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Regulation of progesterone receptor isoforms content in human astrocytoma cell lines

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ABSTRACT

Progesterone regulates several functions through the interaction with its intracellular receptor (PR) which expresses two isoforms with different functions and regulation: PR-A and PR-B. Both PR isoforms have been detected in human astrocytomas, the most common and aggressive primary brain tumours, but their regulation and function are unknown. We studied the effects of estradiol, progesterone and their receptor antagonists (ICI 182,780 and RU 486) on PR isoforms content in U373 and D54 human astrocytoma cell lines, respectively derived from grades III and IV astrocytomas, by Western blot analysis. In U373 cells we also evaluated the effects of PR-A overexpression on cell growth. We observed that in U373 cells estradiol increased the content of both PR isoforms whereas in D54 cells it had no effects. Estradiol effects were blocked by ICI 182,780. In both cell lines, PR isoforms content was down-regulated by progesterone after estradiol treatment. This effect was blocked by RU 486. We observed that overexpression of PR-A significantly diminished the increase in U373 cells number produced after progesterone treatment. Our results suggest a differential PR isoforms regulation depending on the evolution grade of human astrocytoma cells, and an inhibitory role of PR-A on progesterone effects on astrocytomas cell growth.

1. Introduction

Astrocytomas are the most common primary intracerebral neoplasms in humans. They are mainly found in adults between ages 30 and 50, are classified according to their histological characteristics in four groups (I–IV) and the survival of patients is inversely related to the tumours grade [1].

Progesterone is involved in the regulation of several physiological and pathological processes in brain, including tumour cell growth [2,3]. Progesterone regulates several functions through the interaction with its nuclear receptors [4–6]. Two PR isoforms have been reported: a full-length form (PR-B, 114 kDa) and an N-terminal truncated one (PR-A, 94 kDa) which are functionally different [7–9]. PR isoforms are encoded by the same gene, but they are regulated by distinct promoters [9–11]. In general, PR-B is a much stronger activator than PR-A [12,13]. It has been shown that PR isoforms are functionally distinct in terms of their ability to activate target genes in the same cell and regulate different physiological processes [8,14].

PR isoforms have been detected in several human brain tumours such as astrocytomas, meningiomas, chordomas and craniopharyngiomas [15–19]. In astrocytomas, an inverse relation between the content of PR and the tumour grade has been reported [17,18,20,21]. PR isoforms are differentially expressed in biopsies from human astrocytomas: the predominant PR isoform expressed in astrocytomas grades III and IV was PR-B [20]. PR isoforms have been recently detected in U373 and D54 cell lines which are respectively derived from grades III and IV human astrocytomas [22].

It is known that PR isoforms are differentially regulated by estradiol and progesterone in different cells and tissues. In several cell types PR isoforms [23,24] are up-regulated by estradiol. PR up-regulation by estradiol is mediated by estrogen-responsive elements located in the PR promoter [25] whereas PR down-regulation by their own ligand (progesterone) is associated with ligand-dependent proteolysis [26]; progesterone induces receptor phosphorylation, which signals PR to degradation by the ubiquitin–proteasome pathway [27–29].

The regulation and function of PR isoforms in human astrocytoma cells are not known. Therefore, we studied the effects of

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estradiol, progesterone, RU486 and ICI 182,780 on PR isoforms content in U373 and D54 human astrocytoma cell lines, as well as the role of PR-A on U373 cell growth induced by progesterone.

