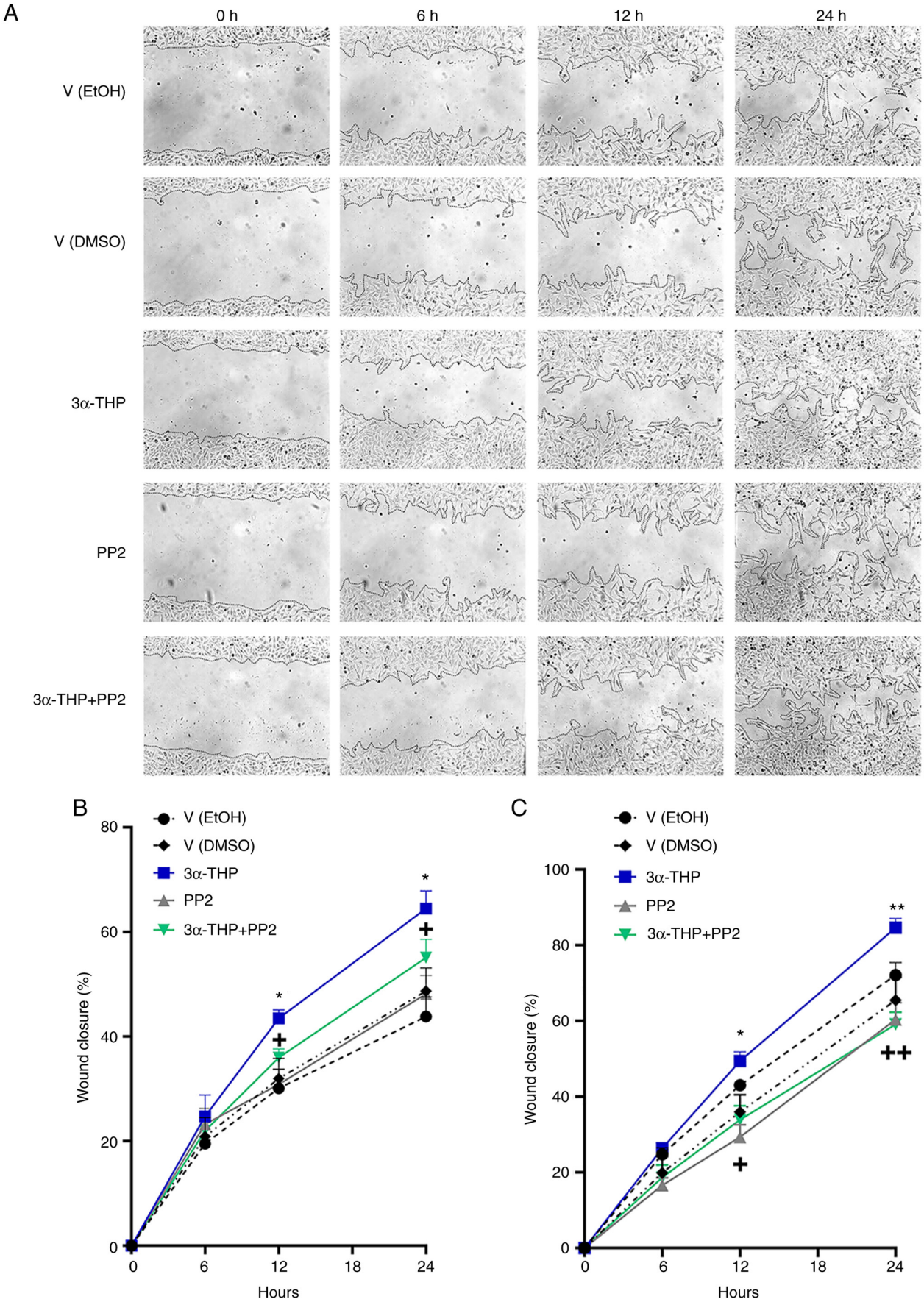

Figure 2. cSrc inhibition blocks the effect of 3α-THP on glioblastoma cell migration. (A) Representative images of U251 cells treated with 3α-THP (100 nM), PP2 (1 μM) and the 3α-THP + PP2 conjunct treatment at 0, 6, 12 and 24 h. Percentage migration graph of (B) U251 cells [*P<0.05, 3α-THP vs. all other treatments ; $^{+}$P<0.05, 3α-THP + PP2 vs. V (EtOH); n=3] and (C) U87 cells [*P<0.05, 3α-THP vs. V (DMSO 0.001%), 3α -THP + PP2, and PP2; **P<0.05, 3α-THP vs. all other treatments; $^{+}$P<0.05, PP2 and 3α-THP + PP2 vs. V (EtOH 0.01%); $^{++}$P<0.05, PP2 vs. V (EtOH), and 3α-THP + PP2 vs. V (EtOH)]; n=4. Each point represents the mean ± SEM. EtOH, ethanol; V, vehicle; PP2, 1-tert-Butyl-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d] pyrimidin-4-amine; 3α-THP, allopregnanolone.

