REVISTA MEXICANA DE ENDOCRINOLOGÍA, METABOLISMO & NUTRICIÓN REVIEW ARTICLE

# Effects of progesterone on human astrocytoma progression

*Liliana Germán-Castelán and Ignacio Camacho-Arroyo**

Unit for Investigation in Human Reproduction, Instituto Nacional de Perinatología-Facultad de Química, Universidad Nacional Autónoma de México, Mexico, D.F., Mexico

## ABSTRACT

Gliomas are the most frequent primary brain tumors in the world population. Astrocytomas are the most common type of glioma, representing 76% of these tumors, and according to the World Health Organization they are classified in four grades (I-IV) based on their histological characteristics, with grade IV tumors being the most aggressive ones. Progesterone (P) promotes cell proliferation, migration, and invasion in human astrocytomas through the interaction with its intracellular receptor (PR), which is a ligand-activated transcription factor involved in the regulation of genes that control cell cycle and metastasis. In astrocytomas, PR expression is directly related with the evolution grade of the tumor, suggesting that PR tumors exhibit a strong oncogenic potential. In this paper, we present an overview about the role of P in the development and progression of human astrocytomas. (REV MEX ENDOCRINOL METAB NUTR. 2015;2:104-8)

*Corresponding author: Ignacio Camacho-Arroyo, camachoarroyo@gmail.com*

**Key words:** Glioma. Astrocytoma. Progesterone. Progesterone receptor.

## RESUMEN

Los gliomas son los tumores cerebrales primarios más frecuentes en la población mundial. Los astrocitomas son el tipo más común de glioma, constituyen el 76% de estos tumores y la Organización Mundial de la Salud los ha clasificado en 4 grados (I-IV) de acuerdo a sus características histológicas, siendo los de grado IV los más agresivos. Se ha informado que la progesterona (P) promueve la proliferación, migración e invasión celulares en los astrocitomas humanos a través de la interacción con su receptor intracelular (RP), el cual es un factor de transcripción activado por su ligando que está involucrado en la regulación de genes relacionados con el control del ciclo celular y la metástasis. La expresión del RP en los astrocitomas está directamente relacionada con el grado de evolución del tumor, lo que sugiere que los tumores que expresan al RP presentan un fuerte potencial oncogénico. En esta revisión se presenta un panorama general acerca del papel de la P en el desarrollo y progresión de los astrocitomas humanos.

**Palabras clave:** Gliomas. Astrocitomas. Progesterona. Receptor a progesterona.

**Correspondence to:**
*Ignacio Camacho-Arroyo
Unidad de Investigación en Reproducción Humana
Instituto Nacional de Perinatología-Facultad de Química
Universidad Nacional Autónoma de México
Av. Universidad, 3000
Ciudad Universitaria, Del. Coyoacán, C.P. 04510, México, D.F., México
E-mail: camachoarroyo@gmail.com

Received for publication: 01-02-2015
Accepted for publication: 05-03-2015

## INTRODUCTION

Progesterone (P) participates in the regulation of several physiological and pathological processes in numerous tissues. This hormone is involved in the growth of brain tumors such as astrocytomas, which are the most common and malignant brain tumors. Progesterone exerts its effects through the interaction with progesterone receptor (PR), which is a ligand-activated transcription factor. The activity of PR is fundamental in the development, growth, and infiltration of these tumors. Two main isoforms of PR have been reported, PR-A and PR-B, each one with different regulation, function, and expression pattern. It has been reported that the expression ratio PR-A:PR-B is determinant in P actions in astrocytoma growth.