2. Materials and methods

2.1. Cell culture and treatments

U373-GB and D54 human astrocytoma cell lines derived from human astrocytoma grades III (ATCC, Manassas, VA) and IV, generously obtained by Dr. Andres Gutiérrez from Dr. Sontheimer (Bringham, AL) Laboratory, and T47D human breast cancer cells (used as positive control of PR expression) were used. 5×10^5 cells were plated in 10 cm dishes and maintained in Dulbecco's modification of Eagle's medium (DMEM) and RPMI medium, respectively, supplemented with 10% fetal bovine serum, 1 mM pyruvate, 2 mM glutamine, 0.1 mM non-essential amino acids (GIBCO, NY) for 24 h. Medium was changed by DMEM or RPMI phenol red free medium supplemented with 10% fetal bovine serum without steroid hormones (Hyclone, Utah), 1 mM pyruvate, 2 mM glutamine, 0.1 mM non-essential amino acids (GIBCO, NY) at 37 °C under a 95% air, 5% CO_2 atmosphere during 24 h. The following treatments of hormones and their antagonists were applied: (1) vehicle (0.02% cyclodextrin in sterile water), 72 h; (2) vehicle, 48 h followed by estradiol (10 nM), 24 h; (3) vehicle, 24 h followed by progesterone (10 nM), 48 h; (4) estradiol, 24 h followed by progesterone, 48 h; (5) estradiol, 24 h followed by progesterone + RU 486 (10 μM), 48 h; (6) vehicle, 48 h followed by ICI 182, 780 (2 μM), 24 h; (7) vehicle, 48 h followed by estradiol + ICI 182,780, 24 h. Each experiment was performed in four independent cultures, each one by duplicate. Cyclodextrin, progesterone, estradiol and RU 486 were purchased from Sigma–Aldrich (St. Louis, MO). ICI 182, 780 was from Tocris Cookson Inc. (Ellisville, MO).

2.2. Protein extraction and Western blotting

After all treatments U373, D54 and T47D cells were homogenized in TDG lysis buffer with protease inhibitors (10 mM Tris–HCl, 1 mM dithiothreitol, 30% glycerol, 1% Triton X-100, 15 mM sodium azide, 1 mM EDTA, 4 μg/ml leupeptin, 22 μg/ml aprotinin, 1 mM PMSF). Proteins were obtained by centrifugation at 20,000 × *g*, at 4 °C for 15 min, and quantified by the method of Bradford (Bio-Rad Laboratories, Hercules, CA). Proteins (70 μg) were separated by electrophoresis on 10% SDS-PAGE at 20 mA. Colored and enhanced chemiluminescence markers (Bio Rad, CA, USA and Gibco-BRL, MD) were included for size determination. Gels were transferred 2 h to nitrocellulose membranes (Amersham, NJ, USA) (60 mA, at room temperature in semi dry conditions), which were blocked at room temperature with 3% non-fat dry milk and 1% bovine serum albumin for 2 h. Membranes were then incubated with 2 μg/ml of mouse-anti-PR polyclonal antibody (NeoMarkers RB-1492-P, Fremont, CA), which recognizes both PR isoforms (PR-A and PR-B), at 4 °C overnight. Blots were then incubated with secondary antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) for 45 min. Signals were detected by enhanced chemiluminescence (ECL) (Amersham, NJ).

To correct for differences in the amount of total protein loaded in each lane, PR protein content was normalized to that of α-tubulin. Blots were stripped with glycine (0.1 M, pH 2.5, 0.5% SDS) at 4 °C overnight and at 37 °C for 30 min, and reproved with a 1:10,000 dilution mouse anti-α-tubulin monoclonal antibody (Sigma T9026, St. Louis, MO) at room temperature for 2 h. Blots were incubated with a 1:3000 dilution goat anti-mouse IgG conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) for 45 min at room temperature. Signals were detected by ECL. The intensity of PR isoforms and α-tubulin signals was quantified by densitometry using Scan Primax 600p apparatus (CO, Utrecht, the Netherlands) and the Scion Image software (Scion Corp., MD).

2.3. Human PR-A transfection in U373 cells

5×10^5 U373 cells were plated on a 10 cm culture dish with 10 ml of DMEM red phenol free medium supplemented with 10% FBS. At a 60% of confluency cells were transiently transfected with 6 μg of plasmid containing the human PR-A using FuGENE (Roche, Indianapolis, IN) following the manufacturer's protocol. PR-A cDNA was inserted in pLEN [30]. After 5 days of transfection a Western blot was performed to determine PR-A overexpression in U373 cells.