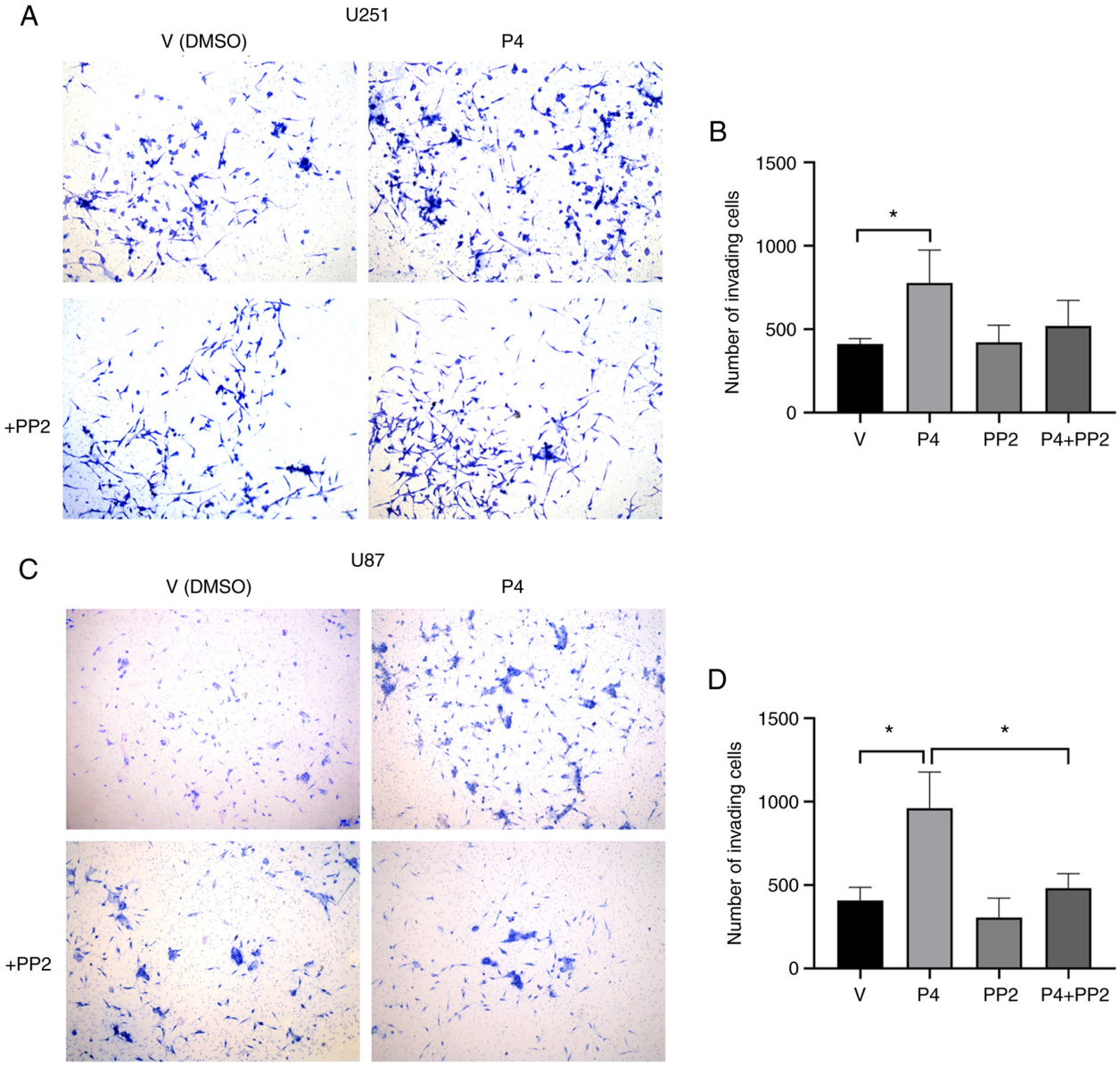

Figure 3. The pharmacological inhibition of cSrc reduces cell invasion induced by P4 in glioblastoma cells. (A) U251 and (C) U87 cells treated with V (0.001% DMSO), P4 (50 nM), PP2 (1 μM) or the P4 + PP2 conjunct treatment for 24 h. Representative images of the Transwell invasion assay are shown. Graphs presented the number of invasive (B) U251 and (D) U87 cells. Data are presented as the mean ± SEM; n=4. *P<0.05. P4, progesterone; V, vehicle.

for 3 or 6 h (Figs. 5 and 6). P4 and 3α-THP did not significantly increase MMP-2 protein expression levels compared with the control in U251 and U87 cells (Figs. 5A and B, and 6A and B). However, P4 significantly increased MMP-9 protein expression levels compared with the control at 3 and 6 h in U251 cells and at 6 h in U87 cells (Fig. 5C and D). 3α-THP significantly increased MMP-9 protein expression levels compared with the control in U87 cells at 3 h (Fig. 6D).

cSrc is involved in the regulation of MMP-9 expression in human glioblastoma-derived cells treated with P4. It has previously been reported that the inactivation of cSrc inhibits triple-negative breast cancer progression through the downregulation of MMP-9 expression (5). Therefore, the role of cSrc in the expression of MMP-9 in P4-treated glioblastoma cells was evaluated. A commercial siRNA against cSrc or a control siRNA were used to transfect U251 and U87 cells. After transfection, cells were treated with 100 nM 3α-THP or 50 nM P4 for 6 h, respectively (Fig. 7). cSrc silencing caused a significant reduction in cSrc protein expression levels compared with the siRNA control (Fig. 7A and B). In cells transfected with siRNA against cSrc, the increase in MMP-9 expression triggered by P4 was significantly reduced compared with cells that received the control siRNA. This result suggested that, at least partially, cSrc participated in the induction of MMP-9 expression by P4 (Fig. 7C and D).

PR regulates the expression of MMP-9. Bello-Alvarez *et al* (15) previously reported that P4 promoted interaction between the PR and cSrc, which led to the activation of cSrc. To assess the effect of PR on MMP-2 and MMP-9 expression, cells were transfected with an siRNA against PR (Fig. 8). MMP-9 protein expression levels were significantly reduced upon PR silencing compared with control siRNA, whereas no significant change was demonstrated for MMP-2 (Fig. 8B and C).

