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Evaluation of progesterone receptor expression in low- and high-grade astrocytomas

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SUMMARY

OBJECTIVE: Gliomas are tumors originating from glial cells. Gliomas are the most common primary neoplasms of the central nervous system, with astrocytomas being the most prevalent glioma subtype. Progesterone regulates several reproductive processes, such as ovulation and sexual behavior, and influences neuronal excitability, learning, and the neoplastic proliferation of glial cells. Progesterone functions mainly by interacting with intracellular progesterone receptors to modify the expression of the genes involved in cell proliferation, angiogenesis, and epidermal growth factor production. As not many studies on the hormone receptors in glial tumors have been reported, the objective of this study was to evaluate the expression of these proteins in astrocytomas and to determine whether their expression levels vary according to the tumor grade.

METHODS: This was a retrospective study using glial tumor paraffin blocks obtained from the São Marcos Hospital Pathology Department archives. Forty cases were divided equally into two groups, based on histological types and the World Health Organization criteria (low- and high-grade tumors). Progesterone receptor expression was analyzed by immunohistochemistry. The data were statistically analyzed using the Mann-Whitney U test and Spearman's correlation coefficient; results with $p<0.05$ were considered statistically significant.

RESULTS: There were no statistically significant differences between the mean nuclear progesterone receptor expression of low-grade (0.1495) and high-grade (0.0937) astrocytomas ($p=0.2$).

CONCLUSION: Progesterone receptors are present in both low- and high-grade gliomas; however, there is no significant difference in the levels of progesterone receptor expression between the tumor grades.

KEYWORDS: Astrocytoma. Glioma. Glioblastoma. Progesterone receptor. Progesterone. Prognosis.

INTRODUCTION

Glioma is the most common primary tumor affecting the central nervous system, and astrocytoma is the most prevalent histological subtype of this tumor. According to the World Health Organization, there are four types of astrocytomas: grades I and II (low-grade or benign) and grades III and IV (high-grade or malignant). Astrocytomas are the most common primary malignant central nervous system tumors in adults, with a reserved prognosis even when treated appropriately[1-4]. To improve patient survival, there is a need to improve knowledge of the pathways that regulate the aberrant growth of these tumors, develop new diagnostic methods, and identify new prognostic biomarkers[5-7].

Progesterone not only regulates ovulation, sexual behavior, and other reproductive processes but also influences learning,

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neuronal excitability, and the neoplastic proliferation of glial cells. Progesterone functions primarily by interacting with intracellular progesterone receptors (PRs)[3].

There are two PR isoforms, A (PR-A) and B (PR-B), which modify the expression of the genes involved in cell proliferation, angiogenesis, and epidermal growth factor production[8]. The presence of these biomarkers in *in vitro* glial tumor models and their response to anti-progesterone drugs piqued our interest in studying the potential mechanisms of action of PRs in glial neoplasms[9-11]. Due to the lack of published studies on hormone receptor expression in glial tumors, the objective of this study was to evaluate the expression of PRs in astrocytomas and determine whether their expression levels differ according to the tumor malignancy grade.

METHODS

Study design

This study used glioma paraffin blocks obtained from the São Marcos Hospital Pathology Department archives (Teresina, Piauí, Brazil). Only astrocytomas obtained from patients who had not undergone treatment before primary surgery and those stored for not more than five years were selected (histopathological samples were collected between June 2013 and June 2018). The Research Ethics Committee of the Federal University of Piauí, Brazil, approved this study (CAAE: 43447015.8.0000.5214). Our methodology also complies with the principles dictated by the Declaration of Helsinki. Forty cases were histologically divided into two groups (low- and high-grade astrocytomas). Each group contained 20 cases that were randomly chosen from the tumors that met the inclusion criteria.

Immunohistochemical method

Tumor tissue samples were fixed in formalin for 12–24 h and cut into 3-µm thick slices. Tissue sections were then processed and stained with hematoxylin and eosin. The slides were dewaxed with xylol at 60°C for 15 min, dehydrated with decreasing concentrations of alcohol (100, 95, 80, and 70%) for 30 s each, and washed with distilled water. To recover antigens, the sections were immersed in buffered citric acid and heated in a microwave for 15 min at maximum power. The samples were subsequently immersed in a buffered solution containing 3% of hydrogen peroxide (twice for 10 min each) and then washed with distilled water and phosphate-buffered saline.

The samples were incubated with a PR-specific monoclonal antibody (PgR 636, IgG1 κ isotype; Dako Corporation, California, United States) overnight at 4–8°C. As per the manufacturer's recommendations, the antibody was diluted to 1:40–1:80 in phosphate-buffered saline containing 1% of bovine serum albumin.

Quantitation method

An optical microscope (Nikon Eclipse E-400, Tokyo, Japan) connected to a color video camera (Samsung, CHC-370N, Seoul, Korea) was used to capture the images, which were transmitted to a computer equipped with the Imagelab software version 2.3 (Softium Informática Ltda., São Paulo, Brazil) for evaluation.

