Expression analysis of progesterone-regulated miRNAs in cells derived from human glioblastoma

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Abstract. Glioblastomas (GBMs) are the most frequent and malignant type of brain tumor. It has been reported that progesterone (P4) regulates the progression of GBMs by modifying the expression of genes that promote cell proliferation, migration and invasion; however, it is not fully understood how these processes are regulated. It is possible that P4 mediates some of these effects through changes in the microRNA (miRNA) expression profile in GBM cells. The present study investigated the effects of P4 on miRNAs expression profile in U-251MG cells derived from a human GBM. U-251MG cells were treated for 6 h with P4, RU486 (an antagonist of the intracellular progesterone receptor), the combined treatment (P4+RU486) and cyclodextrin (vehicle) and then a miRNA microarray analysis conducted. The expression analysis revealed a set of 190 miRNAs with differential expression in the treatments of P4, RU486 and P4+RU486 in respect to the vehicle and P4 in respect to P4+RU486, of which only 16 were exclusively regulated by P4. The possible mRNA targets of the miRNAs regulated by P4 could participate in the regulation of proliferation, cell cycle progression and cell migration of GBMs. The present study provided insight for understanding epigenetic modifications regulated by sex hormones involved in GBM progression, and for identifying potential therapeutic strategies for these brain tumors.

Introduction

Glioblastoma multiforme (GBM), a grade IV astrocytoma, is the most common and aggressive brain tumor in adults. It is characterized by being highly infiltrative, angiogenic and resistant to chemotherapy and radiotherapy. The medical history of patients with GBM is short as few of them survive more than one year (1-3). GBM is mainly diagnosed in adults >50 years old, but it can occur at any age and the incidence is higher in men than in women (3:2) (4).

Studies have focused on the identification of new biomarkers and therapeutic agents in GBM. Of particular interest are the microRNAs (miRNAs), which are single-stranded, short, non-coding RNA sequences with a length between 18 and 25 nucleotides that regulate gene expression at the post-transcriptional level by silencing their mRNA targets through binding to their 3'-untranslated region (3'-UTR). Compelling evidence continues to accumulate that miRNAs are involved in cancer-related signaling pathways associated with gliomagenesis, proliferation, apoptosis, invasion and malignancy of GBM (5-10).

Progesterone (P4), a sex steroid hormone, can exert its effects through a classical mechanism by binding the intracellular progesterone receptor (PR), a ligand-dependent transcription factor. Upon activation, PRs are dimerized and translocated to the nucleus where they recruit coactivators and chromatin-remodeling complexes to activate transcription of progesterone responsive genes (11-13). The participation of PR in P4 actions in GBMs has been documented. In two human astrocytoma cell lines (U373, grade III and D54, grade IV), González-Agüero *et al* (14) studied the effects of progesterone and RU486 on cell growth; at a concentration of 10 nM, P4 increased the number of D54 and U373 cells. Also, it was observed that P4 increased S phase of the cell cycle in U373 cells (14). In scratch-wound and Transwell assays, P4 increases the number of D54 and U-251MG cells migrating and the number of invasive cells, respectively (15). In an *in vivo* study, Germán-Castelán *et al* (16), implanted U87 GBM xenografts into the cerebral cortex of male rats and observed that P4 significantly increases GBM tumor area and infiltration length. RU486, a PR antagonist and an oligonucleotide antisense against PR, blocked the effects of P4.

It has been reported that P4, through PR activation, increases proliferation, migration and invasion of cells derived from human GBMs by regulating the expression of genes

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involved in these processes including TGFβ, COF1, EGFR, VEGF and cyclin D1 (17-20). Although the role of P4 over the expression of miRNAs in GBMs remains to be elucidated, it is known that in breast cancer, P4 regulates the expression of miRNAs with tumor suppressor or oncogenic action, via the classic PR (21-30). The present study characterized the expression profile of P4-regulated miRNAs in the U-251MG cell line, derived from a human GBM and the biological processes that could be regulated by the differentially expressed (DE) miRNAs.

Materials and methods

Cell culture and treatments. The U-251MG cell line derived from a human GBM was acquired from the American Type Culture Collection (ATCC). It was maintained in Dulbecco's modified Eagle's medium (DMEM; Biowest) with high glucose, phenol red, supplemented with 10% fetal bovine serum (FBS; Biowest), 1 mM sodium pyruvate (*In Vitro* S.A.), 0.1 mM of non-essential amino acids (*In Vitro* S.A.) and 1 mM antibiotic (Streptomycin 10 g/l, Penicillin G 6.028 g/l and Amphotericin B 0.025 g/l; *In Vitro* S.A.), at 37˚C in a humid atmosphere with 5% CO_2. The media was replaced 12 h before the treatments by DMEM-no phenol red (cat. no. ME-019; *In Vitro*, MEX) supplemented with 10% charcoal/dextran treated FBS (cat. no. SH30068.03; Thermo Fisher Scientific, Inc.). The treatments consisted of P4 (10 nM), RU486 (10 μM), P4 (10 nM) + RU486 (10 μM; all from Sigma-Aldrich; Merck KGaA) and vehicle (cyclodextrin, 0.02%) for 6 h at 37˚C.