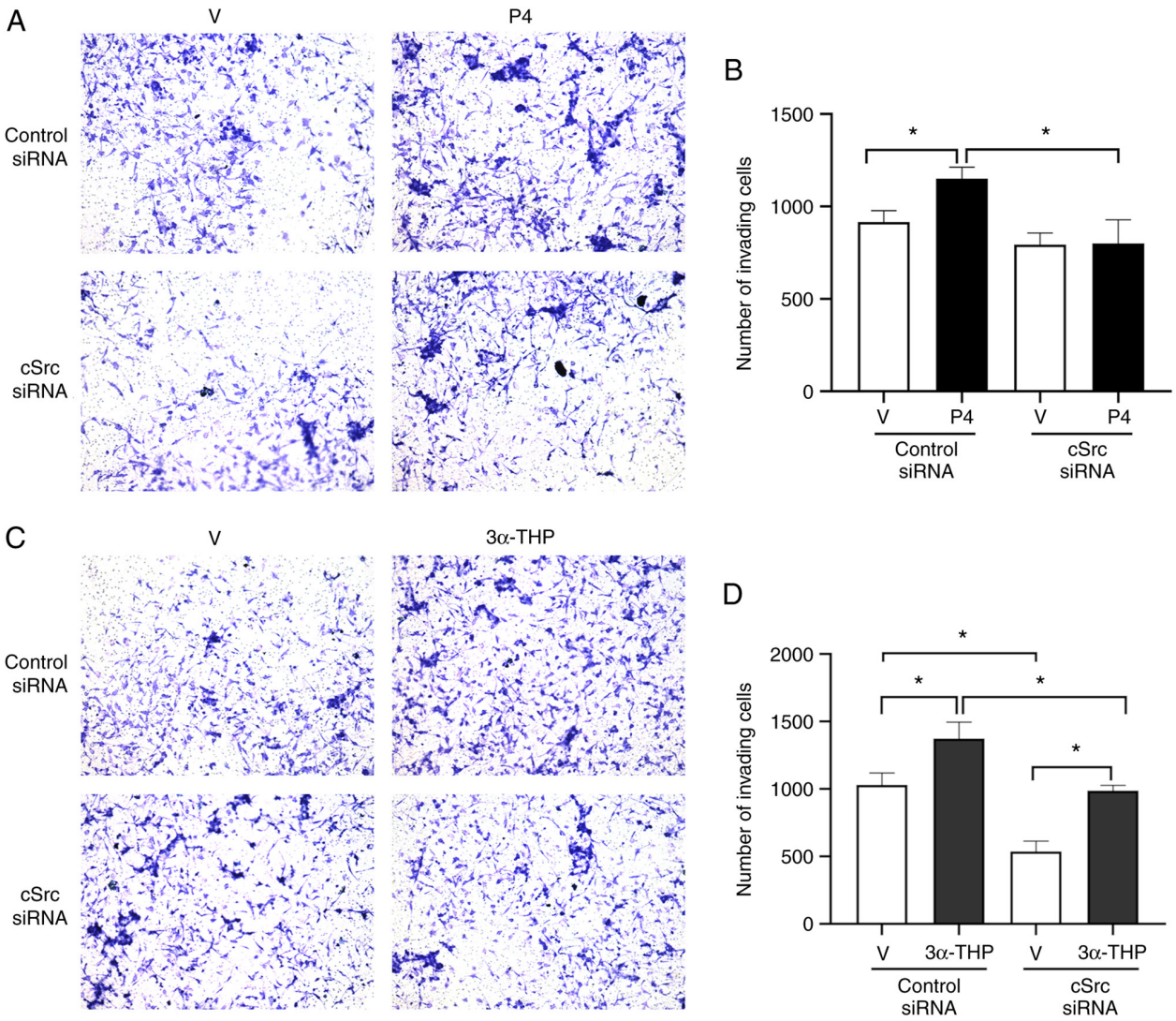

Figure 4. cSrc expression mediates invasion induced by P4 and 3α-THP in U251 cells. cSrc was silenced in the U251 cell line and was treated with P4 (50 nM), 3α-THP (100 nM) or their respective vehicles (0.001% DMSO or 0.01% ethanol) for 24 h. Representative images of cell invasion assays of transfected U251 cells treated with (A) P4 or (C) 3α-THP. Graphs representing the number of invasive U251 cells treated with (B) P4 or (D) 3α-THP. Data are presented as the mean ± SEM; n=3. *P<0.05. P4, progesterone; siRNA, small interfering RNA; V, vehicle; 3α-THP, allopregnanolone.

Discussion

According to the Global Cancer Observatory database (https://gco.iarc.fr/) (22), central nervous system tumors are the 13th leading cause of mortality among all known types of cancer. Glioblastoma is the most malignant and frequent entity among adult brain tumors (1). Due to glioblastoma's migratory and invasive nature, the treatments available are insufficient and tumor recurrence is invariably manifested (23). The migration and subsequent degradation of the ECM is one of the critical events for a malignant cell to colonize areas distant from the tumor's site of origin (24). Proteases are members of the group of molecules that, in a coordinated manner, orchestrate this event to gain cancer aggressiveness (25). MMPs are among the most relevant families of proteases in glioblastoma invasion. The expression of 11 members (MMP-1, -2, -7, -8, -9, -10, -11, -14, -15, -19 and -23) of this group have been reported to be elevated in glioblastomas (26). However, according to the results of several studies (6,27-29), MMP-2 and MMP-9 are essential in the progression of glioblastomas. The expression of these proteins are increased in recurrent gliomas compared with primary gliomas (6). In addition, the expression of both of these MMPs correlates with the tumor grade (7). In the case of MMP-9, its low expression is related to an improved prognosis and response to Temozolomide (30).

The role of P4 at physiological concentrations (10-100 nM) in the development of glioblastomas has been extensively reported. In addition to inducing glioblastoma cell proliferation (23-25), P4 promotes migration and invasion in both *in vitro* and *in vivo* models (15,16,31-33). P4 is one of the most prominent examples of the hormesis effect, a dose-response phenomenon in which stimulation occurs at low concentrations and inhibition at high concentrations (34). In the context of glioblastomas, it has been reported that P4 concentrations >20 μM promoted apoptosis and decreased tumor growth, while concentrations <10 μM promoted the opposite effect (35). Previous results published by our laboratory support this observation as concentrations of 10 and 50 nM promoted glioblastoma cell progression by increasing proliferation, migration and invasion (14-16).

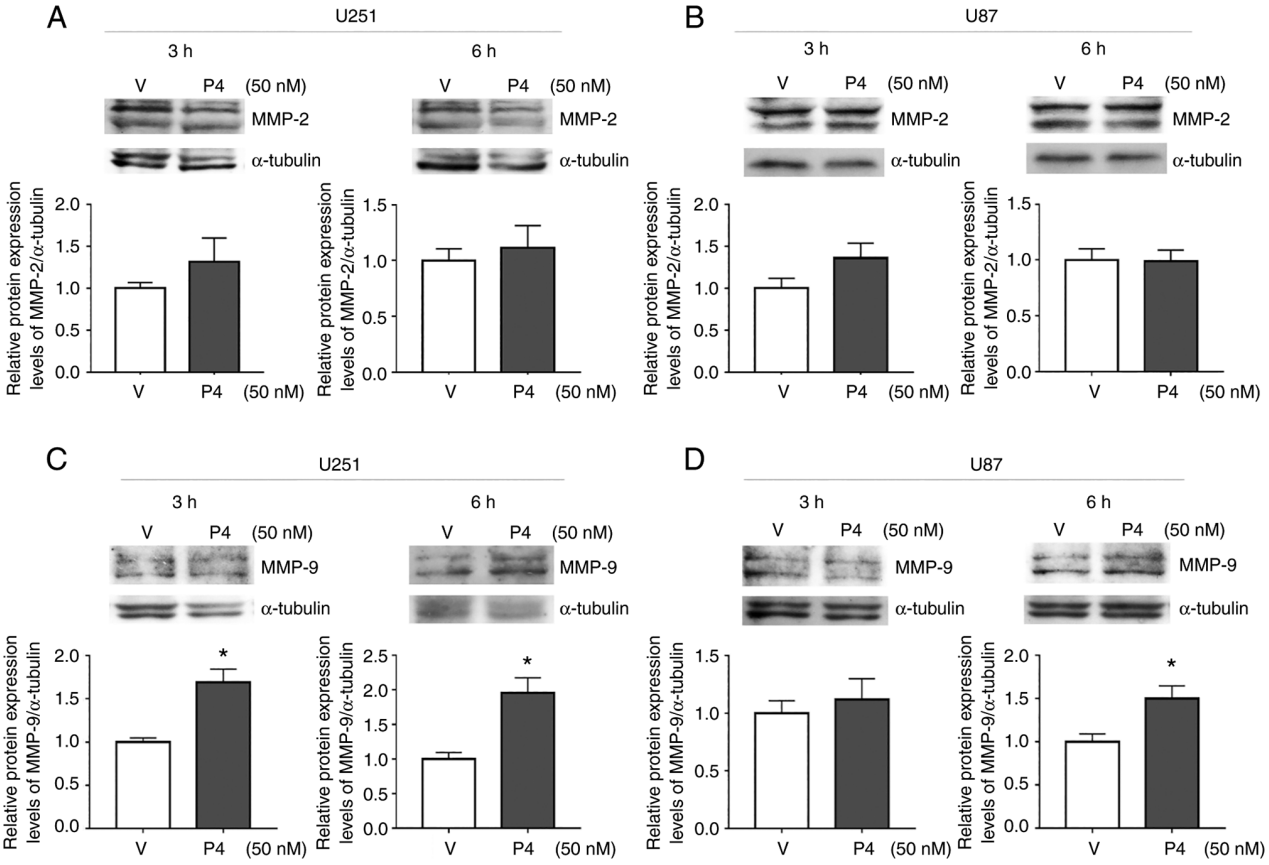

Figure 5. The effect of P4 on MMP-2 and MMP-9 protein expression levels in human glioblastoma-derived cell lines. U251 and U87 cells were treated with P4 (50 nM) or V (0.001% DMSO) for 3 and 6 h. The upper panels show the representative western blots for MMP-2 (proenzyme, 72 kDa; active enzyme, 64 kDa) in (A) U251 and (B) U87 cells, and for MMP-9 (proenzyme, 92 kDa; active enzyme, 84 kDa) in (C) U251 and (D) U87 cells. α-tubulin (55 kDa) was used as the loading control. The lower panels show the densitometric analysis. Data were normalized with respect to V and are presented as the mean ± SEM; n=4. *P<0.05. MMP, metalloproteinase; P4, progesterone; V, vehicle.