2.4. Cell growth

U373 cells were harvested from incubation every day during 6 consecutive days with PBS 1X + EDTA (1 mM). Then, they were centrifuged (3000 rpm/1 min) and the pellet was resuspended in PBS 1X + 10 μM of trypan blue. The number of living cells was measured by trypan blue dye exclusion using an inverted microscope (Olympus CKX41, Center Valley, PA).

2.5. Treatment of U373 transfected cells

U373 transfected cells were plated on 24-well cell culture cluster in 1 ml of DMEM red phenol free medium supplemented with 10% FBS at a cell density of 20×10^3 cells for 24 h. Medium was changed by DMEM red phenol free medium supplemented with SFB 10% without steroid hormones, 1 mM pyruvate, 2 mM glutamine, 0.1 mM non-essential amino at 37 °C under a 95% air, 5% CO_2 atmosphere during 24 h. Then, 10 nM of progesterone or hormone vehicle (0.02% cyclodextrin in sterile water) was added to the culture (day 0). Each experiment was performed in three independent cultures, each one by duplicate, during 6 consecutive days.

2.6. Statistical analysis

Data from protein content densitometry were analysed by using Mann–Whitney test. Prism 2.01 program (Graph Pad, CA) was used for calculating probability values.

3. Results

3.1. Basal expression of PR isoforms in human astrocytoma cell lines

We observed that PR isoforms were detected in human astrocytoma cell lines (U373 and D54) and T47D breast cancer cell line (as a positive control) by Western blot (Fig. 1). In all experiments PR-A and PR-B were detected as bands of 94 and 114 kDa, respectively.

3.2. Regulation of PR isoforms in U373 and D54 cell lines

In U373 cell line PR-B was the predominant isoform (PR-B:PR-A ratio 3:1) (Fig. 2, Western blot) whereas in D54 cells PR-A was the predominant one (PR-B:PR-A ratio 0.66:1) (Fig. 3, Western blot). In U373 cells PR isoforms content was increased by estradiol (Fig. 2), whereas in D54 cells estradiol had no significant effects (Fig. 3). In both cell lines progesterone alone did not modify PR isoforms content but this was down-regulated when progesterone was administered after estradiol treatment (Figs. 2 and 3). PR-A isoform was more sensitive to estradiol + progesterone treatment than PR-B since a greater diminution was observed in PR-A content after estradiol + progesterone treatment (Figs. 2 and 3). PR down-regulation was blocked with PR antagonist, RU 486, in U373 and D54 cells (Figs. 2 and 3).

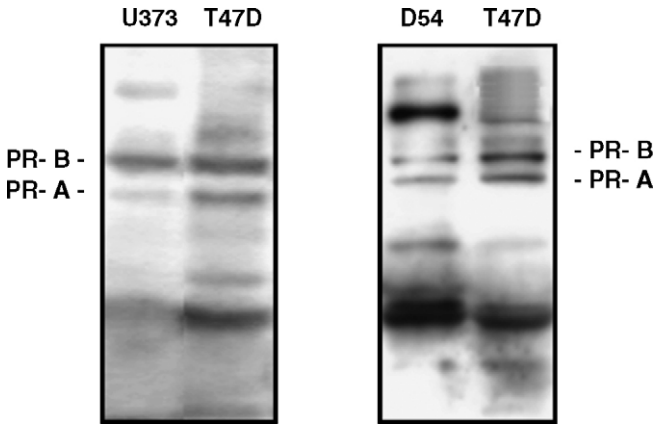

Fig. 1. PR isoforms expression in U373 and D54 human astrocytoma cell lines. D54, U373 and T47D cells were lysed and proteins (70 μg) were separated by electrophoresis on 10% SDS-PAGE. Gels were transferred to nitrocellulose membranes and then incubated with antibodies for PR as described in Section 2. The protein–antibody complexes were detected by ECL.

The ER antagonist, ICI 182,780 did not significantly modify the content of both PR isoforms in U373 cells (Fig. 2) but blocked the increase in PR isoforms content induced by estradiol. In D54 cell line ICI 182,780 administered alone diminished PR-A content (Fig. 3).