## ASTROCYTOMA

Astrocytomas arise from astrocytes, glial progenitor cells, or cancer stem cells[1-5] and are the most common and aggressive primary intracerebral tumor[6]. The World Health Organization has classified them in four grades according to their histological characteristics such as mitotic activity, nuclear atypia, cellularity, and necrosis[7,8]. Pilocytic astrocytoma (grade I) is a well-circumscribed, slow-growing tumor, with minimal variation in shape and size of the nuclei and with the possibility of surgical resection; overall survival usually exceeds 20 years[9,10]. Diffuse astrocytoma (grade II) presents infiltration, low proliferative activity, and it tends to progress to higher grades of malignancy; median overall survival ranges from 6-8 years[11]. Anaplastic astrocytoma (grade III) is characterized by high mitotic activity, nuclear atypia, and infiltrative lesions; survival of patients ranges from 2-3 years[10]. Glioblastoma (grade IV) is the most common and malignant subtype and it exhibits more advanced features of malignancy, including high mitotic activity, neovascular proliferation, infiltrative lesions, and necrosis; overall survival is less than a year[10,11]. Treatment depends on size, localization, extent of resection, and evolution grade. However, present medical treatments such as neurosurgery, radiotherapy and chemotherapy only achieve a modest improvement in patient survival[12-14]. In adults, the incidence of these tumors is 50% higher in men than in women[15], suggesting the involvement of a hormonal component in its development. Sex hormones, particularly P, are involved in the development and progression of astrocytomas, and therefore, they could be useful in the search for therapeutic alternatives against these tumors.

## PROGESTERONE

Progesterone is a steroid hormone derived from cholesterol that participates in the regulation of several physiological and pathological processes in different tissues, such as sexual behavior, brain activity, pregnancy, cell proliferation, and cancer[16-20]. It mainly elicits its effects through the interaction with PR, which is a ligand-activated transcription factor that regulates the expression of several genes involved in metabolism, development, and reproduction[18]. There are two mechanisms through which P acts, called classical and non-classical. The first one involves the interaction of P with PR, which is located in cytoplasm or nucleus. At basal state, PR is associated with heat shock proteins (HSP), and once the hormone enters the cell it interacts with PR and induces conformational changes that allow the dissociation from HSP followed by phosphorylation and dimerization of PR. The activated receptor possesses high affinity for specific sequences in the DNA, known as P response elements, and once bound it regulates the transcription of target genes through the recruitment of co-regulator proteins (co-activators and co-repressors)[21,22]. The second mechanism requires the interaction of P with membrane receptors[23], which activates intracellular signaling pathways through induction of second messenger cascades[24], changes in ionic conductance, and in protein phosphorylation[25].

There are two main isoforms of PR: PR-A (94 kDa) and PR-B (114 kDa). They are encoded by the same gene, but their expression is regulated by different promoters. PR isoforms have diverse functions depending on the type of cell, the target gene, and the promoter[21,26]. Generally, PR-B is a potent transcriptional activator, while PR-A acts as a repressor of transcription mediated by PR-B or by other steroid hormone

receptors[27-29]. This is because, generally, PR-A has higher affinity for co-repressors such as SMRT (silencing mediator for retinoid and thyroid hormone receptor) and PR-B for co-activators such as SRC-1 (steroid receptor co-activator-1)[30].

## PROGESTERONE AND ASTROCYTOMAS

Progesterone has different effects in numerous types of cancer[18-20,31,32]. It has been reported that P administration significantly increases proliferation in U373 and D54 cell lines (derived from human astrocytomas grades III and IV, respectively). In a time-course study using different doses of P (1 nM to 10 µM) it was found that P (10 nM) increased cell number from the second day in D54 cells and from the third in U373 cells, and that this effect persisted until the fifth day of culture[20]. It is important to note that this concentration of P is reached in the luteal phase of the female menstrual cycle[33]. Treatment with P antagonist, mifepristone (RU486) 10 µM co-administered with P blocked the effect of the hormone on days 2 and 4 in both D54 and U373 cells by 42 and 25%, respectively, suggesting that P effects occur through the interaction with PR.

In U373 cell line, PR-B is the predominant isoform (PR-B:PR-A ratio 3:1), whereas in D54 cells, PR-A is the predominant one (ratio 0.66:1.0). It has been reported that the overexpression of PR-A in U373 cells significantly reduces proliferation in these cells when treated with P, suggesting that in this cell line, PR-A has an inhibitory effect on proliferation when PR is activated by its ligand[34].