The previous study by Piña Medina *et al* (16), from our laboratory, reported that PR was partially implicated in the induction of migration and invasion processes in glioblastoma cells, which suggested alternative activation of other regulatory mechanisms mediated by P4 or its metabolites. In that study, when P4 were added to cells treated with antisense oligonucleotides against PR expression, the P4-induced effect was partially reduced. Recently, Bello-Alvarez *et al* (15) reported that, in glioblastoma cells, P4 promoted PR-cSrc interaction, which led to cSrc autophosphorylation and subsequent Fak activation and migration.

Notably, 3α-THP possesses different mechanisms of action than those reported for P4. One of the main differences between these progestins is that 3α-THP lacks affinity for the classical PR (36). This metabolite induces rapid cellular changes by activating membrane PR (mPR) δ and mPRα (37,38). At the genomic level, 3α-THP activates the pregnane X receptor, which is a transcription factor (39,40). The progestin 3α-THP also modulates the action of neurotransmitters due to its interaction with their receptors, such as the ionotropic channel receptor, $GABA_AR$ (41). It has recently been reported that 3α-THP promotes the migration of glioblastoma cells independently of the oxidation of other P4 metabolites with high binding affinity from the PR. In addition, the activation of cSrc kinase by 3a-THP has been reported (18,42). Melfi *et al* (19) reported that, in rat Schwann cells, induced cell migration was dependent on cSrc activation. An additional study reported that 3α-THP promoted cSrc activation at the ventromedial hypothalamus, although the mechanism involved in this activation has not been fully elucidated (43). In the present study, an increase in glioblastoma cell migration induced by P4 is reported. This effect was completely blocked with the addition of the cSrc kinase family inhibitor, PP2, in U251 cells and partially blocked in U87 cells. Glioblastomas are among the tumors with the greatest intra- and inter-tumor heterogeneity (44,45). Therefore, it can be assumed that the established cell lines derived from these tumors have significant differences in their genetic signature. The U87 cell line has a neuronal-like phenotype with a high proliferative capacity, while the U251 cell line has a mesenchymal-like phenotype with a lower proliferative activity (46). The results in the present study demonstrated a difference in the migration rate of both cell lines, which may be related to the aforementioned differences. Considering that, despite the differences in migration rate, the results showed the same trend in both cell lines, it can be hypothesized that the effects of P4, in general, not cell line-dependent but pathology-dependent.

Regarding 3α-THP, in the present study, an increase in cell migration partially blocked by PP2 in U251 and U87 cells was demonstrated. Considering that PP2 is not a specific inhibitor of cSrc, but of the entire Src family, this result suggested that, in addition to cSrc, other members of the Src kinase family may interact with P4 or 3α-THP effectors. For example, the