RNA isolation. Following treatments, total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. The concentration and purity of the extracted RNA were determined by the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.) and the integrity of the RNA was checked by electrophoresis on a 1.5% agarose gel. Only the samples with an RNA Integrity Number of 9-10 were used.

Microarrays analysis. For miRNA expression analysis, a standard protocol was followed. Briefly, for each sample, 250 ng of total RNA were labelled with the FlashTag™ Biotin RNA Labeling kit (Affymetrix®; Thermo Fisher Scientific, Inc.). The labelled RNA was hybridized with the GeneChip miRNA 4.0 Array (Affymetrix®; Thermo Fisher Scientific, Inc.) and then the miRNA microarray chips were washed twice with 1X PBST (0.02% Tween) and stained with FlashTag™ Biotin HSR (Affymetrix; Thermo Fisher Scientific, Inc.) for 5 min at 35˚C. The image was digitized and the CEL files were generated. The fluorescence intensity values in CEL format were loaded into Expression Console™ v1.4.1.46 software (Affymetrix; Thermo Fisher Scientific, Inc.), where they were pre-processed with Robust Multiarray Analysis and normalized by quartiles. The CHP files generated after normalization were loaded into the Transcriptomic Analysis Console™ v4.0.1 software (Affymetrix; Thermo Fisher Scientific, Inc.) for expression analysis through the functions of the limma package (31) and for graphics generation. Heatmaps were generated with pheatmap v1.0.12 (CRAN.R-project.org/package=pheatmap) package for R version 3.5.2 (32).

Prediction of target genes of DE-miRNAs. A search of the target genes of DE-miRNAs was performed in four different open access databases: DIANA-Tarbase v8 (33), miRWalk v2.0 (34), Diana-microT-CDS v5.0 (35) and TargetScan v7.2 (36). From these databases, DIANA-Tarbase v8 and miRWalk reported validated miRNA-mRNA interactions, while Diana-microT-CDS v5.0 and TargetScan v7.2 report only predicted miRNA-mRNA interactions. False positives were avoided by taking the intersections of at least 3 of these databases.

Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. The target genes found in at least 3 databases were used as input for Enrichr 3.0 platform (https://maayanlab.cloud/Enrichr/) (37) to perform a GO functional gene annotation (38) and a KEGG pathway enrichment analysis (39). The resulting GO terms with $P<0.05$ were considered significantly enriched.

Analysis of the protein-protein interaction network (PPI). The PPI network was established using the STRING database (https://string-db.org/) (40). Hub genes were determined with the help of Cytoscape software (v3.7.1) (41). Finally, the expression levels of the hub genes were determined in Gene Expression Profiling Interactive Analysis (GEPIA; v1.0; http://gepia.cancer-pku.cn/) (42), an interactive web server that was developed to perform RNA-seq expression data analysis of 9,736 tumors and 8,587 normal samples of the Cancer Genome Atlas and Genotype-Tissue Expression projects.

Statistical analysis. For the selection of differentially expressed miRNAs between treatments in the microarrays, ANOVA was performed with the t-moderated method of eBayes. The miRNAs with a fold-change (FC) $\geq\pm1.5$ and a P-value <0.05 were selected as differentially expressed in the contrasts of treatments. All data were analyzed and plotted by using GraphPad Prism v5.00 for Windows (GraphPad Software, Inc.).

Results

Identification of miRNAs with differential expression (DE-miRNAs). Global miRNA expression changes in the U-251MG cells were evaluated after 6 h of treatment with vehicle (V), P4, RU486, or P4 + RU486. A total of 190 DE-miRNAs were found after comparing the different treatments. DE-miRNAs were evaluated in the following comparisons (Table I): P4 vs. vehicle (Fig. 1A), RU486 vs. vehicle (Fig. 1B), P4+RU486 vs. vehicle (Fig. 1C) and P4 vs. P4+RU486 (Fig. 1D). The number of upregulated and downregulated DE-miRNAs in each contrast is shown in Table I. The data presented in the present study have been deposited in NCBI's Gene Expression Omnibus (43) and are accessible through GEO Series accession number GSE144204.

To determine the differences among treatments, a heatmap with the DE-miRNAs was generated (Fig. 2). It was identified that P4 treatment decreased the expression of 8 miRNAs; these effects were blocked by RU486 since none of these miRNAs exhibited a significant differential expression in the