3.3. Effect of PR-A transfection on cell growth

We evaluated the effect of PR-A overexpression on cell growth of U373 cells which endogenously express a low amount of PR-A. Therefore, we transfected PR-A to overexpress this PR isoform. We observed an increase of 60% in PR-A content in transfected cells without changes in PR-B (Fig. 4, *upper panel*). A time-course study over a 6-day period with 10 nM of progesterone was performed in

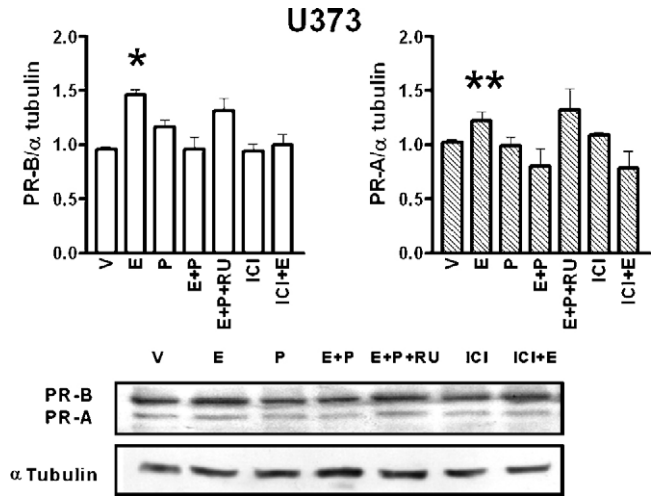

Fig. 2. Regulation of PR isoforms content in U373 human astrocytoma cancer cell line. U373 cells were treated with (1) vehicle (V) (0.02% cyclodextrin in sterile water), 72 h; (2) V, 48 h followed by estradiol (E) (10 nM), 24 h; (3) V, 24 h followed by progesterone (P) (10 nM), 48 h; (4) E, 24 h followed by P, 48 h (E + P); (5) E, 24 h followed by P + RU486 (10 μM), 48 h (E + P + RU); (6) V, 48 h followed by ICI 182, 780 (2 μM), 24 h (ICI); (7) V, 48 h followed by E + ICI 182,780, 24 h (ICI + E). Cells were lysed and proteins (70 μg) were separated by electrophoresis on 10% SDS-PAGE. Gels were transferred to nitrocellulose membranes and then incubated with PR or α-tubulin antibodies as described in Section 2. The protein–antibody complexes were detected by ECL. Proteins detected by Western blot were quantified by densitometric analysis and corrected using data of α-tubulin protein content. The ratio receptor/α-tubulin of V represents the value of 1. A representative assay of four Western blot experiments is shown. Results are expressed as mean ± S.E.M. $n = 4$. $^{*}p < 0.05$ vs. all groups except E + P + RU; $^{**}p < 0.05$ vs. V, P, E + P, ICI + E.

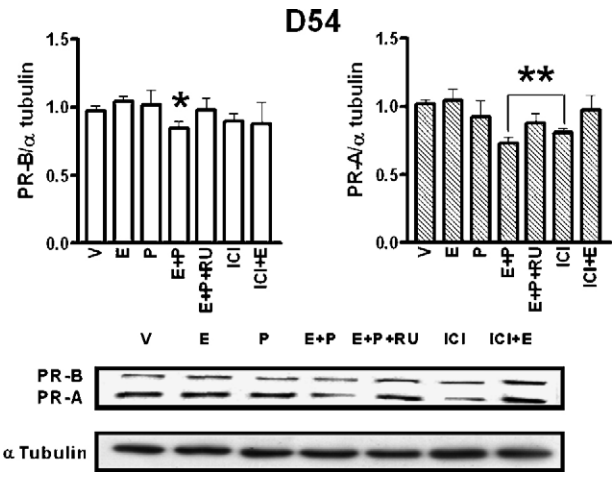

Fig. 3. Regulation of PR isoforms content in D54 human astrocytoma cancer cell line. D54 cells were treated as described in Fig. 2. Cells were lysed and proteins (70 μg) were separated by electrophoresis on 10% SDS-PAGE. Gels were transferred to nitrocellulose membranes and then incubated with PR or α-tubulin antibodies as described in Section 2. Western blot analysis was performed as described in Fig. 2. A representative assay of four Western blot experiments is shown. Results are expressed as mean ± S.E.M. $n = 4$. $^{*}p < 0.05$ vs. E; $^{**}p < 0.05$ vs. V and E.