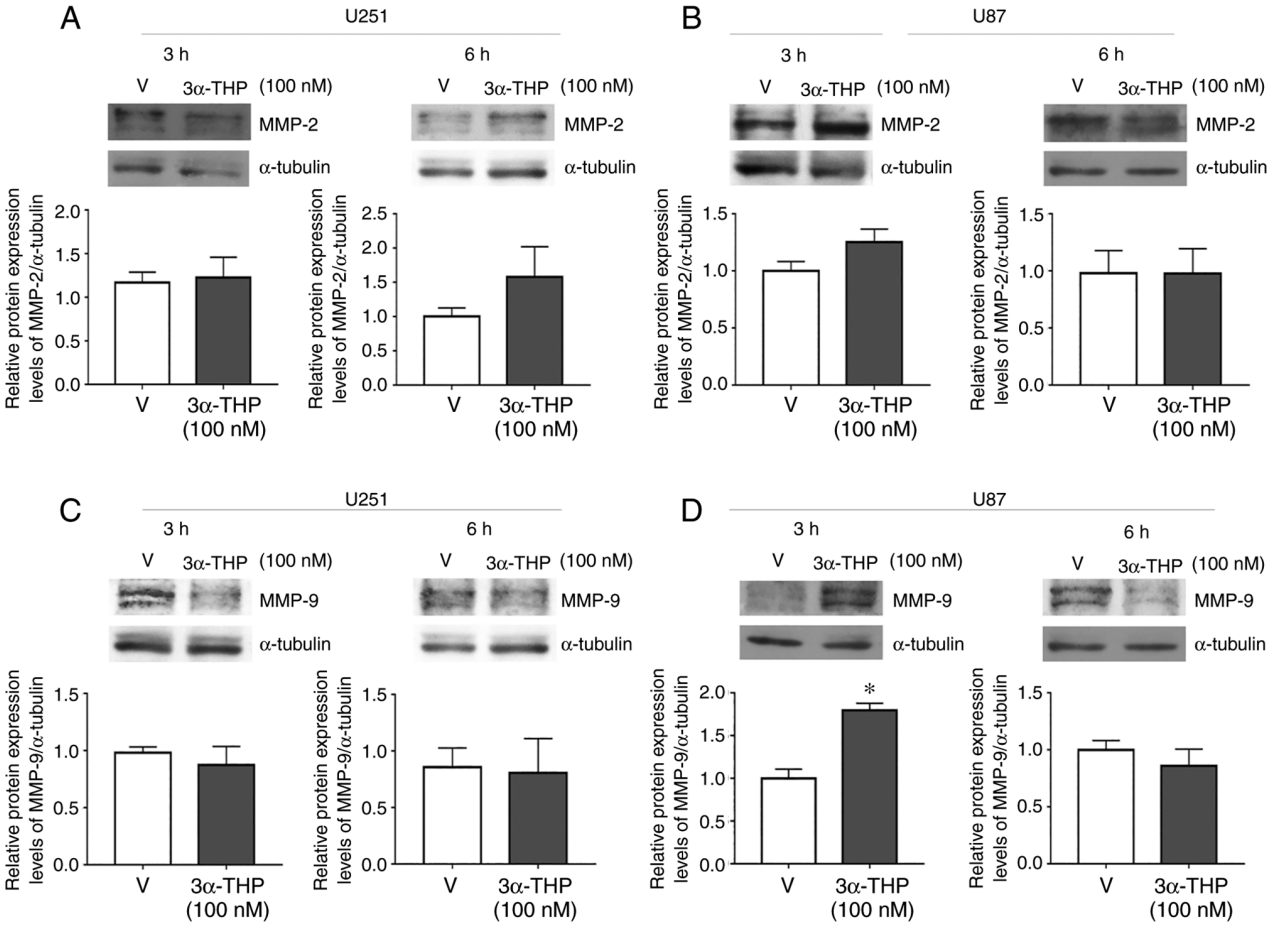

Figure 6. The effect of 3α-THP on MMP-2 and MMP-9 protein expression levels in human glioblastoma-derived cell lines. U251 and U87 cells were treated with 3α-THP (100 nM) or V (0.01% ethanol) for 3 and 6 h. The upper panels show the representative western blots for MMP-2 (proenzyme, 72 kDa; active enzyme, 64 kDa) in (A) U251 and (B) U87 cells, and for MMP-9 (proenzyme, 92 kDa; active enzyme, 84 kDa) in (C) U251 and (D) U87 cells. α-tubulin (55 kDa) was used as the loading control. The lower panels show the densitometric analysis. Data were normalized with respect to V and are presented as the mean ± SEM; n=4. *P<0.05. MMP, metalloproteinase; V, vehicle; 3α-THP, allopregnanolone.

high expression of Lyn, a member of cSrc kinase family, has been reported in glioblastoma cells and is linked to increased tumor progression (47).

The invasiveness of glioblastoma cells depends on the dynamic activation of at least two essential processes: Cell migration and ECM degradation. Until recently, no information on the role of cSrc in the regulation of proteins directly involved in ECM degradation in glioblastoma cells treated with P4 or any of its metabolites was available. In the present study, the effect of P4 and 3α-THP on the expression of MMP-2 and MMP-9 was evaluated. The results demonstrated that in P4-treated cells, the protein expression level of MMP-9 was increased in U251 and U87 cells, and in 3α-THP-treated cells, the protein expression level of MMP-9 was increased in U87 cells. These results indicated that increased MMP-9 expression may be one of the mechanisms implicated in P4- and 3α-THP-induced invasion of glioblastoma cells. MMP-2 upregulation is also associated with poor prognosis and progression of glioblastoma (48). Notably, no significant changes in MMP-2 expression were demonstrated with P4 and 3α-THP treatments. In fibroblast cells, the increase in the protein expression levels of MMP-9 but not MMP-2 were reported to be dependent on the activation of the cSrc-Fak signaling pathway (49). In MCF-7 breast cancer cells, cSrc and Fak activity inhibition blocked MMP-9 secretion (4). A recent article reported the anti-inflammatory property of quercetin that, through the inhibition of TNF-α, decreased MMP-9 expression and activity in the gastric mucosa epithelial GES-1 cell line. This study also reported that the addition of PP1 (a Src family kinase inhibitor) decreased the activity of MMP-9 but not MMP-2 (50). The occurrence of this phenomenon in cells of different lineages suggests that it is independent of cellular context and that it is regulated by highly conserved molecular mechanisms.

Interactions between cSrc and Fak have been widely reported. It is known that cSrc phosphorylates residues Y576 and Y577 of Fak, which are indispensable for full activation of the latter (51,52). Sex steroid receptors are primarily known for their function as transcription factors. However, PR and the androgen receptor are also known to function in the activation of signaling cascades in the cytoplasm through the interaction of their polyproline motifs with the SH3 domains of cytoplasmic molecules, such as cSrc (53). In a recent publication by Bello-Alvarez *et al* (15), it was demonstrated that P4 activates cSrc kinase through the PR, for the first time in glioblastoma-derived cells. This, in turn, induces the phosphorylation of Fak at residues Y397, Y576 and Y577, which provided evidence of the non-genomic function of the PR in this brain tumor.

In the present study to assess the role of cSrc in the regulation of MMP-9 expression by P4 and 3α-THP, an siRNA against