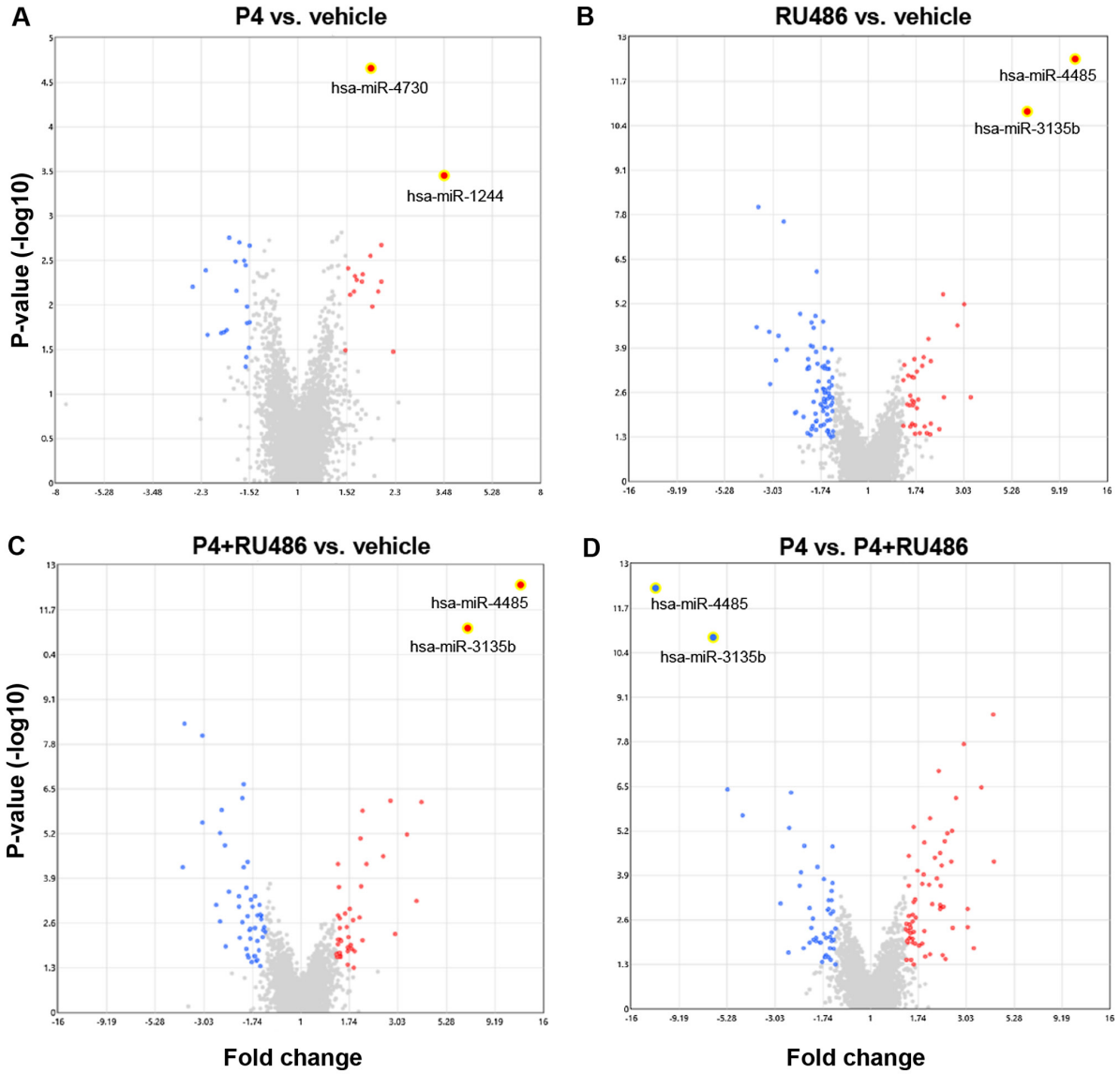

Figure 1. Differential expression analysis of miRNAs in U-251MG cells. The volcano plots show DE-miRNAs in (A) P4 vs. vehicle, (B) RU486 vs. vehicle, (C) P4+RU486 vs. vehicle and (D) P4 vs. P4+RU486. Blue dots represent the miRNAs downregulated and red dots represent the upregulated miRNAs in each comparison (FC <1.5 and P≤0.05). miRNAs, microRNAs; DE, differentially expressed; P4, progesterone; FC, fold-change; has, human.

Table I. Count of DE-miRNAs by comparison.

Comparison	Upregulated	Downregulated	Total
P4 vs. vehicle	16	10	26
RU486 vs. vehicle	27	77	104
P4+RU486 vs. vehicle	38	47	85
P4 vs. P4+RU486	63	39	102

DE, differentially expressed; miRNAs, microRNAs; P4, progesterone.

comparisons RU486 vs. V or P4 vs. P4+RU486; the present study also identified 8 miRNAs upregulated by P4 compared with vehicle, however in this case, the effect of P4 was not blocked by RU486, which can be seen in the comparison of P4 vs. P4+RU486.

Prediction of molecular pathways associated with putative targets of the DE-miRNAs. Bioinformatic analysis for the identification of putative targets of the DE-miRNAs was performed as described in Materials and methods. A total of 367 and 434 putative genes were determined for downregulated and upregulated miRNAs, respectively (Table SI). It was identified that target genes of miRNAs downregulated by P4 participated in cell-cell adhesion, regulation of histone post-translational modifications, cell cycle progression, mRNA binding, transcription regulation, nucleosome assembly (Fig. 3). By contrast, the miRNAs upregulated by P4 participated in the negative regulation of genes involved in transcription, histone H3K4 modifications, regulation of TFGβ pathway, chromatin structure and cytoskeleton conformation (Fig. 3).