U373 cells. As it has been reported by our laboratory in a previous work [22] progesterone increased the number of cells from the third day until the sixth day of treatment (Fig. 4 compares controls between left and right sides of *lower panel*). Interestingly, in U373 cells that overexpress PR-A, the treatment, with progesterone did not induce the increase in cell growth observed from day 4 to day 6 of treatment in wild type cells (Fig. 4, *right panel*).

4. Discussion

Our results demonstrate that PR content is differentially regulated by estradiol and progesterone in U373 cells and D54 cells. In many cell types, PR expression is up-regulated by estradiol at transcriptional level mediated by estrogen-responsive elements located in the PR promoter [10,25]. We found that PR content increased after estradiol treatment in U373 cells but not in D54 cells. The loss of PR regulation by estradiol in D54 cells could be related to the series of changes in the control of metabolic and genetic activities that occur in the more advanced grades of human astrocytomas evolution. Interestingly, in ovarian carcinoma cells the treatment with estradiol (10 nM) induced a decrease of PR-A and PR-B mRNA and protein levels [31].

In rat uterus the activation of ER-β down-regulates both PR isoforms in epithelial cells while ER-α up-regulates PR in the stroma and myometrium [32]. In U373 cells PR isoforms were up-regulated by estradiol and this effect was blocked with ER antagonist, ICI 182,780 (Fig. 2) suggesting that PR regulation by estradiol depends on ER activation. In D54 cells the mechanisms involved in the lack of PR up-regulation by estradiol are not yet known. Differences in ER isoforms content in U373 and D54 cells could be related with the differential PR isoforms regulation by estradiol in both cell lines.

It is known that PR is down-regulated by progesterone after estradiol treatment in different cells and tissues [23,24]. PR down-regulation by their own ligand (progesterone) is associated with ligand-dependent proteolysis [26]; progesterone induces receptor phosphorylation, which signals PR to degradation by the ubiquitin–proteasome pathway [27–29]. In line with these results we observed that in both cell lines, PR isoforms content was down-regulated by progesterone after estradiol treatment and this PR down-regulation was blocked with the PR antagonist, RU 486 (Figs. 2 and 3), suggesting an effect mediated by PR.