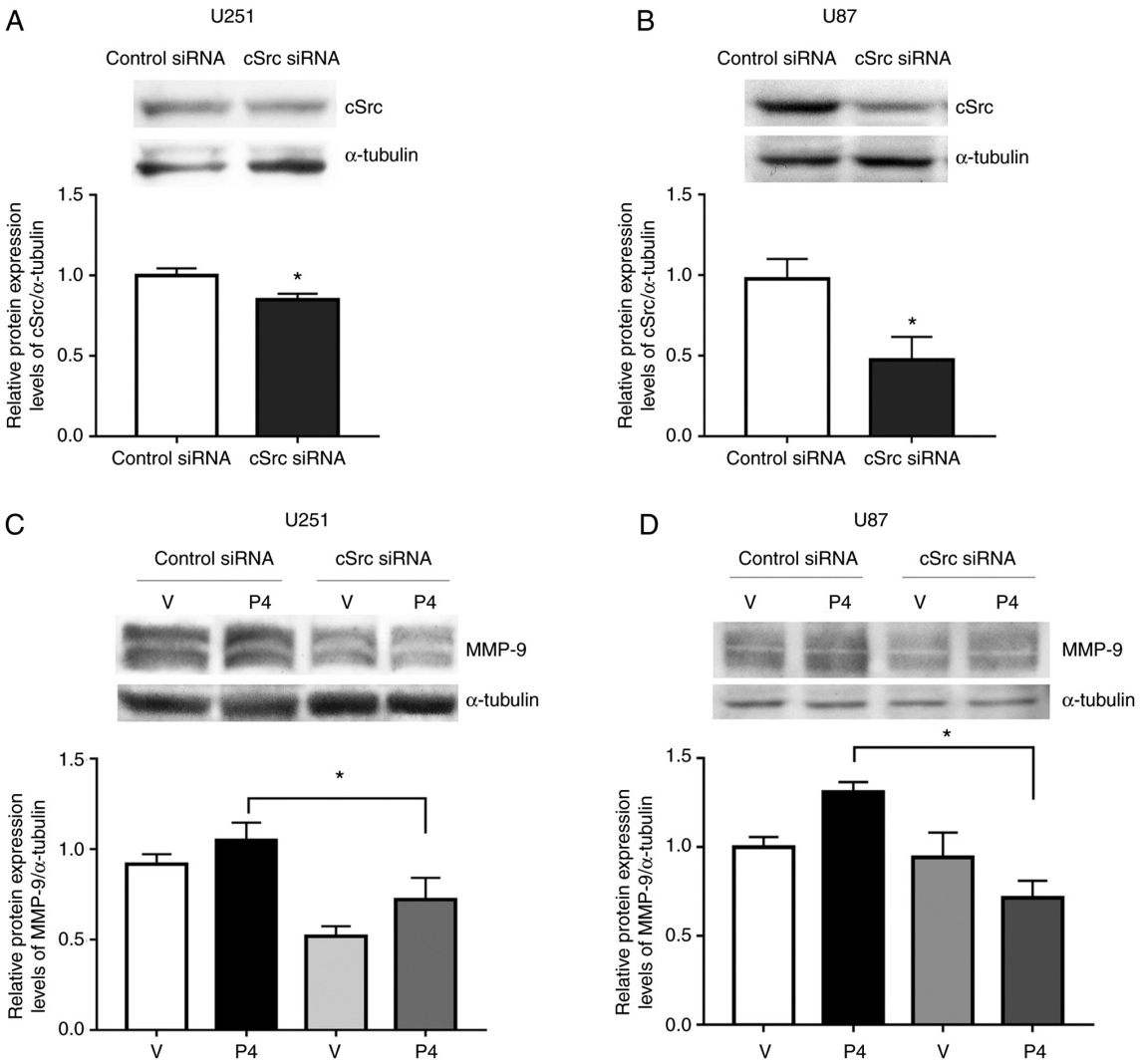

Figure 7. cSrc is involved in the regulation of MMP-9 expression by P4 in glioblastoma cells. (A) U251 and (B) U87 cells were transfected with cSrc siRNA and control siRNA. Transfected cells were treated with (C) P4 (50 nM) or (D) V (0.001% DMSO) for 6 h. The upper panels show the representative western blots for cSrc (60 kDa), MMP-9 (proenzyme, 92 kDa; active enzyme, 84 kDa) and α-tubulin (55 kDa; loading control). The lower panels show the densitometric analysis. Data were normalized with respect to V and are presented as the mean ± SEM; n=4. *P<0.05. MMP, metalloproteinase; P4, progesterone; siRNA, small interfering RNA; V, vehicle.

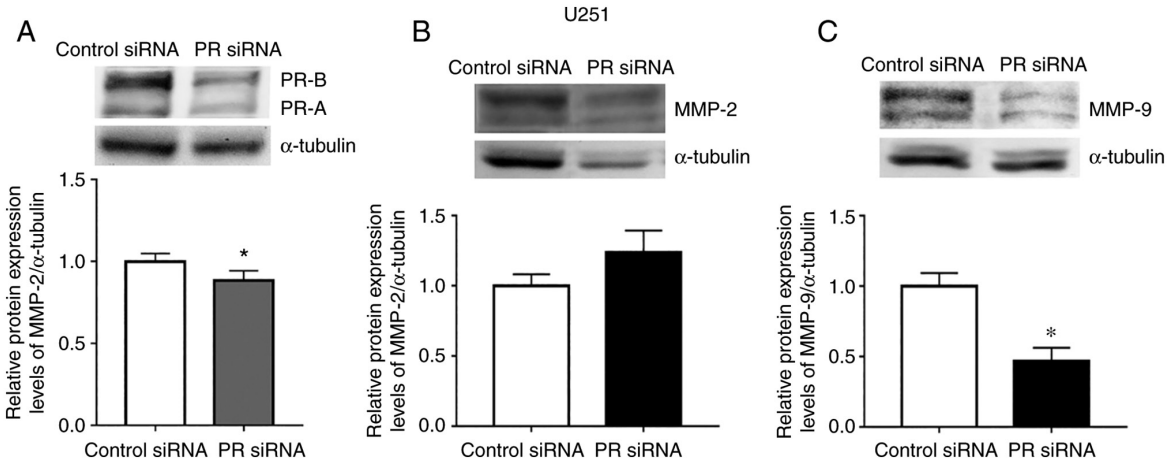

Figure 8. PR silencing reduces the protein expression levels of MMP-9 but not MMP-2. (A) U251 cells were transfected with a PR siRNA and a control siRNA (100 nM). (B) MMP-2 and (C) MMP-9 protein expression levels were determined in cells transfected with PR siRNA at 6 h. The upper panels show the representative western blots for PR (PR-B 110 kDa, 90 PR-A kDa), MMP-2 (proenzyme, 72 kDa; active enzyme, 64 kDa), MMP-9 (proenzyme, 92 kDa; active enzyme, 84 kDa), and α-tubulin (55 kDa, loading control). The lower panels show the densitometric analysis. Data were normalized with respect to control siRNA and are presented as the mean ± SEM; n=3. *P<0.05. MMP, metalloproteinase; P4, progesterone; PR, progesterone receptor; siRNA, small interfering RNA; V, vehicle.

cSrc expression was used. The silencing of cSrc blocked the increase in MMP-9 protein expression levels induced by P4 in U251 and U87 cells. Only a partial effect was demonstrated in U251 cells, possibly due to lower efficiency of the silencing. These results indicated that cSrc mediates P4-induced MMP-9 expression and suggest that, in the context of glioblastoma cells, P4 treatment activates the cSrc-Fak signaling pathway, which modifies the expression of MMP-9 but not that of MMP-2.

Notably, Liu *et al* (54) reported that the $PR^{-/-}$ condition in zebrafish follicular cells decreased the expression of MMP-9 but not MMP-2. In the present study, transfection with an siRNA against the PR decreased MMP-9 but not MMP-2 protein expression levels, which suggested that the difference in the effect of P4 on the regulation of MMP-9 and MMP-2 also involved the PR. However, another study reported that, in a rat model of blood-brain barrier breakdown, P4 and 3α-THP downregulated the expression of MMP-9 and MMP-2 (55); this could be related to differences in the progestin concentration. At the level of transcriptional regulation, these differences could be related to the composition of the MMP-2 and MMP-9 promoters (56).

In the present study, PP2 was used to evaluate the effect of the cSrc family of kinases on P4-induced invasion in human glioblastoma-derived cells. The results of the present study demonstrated that inhibition of cSrc activity decreased invasiveness in both U251 and U87 cell lines, as measured using a Matrigel Boyden chamber assay. A similar effect of 3α-THP has also been recently reported by our laboratory (18).