Pathway enrichment analysis showed that the target genes of the miRNAs downregulated by P4 regulate signaling pathways participating in diseases such as systemic lupus erythematosus and alcoholism, but also in long-term potentiation of synaptic

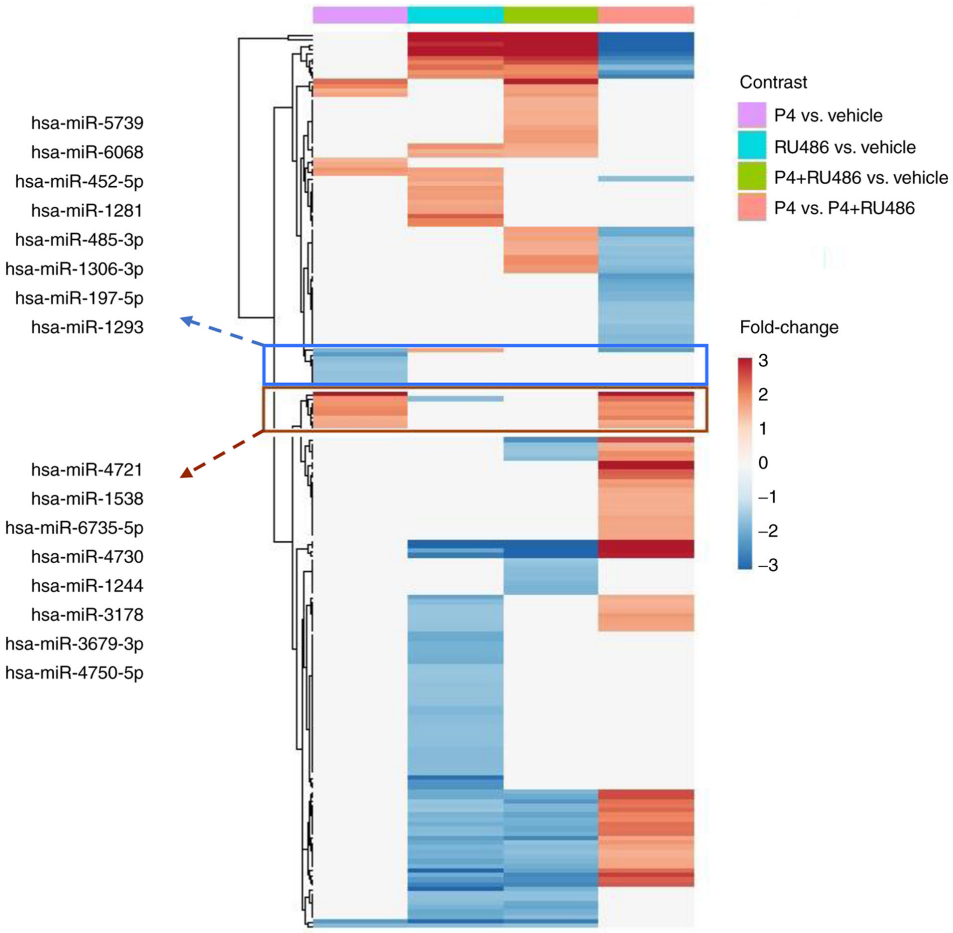

Figure 2. miRNA expression profile. The heatmap shows the FC of 190 significant miRNAs (FC≥ ±1.5 and P≤0.05) from the analyses of differential expression. Each row represents a miRNA and each column a treatment. miRNAs downregulated by P4 are denoted by a blue box, miRNAs upregulated by P4 are enclosed in a red box. miRNA, microRNA; P4, progesterone; FC, fold-change; has, human.

Table II. Pathway enrichment analysis of the target genes of miRNAs downregulated by P4.

Term	Overlap	P-value
Systemic lupus erythematous	14/133	0.0000001245
Alcoholism	16/180	0.0000001687
Viral carcinogenesis	12/201	0.000291301
Cushing syndrome	10/155	0.000503219
Bladder cancer	5/41	0.000798635
Long-term potentiation	6/67	0.001274163
Cell cycle	8/124	0.001804709
Mineral absorption	5/51	0.002163061
MAPK signaling pathway	13/295	0.002737286
Signaling pathways regulating pluripotency of stem cells	8/139	0.003665340

miRNAs, microRNAs; P4, progesterone.

Table III. Pathway enrichment analysis of the target genes of miRNAs upregulated by P4.

Term	Overlap	P-value
Synaptic vesicle cycle	6/78	0.0028211
Fluid shear stress and atherosclerosis	8/139	0.0037271
Tight junction	8/170	0.0120564
Colorectal cancer	5/86	0.0195424
TGFβ signaling pathway	5/90	0.0232857
IL-17 signaling pathway	5/93	0.0263776
Ferroptosis	3/40	0.0349887
Renal cell carcinoma	4/69	0.0357799
Adherent junction	4/72	0.0408592
Bacterial invasion of epithelial cells	4/74	0.0444609

miRNAs, microRNAs; P4, progesterone.

activity, cell cycle and maintenance of stem cells pluripotency (Table II). The target genes of the miRNAs upregulated by P4 participated in synaptic vesicle cycle, fluid shear stress and atherosclerosis, in addition to in TGF-β and IL-17 signaling pathways (Table III).