The number of PR-positive and PR-negative cells were counted (out of 600 cells) from the images taken at 400× magnification and beginning with the areas of higher expressions. The percentage of PR-positive cells on each slide was obtained from the ratio of cells with positively stained nuclei relative to the total number of cells multiplied by 100.

Statistical method

The data were stored in Microsoft Excel spreadsheets, and the statistical analysis was performed using the SPSS 20.0 software. The Kolmogorov–Smirnov normality test was used to analyze the data obtained. As the samples were independent and not normally distributed, the Mann-Whitney U test was used to compare the mean percentage of PR-positive nuclei in low- and high-grade gliomas. The significance level was set at $p<0.05$.

RESULTS

There was a difference in the percentage of cells expressing nuclear PR in low-grade (0.1495) *versus* high-grade (0.0937) astrocytomas. However, this difference was not statistically significant (p=0.2; Table 1; Figure 1).

Table 1. The mean percentage of progesterone receptor-positive nuclei in high- and low-grade gliomas.

Groups	n	Mean	SE	SD	Minimum	Maximum	Median
High-grade progesterone receptor	20	0.0937	0.0237	0.1062	0	0.37	0.0459
Low-grade progesterone receptor	20	0.1495*	0.0330	0.1479	0	0.55	0.0965

SE: standard error; SD: standard deviation. *p=0.2.

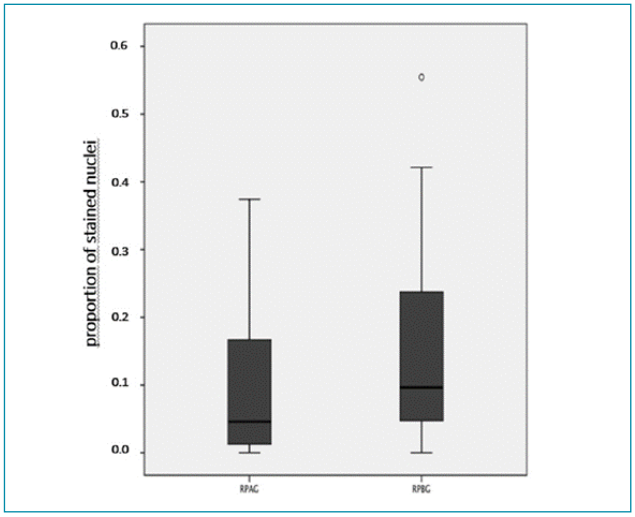

Figure 1. The mean percentage of progesterone receptor-positive nuclei in high- and low-grade gliomas. Progesterone receptor in high-grade astrocytomas; Progesterone receptor in low-grade astrocytomas.

DISCUSSION

Both *in vitro* and *in vivo* studies have shown that progesterone can stimulate astrocyte infiltration and migration into the cortex of the rat brain as well as the expression of the genes that are important for the growth and dissemination of these neoplasms[11,12]. Progesterone is derived from cholesterol and acts *via* two main mechanisms, namely, classical and nonclassical signaling pathways. The first mechanism involves interaction with intracellular PRs, and the second mechanism involves the participation of membrane receptors and ion channels[8,13].

The PR is composed of a central DNA-binding domain and a carboxyl-terminal containing multiple regions, which is important for the activation or inhibition of this protein. In humans, the PR is encoded by a single gene located on chromosome 11q22. Different promoters in different organs and tissues (including the central nervous system) transcribe the two PR isoforms (i.e., PR-A and PR-B). These may have different functions as they are regulated by different promoters[8,11,12,14-16].

The receptors, such as meningiomas, chordomas, craniopharyngiomas, and gliomas, are also found in brain tumors. According to some studies, PR expression increases with malignancy, with the B isoform predominantly being found in malignant gliomas[8,11,15,17-23].

In an immunohistochemical study by Khalid et al.[23] there were significantly more PR-positive cells in glioblastoma multiformes than in low-grade or anaplastic astrocytomas; they also reported that PR-positive glial tumors had a higher rate of proliferation than PR-negative tumors.

Assimakopoulou et al.[18] demonstrated that PRs were expressed in 59% of glioblastomas, 45% of anaplastic astrocytomas, and 8% of low-grade glial tumors and were absent in normal astrocytes. Carroll et al.[24] observed that PR expression was higher in high-grade tumors (62% of glioblastomas, 37% of anaplastic tumors, and 25% of low-grade astrocytomas).

Conversely, Khalid et al.[23] reported that all of their anaplastic astrocytoma samples were negative for PRs, while all of their glioblastoma samples expressed PRs.

This study found that low-grade tumors expressed more PRs than high-grade astrocytomas, but the difference was not statistically significant (Table 1; Figure 1). Our findings did not corroborate with the literature, possibly due to the small sample size used in this study. A larger number of tumors may generate results similar to those in the studies previously mentioned.

CONCLUSION

PRs are expressed by both low- and high-grade gliomas; although there was a difference in the level of expression between the two tumor grades, this difference was not statistically significant.

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AUTHORS' CONTRIBUTIONS

CBT: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **FCSAGB:** Conceptualization, Data curation, Formal Analysis, Writing – original draft. **EBS:** Writing – original draft, Writing – review & editing. **HACSM:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **JNPOB:** Writing – original draft.

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