In conclusion, the results of the present study suggest that the effect of P4 and 3α-THP on the invasion of human glioblastoma-derived cells involves the activation of cSrc and its regulatory role in MMP-9 expression. Experimentation on cell lines is an important limitation of this study since it implies a study model that is far from reality. However, the results obtained broaden the knowledge for the future development of targeted therapies against migration and invasion processes in an underestimated area in the study of glioblastomas, namely, signaling through sex hormones and their receptors.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CBA, CJZS and ICA conceptualized the study. CBA, CJZS and KMPG performed the experiments. CBA and CJZS prepared the first draft of the manuscript and ICA contributed to the revision and editing of the manuscript. ICA obtained the funding. CBA, CJZS, KMPG and ICA confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Ostrom QT, Gittleman H, Truitt G, Boscia A, Kruchko C and Barnholtz-Sloan JS: CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2011-2015. Neuro Oncol 20: iv1-iv86, 2018.
2. Giese A, Bjerkvig R, Berens ME and Westphal M: Cost of migration: Invasion of malignant gliomas and implications for treatment. J Clin Oncol 21: 1624-1636, 2003.
3. Patel A, Sabbineni H, Clarke A and Somanath PR: Novel roles of Src in cancer cell epithelial-to-mesenchymal transition, vascular permeability, microinvasion and metastasis. Life Sci 157: 52-61, 2016.
4. Cortes-Reynosa P, Robledo T, Macias-Silva M, Wu SV and Salazar EP: Src kinase regulates metalloproteinase-9 secretion induced by type IV collagen in MCF-7 human breast cancer cells. Matrix Biol 27: 220-231, 2008.
5. Gautam J, Banskota S, Lee H, Lee YJ, Jeon YH, Kim JA and Jeong BS: Down-regulation of cathepsin S and matrix metalloproteinase-9 via Src, a non-receptor tyrosine kinase, suppresses triple-negative breast cancer growth and metastasis. Exp Mol Med 50: 1-14, 2018.
6. Zhou W, Yu X, Sun S, Zhang X, Yang W, Zhang J, Zhang X and Jiang Z: Increased expression of MMP-2 and MMP-9 indicates poor prognosis in glioma recurrence. Biomed Pharmacother 118: 109369, 2019.
7. Forsyth PA, Wong H, Laing TD, Rewcastle NB, Morris DG, Muzik H, Leco KJ, Johnston RN, Brasher PM, Sutherland G and Edwards DR: Gelatinase-A (MMP-2), gelatinase-B (MMP-9) and membrane type matrix metalloproteinase-1 (MT1-MMP) are involved in different aspects of the pathophysiology of malignant gliomas. Br J Cancer 79: 1828-1835, 1999.
8. Kondraganti S, Mohanam S, Chintala SK, Kin Y, Jasti SL, Nirmala C, Lakka SS, Adachi Y, Kyritsis AP, Ali-Osman F, *et al*: Selective suppression of matrix metalloproteinase-9 in human glioblastoma cells by antisense gene transfer impairs glioblastoma cell invasion. Cancer Res 60: 6851-6855, 2000.
9. Trabert B, Sherman ME, Kannan N and Stanczyk FZ: Progesterone and breast cancer. Endocr Rev 41: 320-344, 2020.
10. Grindstad T, Richardsen E, Andersen S, Skjefstad K, Khanehkenari MR, Donnem T, Ness N, Nordby Y, Bremnes RM, Al-Saad S and Busund LT: Progesterone receptors in prostate cancer: Progesterone receptor B is the isoform associated with disease progression. Sci Rep 8: 1-11, 2018.
11. Bello-Alvarez C and Camacho-Arroyo I: Impact of sex in the prevalence and progression of glioblastomas: The role of gonadal steroid hormones. Biol Sex Differ 12: 28, 2021.
12. Fu XD, Goglia L, Sanchez AM, Flamini M, Giretti MS, Tosi V, Genazzani AR and Simoncini T: Progesterone receptor enhances breast cancer cell motility and invasion via extranuclear activation of focal adhesion kinase. Endocr Relat Cancer 17: 431-443, 2010.
13. Grochans S, Cybulska AM, Simińska D, Korbecki J, Kojder K, Chlubek D and Baranowska-Bosiacka I: Epidemiology of glioblastoma multiforme-literature review. Cancers (Basel) 14: 2412, 2022.
14. Germán-Castelán L, Manjarrez-Marmolejo J, González-Arenas A and Camacho-Arroyo I: Intracellular progesterone receptor mediates the increase in glioblastoma growth induced by progesterone in the rat brain. Arch Med Res 47: 419-426, 2016.