Screening of hub Genes and protein-protein interaction network (PPI). The target genes of the miRNAs regulated by

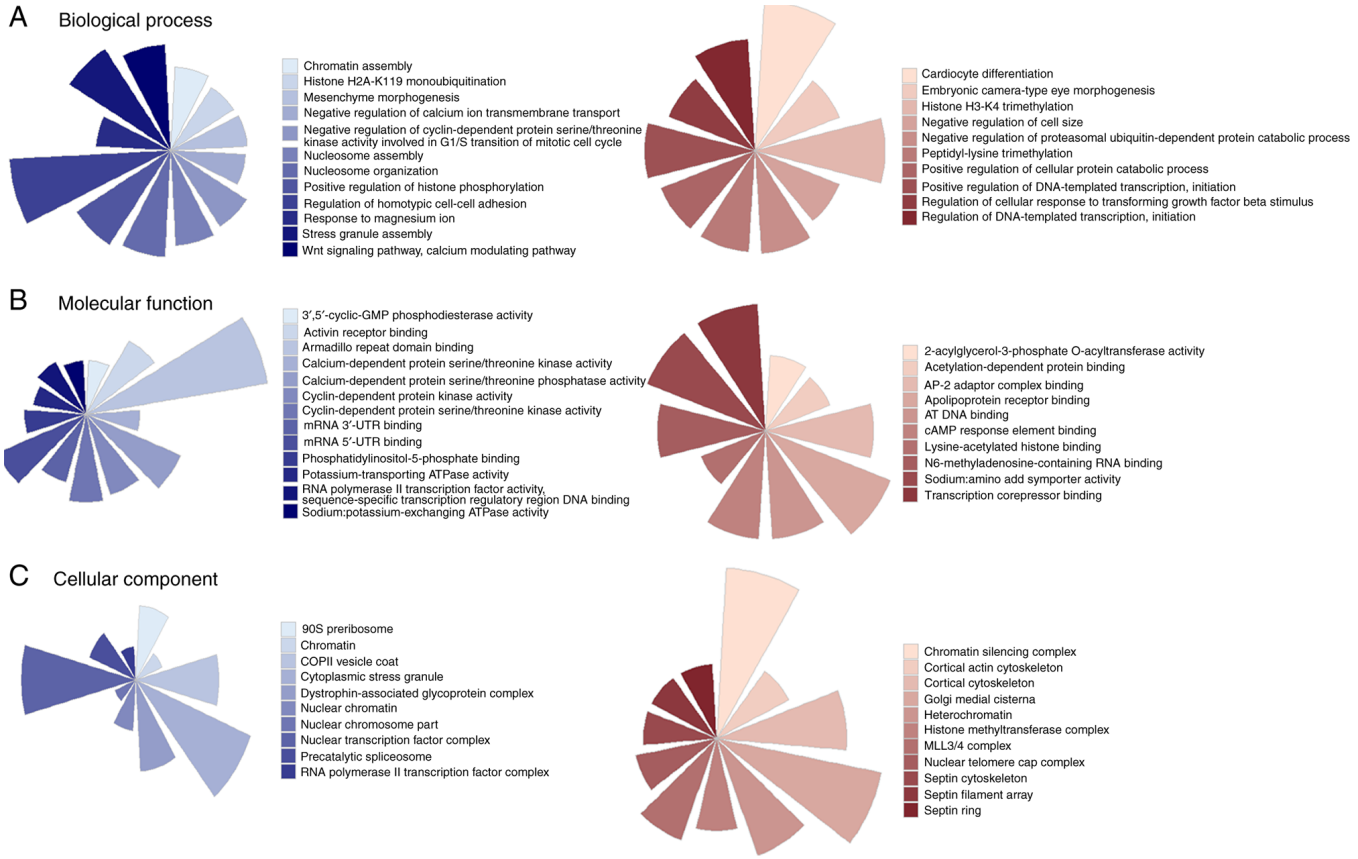

Figure 3. Functional annotation of miRNA-target genes regulated by P4. (A) Biological processes, (B) Molecular functions and (C) Cellular components enriched by candidate genes of downregulated miRNA (blue bars) and upregulated miRNAs (red bars). Gene Ontology terms with P<0.05 were considered significantly enriched. Putative targets of downregulated miRNAs by P4: 367 genes; putative targets of upregulated miRNAs by P4: 434 genes. miRNA, microRNA; P4, progesterone.

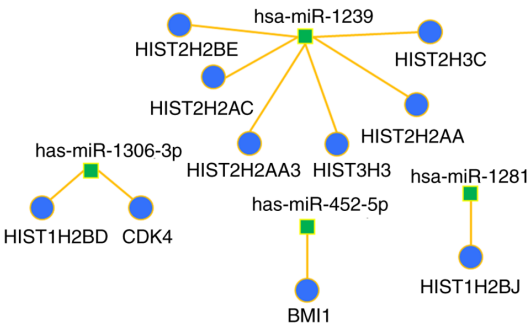

Figure 4. Regulatory network miRNA-mRNA for the hub genes of the miRNAs downregulated by P4. miRNA, microRNA; P4, progesterone; has, human.