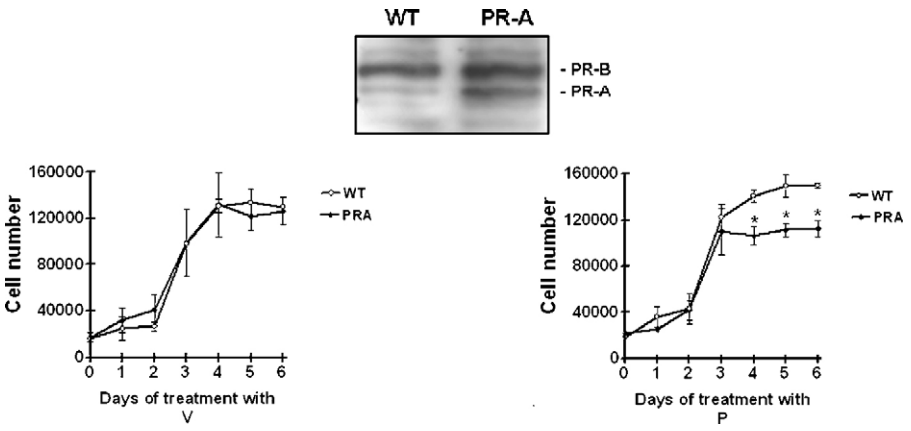

Fig. 4. Effects of PR-A transfection on U373 cell growth. *Upper panel*, U373 wild type (WT) and U373 cells transfected with PR-A (PR-A) were lysed, proteins (70 μg) were separated by electrophoresis, and gels were electrotransferred for Western blot detection of both PR isoforms (PR-A and PR-B). *Lower panel*, U373 cells WT (○) or transfected with PR-A (♦) were treated with hormone vehicle (0.02% cyclodextrin) (*left*) or with 10 nM of P (*right*) (day 0). Each experiment was performed in three independent cultures, each one by duplicate, during 6 days. Every day cells were removed from incubation and the number of cells was measured by trypan blue dye exclusion. Data are mean ± E.S. $^{*}p < 0.05$ vs. wild type.

PR isoforms have been detected in several human brain tumours such as meningiomas, chordomas, craniopharyngiomas and astrocytomas [15–20]. PR isoforms have also been recently detected in human astrocytoma cell lines (U373 and D54) [22]. PR isoforms are differentially expressed in human astrocytomas, González-Agüero et al. [20] observed that the PR predominant isoform expressed in astrocytomas grades III and IV was PR-B. In this work we observed that in U373 cell line PR-B was the predominant isoform (PR-B:PR-A ratio 3:1) (Fig. 2, Western blot) whereas in D54 cells PR-A was the predominant one (PR-B:PR-A ratio 0.66:1) (Fig. 3, Western blot).

PR-A and PR-B exhibit different activities in vitro; PR-B is a much stronger activator than PR-A and this one exhibits a dominant negative inhibitory effect on the activity of PR-B [12,13,33]. We observed that overexpression of PR-A significantly diminished the cell number of U373 cells treated with progesterone, suggesting that PR-A has an inhibitory effect on cell growth when it is activated by its ligand (Fig. 4). In human breast cancer cells (T47D) with overexpression of PR-A, McGowan and Clarke [34] observed marked changes in cell morphology, consistent with loss of adherent properties, suggesting PR-A participation in metastasis. Studies in MCF-7 breast cancer cells showed PR-B participation in the reinitiation of cell cycle progression induced by progesterone [35]. All these differences in PR isoforms ratio have been associated with a metastatic state of breast cancer cells. Thus, the activation of PR-A or PR-B may exert different effects on tumour progression and metastasis.

Differences in PR regulation are important because progesterone can exert different functions in a cell, depending on the expression pattern of their isoforms [9,14]. In a recent work we have demonstrated that progesterone induces cell growth of U373 and D54 cell lines, through the interaction with PR [22]. Indeed, PR isoforms can regulate different genes in the same tissue. Thus, modifications in PR regulation could be related with tumour progression or metastasis.

In conclusion, our data indicate that PR isoforms regulation depends on human astrocytomas evolution grade and that PR-A inhibits the effects of progesterone on astrocytomas cell growth.