Several studies have shown that PR expression is regulated by P and estradiol (E2). In U373 cells, PR isoform content increases after treatment with E2 (10 nM), whereas in D54 cells E2 had no significant effects[34]. This could be related with the genetic and metabolic changes that occur in the more advanced grade of malignancy of astrocytomas. Meanwhile, P acts as a down-regulator of PR through a mechanism of ligand-dependent proteolysis[35], since when P binds its receptor it induces its phosphorylation, which signals PR to degradation by the ubiquitin-proteasome pathway[36-38].

It was found that P increased the S phase of cell cycle in U373 cells on day 5[20], which is correlated with the induction of genes associated with cell cycle progression, such as cyclin D1[39]. The same time-course study was performed with the U87 cell line (derived from grade IV astrocytoma) and it was observed that P (10 nM) significantly increased cell number from day 4 to 6 of culture and that co-administration with RU486 (10 µM) blocked P effects on these days (unpublished data).

Similar results were observed in an *in vivo* model where U373 cells were implanted in the cerebral motor cortex of male rats and the effects of P were studied under two treatment schemes. In the first one, 15 days after U373 cell implantation, rats were treated daily subcutaneously with vehicle (propylene glycol), P (1 mg), RU486 (5 mg) or P plus RU486 (1 and 5 mg, respectively) for 21 days. In the second schedule, treatments started eight weeks after cells implantation and lasted 14 days. In both schemes, it was observed that P significantly increased both the area and length of infiltration of the tumors compared to all other treatments and that RU486 blocked the effects of P. Similarly, it was observed that all rats treated with P showed tumor infiltration, while 29 and 43% of the animals treated with RU486 and P plus RU486, respectively, presented it[40].

Also, in another study using an *in vivo* model, U87 cells were implanted in the cerebral motor cortex of male rats. Eight weeks after cell implantation, rats were daily administered intracerebrally with sense or antisense oligodeoxynucleotides (ODN) in order to silence PR expression. The subcutaneous administration of vehicle (propylene glycol) or P (1 mg) started one day after the beginning of ODN administration. The ODN and hormone treatments lasted 15 and 14 days, respectively. Similarly to the results described above in U373 cells, we observed that P increased the area and length of infiltration of the tumors, and that these effects were blocked by the administration of antisense ODN (unpublished data). These results support the proposal that P effects on human astrocytomas occur through PR.

Additionally, recent studies by our lab have shown that P significantly increases migration and invasion in D54 cells and that RU486 administration blocks these effects.

Table 1. Effects of progesterone on human astrocytoma cell lines

| | U373 cells | D54 cells | U87 cells | Reference |
|---|---|---|---|---|
| Cell number | Increase | Increase | Increase | González-Agüero, et al.[20], U.D. |
| Cell proliferation *(in vivo)* | Increase | Not studied | Increase | Germán-Castelán, et al.[40], U.D. |
| Cell infiltration *(in vivo)* | Increase | Not studied | Increase | |
| PR regulation | Down- regulation of PR-A and PR-B (after E2 treatment) | Down-regulation of PR-A and PR-B (after E2 treatment) | Not studied | Camacho-Arroyo, et al.[31] |
| Gene expression: | | | | |
| Cyclin D1 | Increase | No effect | Not studied | Hernández-Hernández, et al.[39,49] |
| VEGF | No effect | Increase | Not studied | |
| EGFR | No effect | Increase | Not studied | |
| SRC-1 | No effect | Increase | Not studied | Hernández-Hernández, et al.[50] |
| GLIPR2 | Increase (+ RU486) | Not studied | Not studied | González-Arenas, et al.[48] |
| IL7R | Decrease (+ RU486) | Not studied | Not studied | |
| AOC3 | Increase | Not studied | Not studied | |