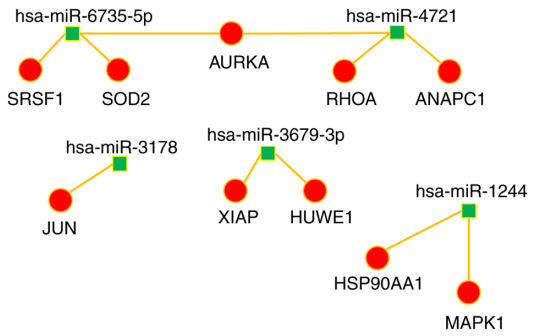

Figure 5. Regulatory network miRNA-mRNA for the hub genes of the miRNAs upregulated by P4. miRNA, microRNA; P4, progesterone; has, human.

P4 were mapped in the STRING database to obtain a clearer idea of their interactions and their global action. The analysis of PPI networks in Cytoscape, resulted in 259 and 267 pairs of nodes for the target genes of the miRNAs downregulated and upregulated by P4, respectively. The genes with a high degree of interactions in the PPI network were defined as hub genes since they could serve a critical role in the module (Table IV). A miRNA-target interaction network was performed to visualize the interaction between the calculated hub genes and their corresponding miRNA (Figs. 4 and 5). The majority of the hub genes of miRNAs downregulated by P4 were histones; whereas the principal hub gene of the miRNAs upregulated by P4 was HSP90AA1, a gene that encodes an inducible molecular chaperone.

The GEPIA database was used to compare the expression levels of the hub genes in biopsies of GBM compared with normal tissue. The expression of 7 hub genes (HIST2H2BE, HIST2H2AC, HIST1H2BJ, HIST1H2BD, CDK4, HIST2H2AA3 and HIST2H2AA) modulated by the DE-miRNAs downregulated by P4 was significantly higher in GBM compared with normal tissue (Fig. 6), as was expected given the negative association between a miRNA and its target

Table IV. Hub genes of the miRNAs regulated by P4.

Target genes of the miRNAs downregulated by P4		
miRNA	Gene	Degree
hsa-miR-1239	HIST2H2BE	34
hsa-miR-1239	HIST2H2AC	32
hsa-miR-1281	HIST1H2BJ	24
hsa-miR-1306-3p	HIST1H2BD	24
hsa-miR-1306-3p	CDK4	20
hsa-miR-1239	HIST2H2AA3	20
hsa-miR-1239	HIST3H3	20
hsa-miR-1239	HIST2H2AA	20
hsa-miR-452-5p	BMI1	20
hsa-miR-1239	HIST2H3C	17
Target genes of the miRNAs upregulated by P4		
hsa-miR-1244	HSP90AA1	30
hsa-miR-1244	MAPK1	29
hsa-miR-3178	JUN	27
hsa-miR-4721	RHOA	25
hsa-miR-6735-5p	SRSF1	15
hsa-miR-4721	ANAPC1	15
hsa-miR-3679-3p	HUWE1	14
hsa-miR-3679-3p	XIAP	14
hsa-miR-6735-5p	SOD2	14
hsa-miR-4721	AURKA	13

miRNAs, microRNAs; P4, progesterone; has, human.

genes. Notably, the target genes of the upregulated miRNAs, JUN, RHOA, SOD2 and AURKA exhibited a significantly higher expression compared with normal healthy tissue, contrary to the expected result; the remaining target genes did not present significant differences (Fig. 7).

Discussion

GBM, a grade IV astrocytoma, is the commonest and most aggressive brain tumor in adults (1-3). Previous studies have suggested that alterations in miRNAs expression are involved in GMB progression (5-10). Changes in the expression profile of miRNAs by steroid hormones have been noted in hormone-responsive cancers (44-47) but not in GBM. Since it has been suggested that sex hormones, such as P4, are involved in the several processes that contribute to GBM progression (14-20), the objective of the present study was to determine the expression profile of miRNAs regulated by P4 in GBM cells and identify the pathways regulated by them. The effect of P4 on increased cell proliferation of GBMs was observed at a concentration of 10 nM (14), the same concentration that increases migration and invasion (15). In all previous cases, 10 μM of RU486 was determined to be the concentration that significantly blocked the effects of P4 on GBM malignancy (14,15).

In the present study, the expression of 190 new miRNAs was altered by P4, RU486 and P4+RU486 in U-251MG cells. Of the 190 miRNAs, only 16 were regulated by P4 and this effect was blocked by RU486 (an antagonist of PR). However, some miRNAs exhibited changes in their expression upon treatment with of RU486 compared with the vehicle, suggesting that RU486 alone should exert an effect on miRNA expression, probably due to the affinity that RU486 also has for the glucocorticoid receptor (48,49). It was also found that P4+RU486 treatment had several DE-miRNAs respect to the vehicle, which suggests that RU486 does not entirely block P4 actions, and that P4 and RU486 may exert their effects via different mechanisms.

Of the 16 miRNAs reported in the present study, only the human (has)-miR-485-3p miRNA, downregulated by P4, has been previously evaluated in GBM cells. Agreeing with the present study, Zhang *et al* (50) found that the expression of hsa-miR-485-3p is downregulated in biopsies from patients with compared with healthy brain tissue; these authors determined that its target gene is the ring finger protein 35 (RNF135). By silencing RNF135, the hsa-miR-485-3p inactivated the MAPK/ERK1/2 pathway in GBM cells, while functional assays showed that hsa-miR-485-3p inhibited proliferation and migration of GBM cells, which was reversed by the overexpression of RNF135; all suggesting that the hsa-miR-485-3p miRNA has a tumor suppressor function in GBMs (50).