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References

[1] C. Daumas-Duport, B. Scheithauer, J. O'Fallon, P. Kelly, Grading of astrocytomas—a simple and reproducible method, Cancer 62 (1988) 2152–2165.
[2] U.M. Schrell, E.F. Adams, R. Fahlbusch, R. Greb, G. Jirikowski, R. Prior, F.J. Ramalho-Ortigao, Hormonal dependency of cerebral meningiomas. Part 1. Female sex steroid receptors and their significance as specific markers for adjuvant medical therapy, J. Neurosurg. 73 (1990) 743–749.
[3] S.M. Grunberg, M.H. Weiss, I.M. Spitz, J. Ahmadi, A. Sadun, C.A. Russell, L. Lucci, L.L. Stevenson, Treatment of unresectable meningiomas with the antiprogesterone agent mifepristone, J. Neurosurg. 74 (1991) 861–866.
[4] J.E. Levine, P.E. Chappell, J.S. Schneider, N.C. Sleiter, M. Szabo, Progesterone receptors as neuroendocrine integrators, Front. Neuroendocrinol. 22 (2001) 69–106.
[5] M. Schumacher, H. Coirini, F. Robert, R. Guennoun, M. El-Etr, Genomic and membrane actions of progesterone: implications for reproductive physiology and behavior, Behav. Brain Res. 105 (1999) 37–52.
[6] O. Villamar-Cruz, J. Manjarrez-Marmolejo, R. Alvarado, I. Camacho-Arroyo, Regulation of the content of progesterone and estrogen receptors, and their cofactors src-1 and smrt by the 26s proteasome in the rat brain during the estrous cycle, Brain Res. Bull. 69 (2006) 276–281.
[7] I. Camacho-Arroyo, A. González-Arenas, G. González-Moran, Ontogenic variations in the content and distribution of progesterone receptor isoforms in the reproductive tract and brain of chicks, Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 146 (2007) 644–652.
[8] B.M. Jacobsen, S.A. Schittone, J.K. Richer, K.B. Horwitz, Progesterone-independent effects of human progesterone receptors (PRs) in estrogen receptor-positive breast cancer: PR isoform-specific gene regulation and tumor biology, Mol. Endocrinol. 19 (2005) 574–587.
[9] B. Mulac-Jericevic, R.A. Mullinax, F.J. DeMayo, J.P. Lydon, O.M. Conneely, Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform, Science 289 (2000) 1751–1754.
[10] P. Kastner, A. Krust, B. Turcotte, U. Stropp, L. Tora, H. Gronemeyer, P. Chambon, Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B, EMBO J. 9 (1990) 1603–1614.
[11] W.L. Kraus, M.M. Montano, B.S. Katzenellenbogen, Cloning of the rat progesterone receptor gene 5′-region and identification of two functionally distinct promoters, Mol. Endocrinol. 7 (1993) 1603–1616.
[12] P.H. Giangrande, D.P. McDonnell, The A and B isoforms of the human progesterone receptor: two functionally different transcription factors encoded by a single gene, Recent Prog. Horm. Res. 54 (1999) 291–313.
[13] S.A. Leonhardt, V. Boonyaratanakornkit, D.P. Edwards, Progesterone receptor transcription and non-transcription signaling mechanisms, Steroids 68 (2003) 761–770.
[14] J.K. Richer, B.M. Jacobsen, N.G. Manning, M.G. Abel, D.M. Wolf, K.B. Horwitz, Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells, J. Biol. Chem. 277 (2002) 5209–5218.
[15] C. Bozzetti, R. Camisa, R. Nizzoli, L. Manotti, A. Guazzi, N. Naldi, S. Mazza, V. Nizzoli, G. Cocconi, Estrogen and progesterone receptors in human meningiomas: biochemical and immunocytochemical evaluation, Surg. Neurol. 43 (1995) 230–233.