U.D.: unpublished data

Recently, in order to analyze the role of P and RU486 in gene regulation and to identify some mediators of P effects, a microarray study was performed in our lab in U373 cells. It was found that these steroids regulate genes that encode for proteins involved in metabolism, transport, cell cycle, proliferation, metastasis, apoptosis, processing of nucleic acids and proteins, adhesion, pathogenesis, immune response, cytoskeleton and membrane receptors. Specifically for P, it was found that after a 12-hour treatment, this hormone regulates, positively or negatively, the expression of various genes involved in proliferation, adhesion, metabolism, and immunological processes that may play an important role in the development and progression of tumors. Some of the genes whose expression was altered by P have already been described in other types of cancer[41-43], however, new P-regulated genes were also identified, such as GLIPR2 which can induce epithelial mesenchymal transition and cancer progression[44,45], IL7R, that has been associated with decreased immune response responsible for preventing the development of tumor in high-grade gliomas[46], and AOC3, which has been involved in cell migration and extravasation induced by inflammatory processes[47]. In total, it was determined that 30 genes were regulated by P, 41 by RU486, and 13 by the co-treatment P plus RU486, suggesting that there are many genes regulated by intracellular PR or through other signaling pathways modulated by P that can modify astrocytoma development and growth[48]. Some growth factors and their receptors have also been proposed as mediators of P effects. The mRNA and protein expression of vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) were increased by P in astrocytoma cells, and this increase was blocked by RU486[39]. A summary of the actions mediated by P in astrocytoma cell lines is presented in table 1.

## CONCLUSIONS

Progesterone increases proliferation, migration, invasion, and infiltration in different human astrocytoma cell lines, and this occurs through its interaction with its intracellular receptor, which involves changes in the expression of genes involved in cell-cycle regulation, proliferation, angiogenesis, metabolism, metastasis, etc.

Astrocytomas are the most frequent primary brain tumors and constitute a leading cause of brain cancer-related deaths. Both P and PR influence the development and growth of this type of cancer, and thus, the understanding of the hormonal dependence of this condition can provide tools for the development of new therapies against astrocytomas progression, one of which could be based in the use of anti-progestins such as RU486, since we have observed that it can efficiently block P effects both *in vitro* and *in vivo*.

## AKNOWLEDGEMENTS

This work was supported by grant IN201414 from PAPIIT-DGAPA, UNAM.