To evaluate the possible targets of the DE-miRNAs, the present study conducted functional annotation and pathway analysis. A set of target genes for the miRNAs downregulated by P4 and another set for the ones upregulated were defined. The functional annotation demonstrated that the target genes of the downregulated miRNAs could be involved in post-transcriptional histone modification, chromatin assembly, nucleosome assembly, cell cycle progression, RNA binding and regulation of transcription. The pathway analysis revealed that these genes could participate in some pathways not related to cancer (Systemic lupus erythematosus and alcoholism) as well as with pathways that are well characterized in cancer and specifically in GBM, including cell cycle (51), MAPK signaling pathway (52,53) and regulation of stem cells pluripotency (54-56).

Regarding the target genes of the upregulated miRNAs, the results of the functional notation matched the results of the pathway analysis as the regulation of transcription, modifications of histone H3K4, regulation of the TGFβ pathway, chromatin structure and conformation of the cytoskeleton are related to the pathways of synaptic vesicles formation, maintenance of tight junctions, TGFβ and the IL signaling pathways. Particularly in GBMs, the secretion of VEGF by cancer cells inhibits the formation of tight junctions (57,58) and notably, P4 increases the expression of VEGF in cell lines derived from GBMs (20). Based on these data, it could be hypothesized that P4 increases the malignancy of GBMs through the regulation of miRNAs that affect the availability of VEGF and the maintenance of tight junctions. Regarding the TGFβ pathway, it is known that TGFβ expression is directly upregulated by P4 through PR (17). This suggests that the increase of TGFβ expression should be due to the silencing of repressors

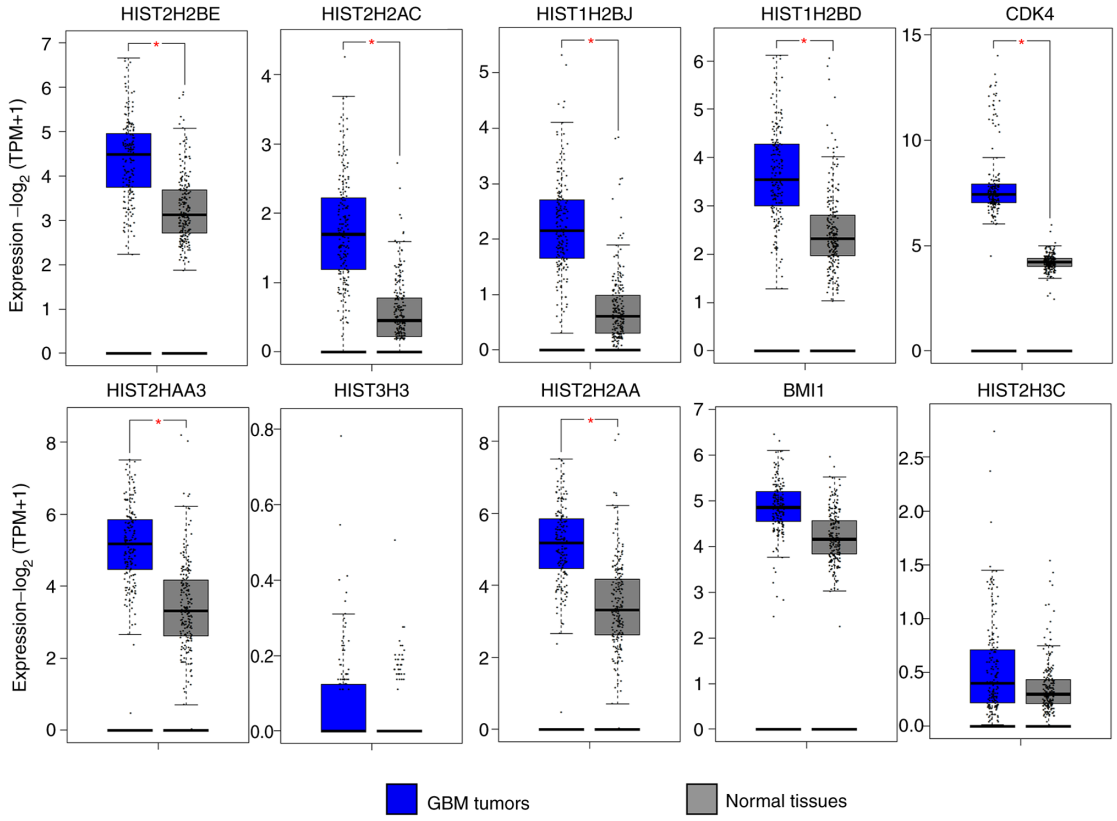

Figure 6. Expression levels of the 10 hub genes of the downregulated miRNAs by P4 in 163 tumors (blue) and 207 normal tissues (gray) obtained from The Cancer Genome Atlas and Genotype-Tissue Expression in Gene Expression Profiling Interactive Analysis database (*P<0.05 vs. normal tissue). miRNA, microRNA; P4, progesterone.