15. Bello-Alvarez C, Moral-Morales AD, González-Arenas A and Camacho-Arroyo I: Intracellular progesterone receptor and cSrc protein working together to regulate the activity of proteins involved in migration and invasion of human glioblastoma cells. Front Endocrinol (Lausanne) 12: 640298, 2021.
16. Piña-Medina AG, Hansberg-Pastor V, González-Arenas A, Cerbón M and Camacho-Arroyo I: Progesterone promotes cell migration, invasion and cofilin activation in human astrocytoma cells. Steroids 105: 19-25, 2016.
17. Zamora-Sánchez CJ, Hansberg-Pastor V, Salido-Guadarrama I, Rodríguez-Dorantes M and Camacho-Arroyo I: Allopregnanolone promotes proliferation and differential gene expression in human glioblastoma cells. Steroids 119: 36-42, 2017.
18. Zamora-Sánchez CJ, Bello-Alvarez C, Rodríguez-Dorantes M and Camacho-Arroyo I: Allopregnanolone promotes migration and invasion of human glioblastoma cells through the protein tyrosine kinase c-Src activation. Int J Mol Sci 23: 4996, 2022.
19. Melfi S, Guevara MM, Bonalume V, Ruscica M, Colciago A, Simoncini T and Magnaghi V: Src and phospho-FAK kinases are activated by allopregnanolone promoting Schwann cell motility, morphology and myelination. J Neurochem 141: 165-178, 2017.
20. Pirkmajer S and Chibalin AV: Serum starvation: Caveat emptor. Am J Physiol Cell Physiol 301: C272-C279, 2011.
21. Jabłońska-Trypuć A, Matejczyk M and Rosochacki S: Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anti-cancer drugs. J Enzyme Inhib Med Chem 31: 177-183, 2016.
22. Global Cancer Observatory.
23. van Linde ME, Brahm CG, de Witt Hamer PC, Reijneveld JC, Bruynzeel AME, Vandertop WP, van de Ven PM, Wagemakers M, van der Weide HL, Enting RH, *et al*: Treatment outcome of patients with recurrent glioblastoma multiforme: A retrospective multicenter analysis. J Neurooncol 135: 183-192, 2017.
24. Gkretsi V and Stylianopoulos T: Cell adhesion and matrix stiffness: Coordinating cancer cell invasion and metastasis. Front Oncol 8: 145, 2018.
25. Quintero-Fabián S, Arreola R, Becerril-Villanueva E, Torres-Romero JC, Arana-Argáez V, Lara-Riegos J, Ramírez-Camacho MA and Alvarez-Sánchez ME: Role of matrix metalloproteinases in angiogenesis and cancer. Front Oncol 9: 1370, 2019.
26. Quesnel A, Karagiannis GS and Filippou PS: Extracellular proteolysis in glioblastoma progression and therapeutics. Biochim Biophys Acta Rev Cancer 1874: 188428, 2020.
27. Cai HP, Wang J, Xi SY, Ni XR, Chen YS, Yu YJ, Cen ZW, Yu ZH, Chen FR, Guo CC, *et al*: Tenascin-c mediated vasculogenic mimicry formation via regulation of MMP2/MMP9 in glioma. Cell Death Dis 10: 1-14, 2019.
28. Mirabdaly S, Komi DE, Shakiba Y, Moini A and Kiani A: Effects of temozolomide on U87MG glioblastoma cell expression of CXCR4, MMP2, MMP9, VEGF, anti-proliferatory cytotoxic and apoptotic properties. Mol Biol Rep 47: 1187-1197, 2020.
29. Chen CJ, Shang HS, Huang YL, Tien N, Chen YL, Hsu SY, Wu RSC, Tang CL, Lien JC, Lee MH, *et al*: Bisdemethoxycurcumin suppresses human brain glioblastoma multiforme GBM 8401 cell migration and invasion via affecting NF-κB and MMP-2 and MMP-9 signaling pathway in vitro. Environ Toxicol 37: 2388-2397, 2022.
30. Li Q, Chen B, Cai J, Sun Y, Wang G, Li Y, Li R, Feng Y, Han B, Li J, *et al*: Comparative analysis of matrix metalloproteinase family members reveals that MMP9 predicts survival and response to temozolomide in patients with primary glioblastoma. PLoS One 11: e0151815, 2016.
31. González-Agüero G, Gutiérrez AA, González-Espinosa D, Solano JD, Morales R, González-Arenas A, Cabrera-Muñoz E and Camacho-Arroyo I: Progesterone effects on cell growth of U373 and D54 human astrocytoma cell lines. Endocrine 32: 129-135, 2007.
32. Hernández-Hernández OT, González-García TK and Camacho-Arroyo I: Progesterone receptor and SRC-1 participate in the regulation of VEGF, EGFR and cyclin D1 expression in human astrocytoma cell lines. J Steroid Biochem Mol Biol 132: 127-134, 2012.
33. González-Arenas A, Valadez-Cosmes P, Jiménez-Arellano C, López-Sánchez M and Camacho-Arroyo I: Progesterone-induced blocking factor is hormonally regulated in human astrocytoma cells, and increases their growth through the IL-4R/JAK1/STAT6 pathway. J Steroid Biochem Mol Biol 144: 463-470, 2014.
34. Strom JO, Theodorsson A and Theodorsson E: Hormesis and female sex hormones. Pharmaceuticals (Basel) 4: 726-740, 2011.
35. Atif F, Yousuf S and Stein DG: Anti-tumor effects of progesterone in human glioblastoma multiforme: Role of PI3K/Akt/mTOR signaling. J Steroid Biochem Mol Biol 146: 62-73, 2015.
36. Diviccaro S, Cioffi L, Falvo E, Giatti S and Melcangi RC: Allopregnanolone: An overview on its synthesis and effects. J Neuroendocrinol 34: e12996, 2022.
37. Thomas P and Pang Y: Membrane progesterone receptors: Evidence for neuroprotective, neurosteroid signaling and neuroendocrine functions in neuronal cells. Neuroendocrinology 96: 162-71, 2012.
38. Pang Y, Dong J and Thomas P: Characterization, neurosteroid binding and brain distribution of human membrane progesterone receptors δ and {epsilon} (mPRδ and mPR{epsilon}) and mPRδ involvement in neurosteroid inhibition of apoptosis. Endocrinology 154: 283-295, 2013.
39. Lamba V, Yasuda K, Lamba JK, Assem M, Davila J, Strom S and Schuetz EG: PXR (NR1I2): Splice variants in human tissues, including brain, and identification of neurosteroids and nicotine as PXR activators. Toxicol Appl Pharmacol 199: 251-265, 2004.
40. Frye CA, Koonce CJ and Walf AA: The pregnane xenobiotic receptor, a prominent liver factor, has actions in the midbrain for neurosteroid synthesis and behavioral/neural plasticity of female rats. Front Syst Neurosci 8: 60, 2014.
41. Belelli D and Gee KW: 5 alpha-pregnan-3 alpha,20 alpha-diol behaves like a partial agonist in the modulation of GABA-stimulated chlride ion uptake by synaptoneurosomes. Eur J Pharmacol 167: 173-176, 1989.
42. Zamora-Sánchez CJ, Hernández-Vega AM, Gaona-Domínguez S, Rodríguez-Dorantes M and Camacho-Arroyo I: 5alpha-dihydroprogesterone promotes proliferation and migration of human glioblastoma cells. Steroids 163: 108708, 2020.
43. González-Flores O, Beyer C, Gómora-Arrati P, García-Juárez M, Lima-Hernández FJ, Soto-Sánchez A and Etgen AM: A role for Src kinase in progestin facilitation of estrous behavior in estradiol-primed female rats. Horm Behav 58: 223-229, 2010.
44. Bergmann N, Delbridge C, Gempt J, Feuchtinger A, Walch A, Schirmer L, Bunk W, Aschenbrenner T, Liesche-Starnecker F and Schlegel J: The intratumoral heterogeneity reflects the intertumoral subtypes of glioblastoma multiforme: A regional immunohistochemistry analysis. Front Oncol 10: 494, 2020.
45. Becker AP, Sells BE, Haque SJ and Chakravarti A: Tumor heterogeneity in glioblastomas: From light microscopy to molecular pathology. Cancers (Basel) 13: 1-25, 2021.
46. Motaln H, Koren A, Gruden K, Ramšak Ž, Schichor C and Lah TT: Heterogeneous glioblastoma cell cross-talk promotes phenotype alterations and enhanced drug resistance. Oncotarget 6: 40998-41017, 2015.
47. Stettner MR, Wang W, Nabors LB, Bharara S, Flynn DC, Grammer JR, Gillespie GY and Gladson CL: Lyn kinase activity is the predominant cellular Src kinase activity in glioblastoma tumor cells. Cancer Res 65: 5535-5543, 2005.
48. Ramachandran RK, Sørensen MD, Aaberg-Jessen C, Hermansen SK and Kristensen BW: Expression and prognostic impact of matrix metalloproteinase-2 (MMP-2) in astrocytomas. PLoS One 12: e0172234, 2017.
49. Hsia DA, Mitra SK, Hauck CR, Streblow DN, Nelson JA, Ilic D, Huang S, Li E, Nemerow GR, Leng J, *et al*: Differential regulation of cell motility and invasion by FAK. J Cell Biol 160: 753-767, 2003.
50. Hsieh HL, Yu MC, Cheng LC, Chu MY, Huang TH, Yeh TS and Tsai MM: Quercetin exerts anti-inflammatory effects via inhibiting tumor necrosis factor-α-induced matrix metalloproteinase-9 expression in normal human gastric epithelial cells. World J Gastroenterol 28: 1139-1158, 2022.
51. Yeatman TJ: A renaissance for SRC. Nat Rev Cancer 4: 470-480, 2004.
52. Mitra SK and Schlaepfer DD: Integrin-regulated FAK-Src signaling in normal and cancer cells. Curr Opin Cell Biol 18: 516-523, 2006.
53. Boonyaratanakornkit V and Edwards DP: Receptor mechanisms mediating non-genomic actions of sex steroids. Semin Reprod Med 25: 139-153, 2007.
54. Liu DT, Carter NJ, Wu XJ, Hong WS, Chen SX and Zhu Y: Progestin and nuclear progestin receptor are essential for upregulation of metalloproteinase in zebrafish preovulatory follicles. Front Endocrinol (Lausanne) 9: 517, 2018.
55. Ishrat T, Sayeed I, Atif F, Hua F and Stein DG: Progesterone and allopregnanolone attenuate blood-brain barrier dysfunction following permanent focal ischemia by regulating the expression of matrix metalloproteinases. Exp Neurol 226: 183-190, 2010.
56. Yan C and Boyd DD: Regulation of matrix metalloproteinase gene expression. J Cell Physiol 211: 19-26, 2007.