## REFERENCES

1. Friedmann-Morvinski D, Bushong EA, Ke E, et al. Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. Science. 2012;338:1080-4.
2. Alcantara Llaguno S, Chen J, Kwon C-H, et al. Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. Cancer Cell. 2009;15:45-56.
3. Cheng L, Bao S, Rich JN. Potential therapeutic implications of cancer stem cells in glioblastoma. Biochem Pharmacol. 2010;80:654-65.
4. Schonberg DL, Lubelski D, Miller TE, Rich JN. Brain tumor stem cells: Molecular characteristics and their impact on therapy. Mol Aspects Med. 2014;39:82-101.
5. Cho DY, Lin SZ, Yang WK, et al. Targeting cancer stem cells for treatment of glioblastoma multiforme. Cell Transplant. 2013;22:731-9.
6. Agnihotri S, Burrell KE, Wolf A, et al. Glioblastoma, a brief review of history, molecular genetics, animal models and novel therapeutic strategies. Arch Immunol Ther Exp (Warsz). 2013;61:25-41.
7. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 2007;114:97-109.
8. Huse JT, Phillips HS, Brennan CW. Molecular subclassification of diffuse gliomas: seeing order in the chaos. Glia. 2011;59:1190-9.
9. Riemenschneider MJ, Reifenberger G. Astrocytic tumors. Recent Results. Cancer Res. 2009;171:3-24.
10. Tihan T, Bloomer MM. Astrocytic neoplasms of the central nervous system and orbit: a morphologic perspective. Semin Diagn Pathol. 2010;27:114-21.
11. Arko L, Katsyv I, Park GE, Luan WP, Park JK. Experimental approaches for the treatment of malignant gliomas. Pharmacol Ther. 2010;128:1-36.
12. Osoba D, Brada M, Yung WK, Prados MD. Health-related quality of life in patients with anaplastic astrocytoma during treatment with temozolomide. Eur J Cancer. 2000;36:1788-95.
13. Sahebjam S, McNamara M, Mason WP. Management of glioblastoma in the elderly. Clin Adv Hematol Oncol. 2012;10:379-86.
14. Anton K, Baehring JM, Mayer T. Glioblastoma multiforme: overview of current treatment and future perspectives. Hematol Oncol Clin North Am. 2012;26:825-53.
15. Kabat GC, Etgen AM, Rohan TE. Do steroid hormones play a role in the etiology of glioma? Cancer Epidemiol Biomarkers Prev. 2010;19:2421-7.
16. Brinton RD, Thompson RF, Foy MR, et al. Progesterone receptors: form and function in brain. Front Neuroendocrinol. 2008;29:313-39.
17. Cabrera-Muñoz E, Hernández-Hernández OT, Camacho-Arroyo I. Role of progesterone in human astrocytomas growth. Curr Top Med Chem. 2011;11:1663-7.
18. Graham JD, Clarke CL. Physiological action of progesterone in target tissues. Endocr Rev. 1997;18:502-19.
19. Kariagina A, Xie J, Langohr IM, Opreanu RC, Basson MD, Haslam SZ. Progesterone stimulates proliferation and promotes cytoplasmic localization of the cell cycle inhibitor p27 in steroid receptor positive breast cancers. Horm Cancer. 2013;4:381-90.
20. González-Agüero G, Gutiérrez AA, González-Espinosa D, et al. Progesterone effects on cell growth of U373 and D54 human astrocytoma cell lines. Endocrine. 2007;32:129-35.
21. Jacobsen BM, Horwitz KB. Progesterone receptors, their isoforms and progesterone regulated transcription. Mol Cell Endocrinol. 2012;357:18-29.
22. Edwards DP, Wardell SE, Boonyaratanakornkit V. Progesterone receptor interacting coregulatory proteins and cross talk with cell signaling pathways. J Steroid Biochem Mol Biol. 2002;83:173-86.
23. Burger K, Fahrenholz F, Gimpl G. Non-genomic effects of progesterone on the signaling function of G protein-coupled receptors. FEBS Lett. 1999;464:25-9.
24. Lösel R, Wehling M. Nongenomic actions of steroid hormones. Nat Rev Mol Cell Biol. 2005;4:46-56.
25. Blackmore PF. Rapid non-genomic actions of progesterone stimulate Ca2+ influx and the acrosome reaction in human sperm. Cell Signal. 1993;5:531-8.
26. Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM, Horwitz KB. Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. J Biol Chem. 2002;277:5209-18.
27. Tung L, Mohamed MK, Hoeffler JP, Takimoto GS, Horwitz KB. Antagonist-occupied human progesterone B-receptors activate transcription without binding to progesterone response elements and are dominantly inhibited by A-receptors. Mol Endocrinol. 1993;7:1256-65.