[16] I. Camacho-Arroyo, G. González-Agüero, A. Gamboa-Dominguez, M.A. Cerbon, R. Ondarza, Progesterone receptor isoforms expression pattern in human chordomas, J. Neurooncol. 49 (2000) 1–7.
[17] R.S. Carroll, J. Zhang, K. Dashner, M. Sar, P.M. Black, Steroid hormone receptors in astrocytic neoplasms, Neurosurgery 37 (1995) 496–503.
[18] J. Honegger, C. Renner, R. Fahlbusch, E.F. Adams, Progesterone receptor gene expression in craniopharyngiomas and evidence for biological activity, Neurosurgery 41 (1997) 1354–1363.
[19] A. Omulecka, W. Papierz, A. Nawrocka-Kunecka, I. Lewy-Trenda, Immunohistochemical expression of progesterone and estrogen receptors in meningiomas, Folia Neuropathol. 44 (2006) 111–115.
[20] G. González-Agüero, R. Ondarza, A. Gamboa-Dominguez, M.A. Cerbon, I. Camacho-Arroyo, Progesterone receptor isoforms expression pattern in human astrocytomas, Brain Res. Bull. 56 (2001) 43–48.
[21] J. Halper, D.S. Colvard, B.W. Scheithauer, N.S. Jiang, M.F. Press, M.L. Graham 2nd, E. Riehl, E.R. Laws Jr., T.C. Spelsberg, Estrogen and progesterone receptors in meningiomas: comparison of nuclear binding, dextran-coated charcoal, and immunoperoxidase staining assays, Neurosurgery 25 (1989) 546–552.
[22] G. González-Agüero, A.A. Gutiérrez, D. González-Espinosa, J.D. Solano, R. Morales, A. González-Arenas, E. Cabrera-Muñoz, I. Camacho-Arroyo, Progesterone effects on cell growth of U373 and D54 human astrocytoma cell lines, Endocrine 32 (2007) 129–135.
[23] I. Camacho-Arroyo, C. Guerra-Araiza, M.A. Cerbon, Progesterone receptor isoforms are differentially regulated by sex steroids in the rat forebrain, Neuroreport 9 (1998) 3993–3996.
[24] C. Guerra-Araiza, O. Villamar-Cruz, A. González-Arenas, R. Chavira, I. Camacho-Arroyo, Changes in progesterone receptor isoforms content in the rat brain during the oestrous cycle and after oestradiol and progesterone treatments, J. Neuroendocrinol. 15 (2003) 984–990.
[25] W.L. Kraus, M.M. Montano, B.S. Katzenellenbogen, Identification of multiple, widely spaced estrogen-responsive regions in the rat progesterone receptor gene, Mol. Endocrinol. 8 (1994) 952–969.
[26] C.A. Lange, T. Shen, K.B. Horwitz, Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26s proteasome, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 1032–1037.
[27] J.L. Turgeon, D.W. Waring, Progesterone regulation of the progesterone receptor in rat gonadotropes, Endocrinology 141 (2000) 3422–3429.
[28] N.L. Weigel, Steroid hormone receptors and their regulation by phosphorylation, Biochem. J. 319 (Pt 3) (1996) 657–667.
[29] I. Camacho-Arroyo, O. Villamar-Cruz, A. González-Arenas, C. Guerra-Araiza, Participation of the 26s proteasome in the regulation of progesterone receptor concentrations in the rat brain, Neuroendocrinology 76 (2002) 267–271.
[30] R. García-Becerra, E. Borja-Cacho, A.J. Cooney, K.J. Jackson, A.E. Lemus, G. Pérez-Palacios, F. Larrea, The intrinsic transcriptional estrogenic activity of a nonphenolic derivative of levonorgestrel is mediated via the estrogen receptor-alpha, J. Steroid Biochem. Mol. Biol. 82 (2002) 333–341.
[31] K. Mukherjee, V. Syed, S.M. Ho, Estrogen-induced loss of progesterone receptor expression in normal and malignant ovarian surface epithelial cells, Oncogene 24 (2005) 4388–4400.
[32] L. Sahlin, B. Masironi, S. Akerberg, H. Eriksson, Tissue- and hormone-dependent progesterone receptor distribution in the rat uterus, Reprod. Biol. Endocrinol. 4 (2006) 47.
[33] A.R. Hovland, R.L. Powell, G.S. Takimoto, L. Tung, K.B. Horwitz, An N-terminal inhibitory function, if, suppresses transcription by the A-isoform but not the B-isoform of human progesterone receptors, J. Biol. Chem. 273 (1998) 5455–5460.
[34] E.M. McGowan, C.L. Clarke, Effect of overexpression of progesterone receptor a on endogenous progestin-sensitive endpoints in breast cancer cells, Mol. Endocrinol. 13 (1999) 1657–1671.
[35] E.M. McGowan, A.J. Russell, V. Boonyaratanakornkit, D.N. Saunders, G.M. Lehrbach, C.M. Sergio, E.A. Musgrove, D.P. Edwards, R.L. Sutherland, Progestins reinitiate cell cycle progression in antiestrogen-arrested breast cancer cells through the B-isoform of progesterone receptor, Cancer Res. 67 (2007) 8942–8951.