28. Vegeto E, Shahbaz MM, Wen DX, Goldman ME, O'Malley BW, McDonnell DP. Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. Mol Endocrinol. 1993;7:1244-55.
29. Conneely OM, Lydon JP. Progesterone receptors in reproduction: functional impact of the A and B isoforms. Steroids. 2000;65:571-7.
30. Giangrande PH, McDonnell DP. The A and B isoforms of the human progesterone receptor: two functionally different transcription factors encoded by a single gene. Recent Prog Horm Res. 1999;54:291-313; discussion 313-4.
31. Camacho-Arroyo I, Hansberg-Pastor V, Cabrera-Muñoz E, Hernández-Hernández OT, González-Arenas A. Role of progesterone receptor isoforms in human astrocytomas growth. In: Hayat MA (ed) Tumors Cent. Nerv. Syst. Vol. 5. Springer Netherlands. 2012;57-63.
32. Olson JJ, Beck DW, Schlechte J, Loh PM. Hormonal manipulation of meningiomas in vitro. J Neurosurg. 1986;65:99-107.
33. Stening K, Eriksson O, Wahren L, Berg G, Hammar M, Blomqvist A. Pain sensations to the cold pressor test in normally menstruating women: comparison with men and relation to menstrual phase and serum sex steroid levels. Am J Physiol Regul Integr Comp Physiol. 2007;293:R1711-6.
34. Cabrera-Muñoz E, González-Arenas A, Saqui-Salces M, et al. Regulation of progesterone receptor isoforms content in human astrocytoma cell lines. J Steroid Biochem Mol Biol. 2009;113:80-4.
35. Lange CA, Shen T, Horwitz KB. Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome. Proc Natl Acad Sci USA. 2000;97:1032-7.
36. Turgeon JL, Waring DW. Progesterone regulation of the progesterone receptor in rat gonadotropes. Endocrinology. 2000;141:3422-9.
37. Weigel NL. Steroid hormone receptors and their regulation by phosphorylation. Biochem J. 1996;319:657-67.
38. Camacho-Arroyo I, Villamar-Cruz O, González-Arenas A, Guerra-Araiza C. Participation of the 26S proteasome in the regulation of progesterone receptor concentrations in the rat brain. Neuroendocrinology. 2002;76:267-71.
39. Hernández-Hernández OT, González-García TK, 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. 2012;132:127-34.
40. Germán-Castelán L, Manjarrez-Marmolejo J, González-Arenas A, González-Morán MG, Camacho-Arroyo I. Progesterone induces the growth and infiltration of human astrocytoma cells implanted in the cerebral cortex of the rat. Biomed Res Int. 2014;2014:393174.
41. Friedl P, Wolf K. Tumour-cell invasion and migration: diversity and escape mechanisms. Nat Rev Cancer. 2003;3:362-74.
42. Paulssen RH, Moe B, Grønaas H, Orbo A. Gene expression in endometrial cancer cells (Ishikawa) after short time high dose exposure to progesterone. Steroids. 2008;73:116-28.
43. Lapp CA, Thomas ME, Lewis JB. Modulation by progesterone of interleukin-6 production by gingival fibroblasts. J Periodontol. 1995;66:279-84.
44. Fan J, Shimizu Y, Chan J, et al. Hormonal modulators of glial ABCA1 and apoE levels. J Lipid Res. 2013;54:3139-50.
45. Groves MR, Kuhn A, Hendricks A, et al. Crystallization of a Golgi-associated PR-1-related protein (GAPR-1) that localizes to lipid-enriched microdomains. Acta Crystallogr D Biol Crystallogr. 2004;60:730-2.
46. Al-Rawi MAA, Rmali K, Watkins G, Mansel RE, Jiang WG. Aberrant expression of interleukin-7 (IL-7) and its signalling complex in human breast cancer. Eur J Cancer. 2004;40:494-502.
47. Ardon H, Verbinnen B, Maes W, Beez T, Van Gool S, De Vleeschouwer S. Technical advancement in regulatory T cell isolation and characterization using CD127 expression in patients with malignant glioma treated with autologous dendritic cell vaccination. J Immunol Methods. 2010;352:169-73.
48. González-Arenas A, Cabrera-Wrooman A, Díaz NF, et al. Progesterone receptor subcellular localization and gene expression profile in human astrocytoma cells are modified by progesterone. Nucl Recept Res. 2014. [Epub ahead of print].
49. Hernández-Hernández OT, Camacho-Arroyo I (2013) Regulation of gene expression by progesterone in cancer cells: effects on cyclin D1, EGFR and VEGF. Mini Rev Med Chem. 2013;13:635-42.
50. Hernández-Hernández OT, Rodríguez-Dorantes M, González-Arenas A, Camacho-Arroyo I. Progesterone and estradiol effects on SRC-1 and SRC-3 expression in human astrocytoma cell lines. Endocrine. 2010;37:194-200.