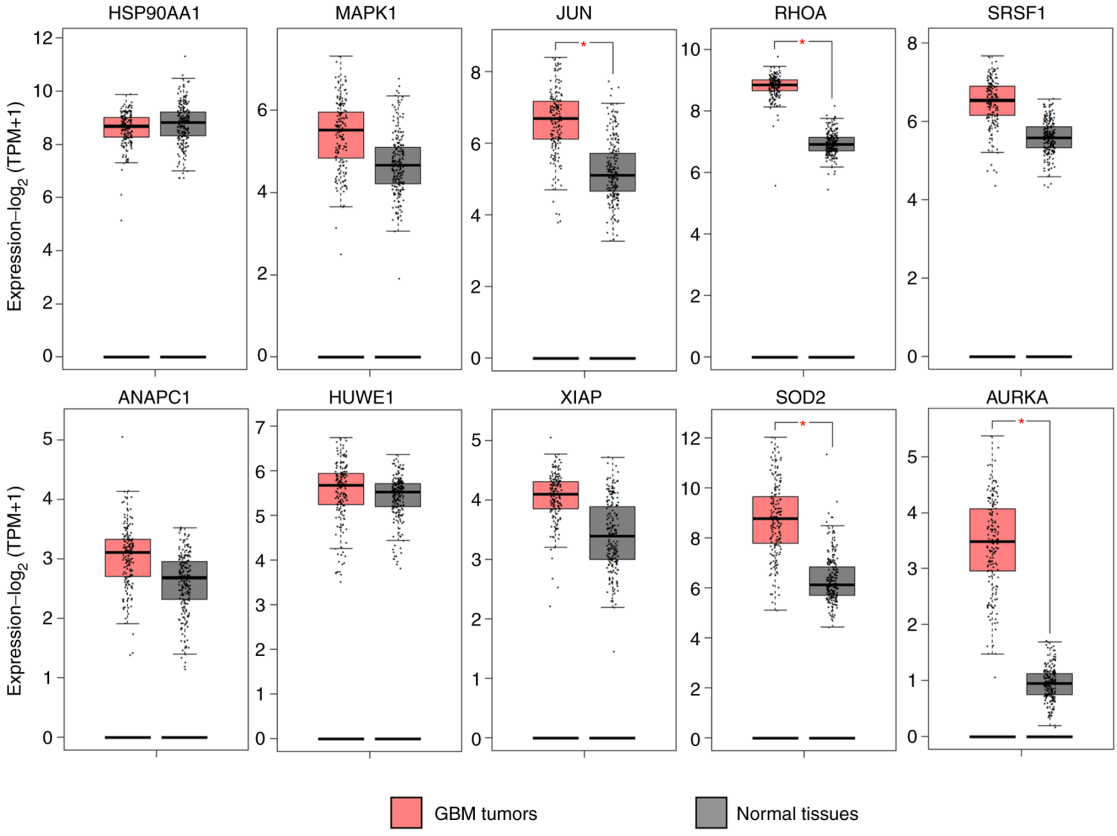

Figure 7. Expression levels of the 10 hub genes of the upregulated miRNAs by P4 in 163 tumors (red) and 207 normal tissues (gray) obtained from The Cancer Genome Atlas and Genotype-Tissue Expression in Gene Expression Profiling Interactive Analysis database (*P<0.05 vs. Normal tissue). miRNA, microRNA; P4, progesterone.

pathways, due to the action of miRNAs upregulated by P4. Finally, when looking for an association between the IL-17 pathway and GBMs, it was noted that IL-17 expression has been positively related to the survival rates of patients with GBM (59); in this case, it is possible that P4 decreases the expression of IL-17 by the upregulation of miRNAs directed to activators of this pathway.

Analysis of the protein-protein interaction network, carried out with the target genes of those miRNAs regulated by P4, determined a list of hub genes that could be important in the regulation of the malignancy of GBMs. The results indicated that eight of the 10 hub target genes of miRNAs downregulated by P4 correspond to histones; therefore, these histones would be expected to be upregulated in GBMs. One way to confirm this deduction was through the analysis of the expression data from the GEPIA database. Based on the analysis of these data, 7 of the 8 histones matched their miRNA regulators; markedly, only the expression of histone HIST2H2BE has been observed in GBMs under the treatment of OTX015, an inhibitor of bromodomain and extraterminal bromodomain proteins (60). In addition to histones, BMI1 and CDK4 were chosen as hub genes in the present study's analysis of the target genes for the miRNAs downregulated by P4 and, according to the results of their expression in GEPIA, only CDK4 was significantly upregulated in GBMs biopsies; however, according to literature (56,61-63), BMI1 is also highly expressed in GBM. There is evidence that CDK4 and BMI1 are oncogenes involved in the regulation of the cell cycle and the increase in proliferation and invasion of GBMs cells (56,61-63). In addition, the function of these hub genes coincided with the results of functional gene notation and pathway enrichment analysis.

In the case of the expression of hub genes of the miRNAs upregulated by P4, genes are involved in the cell cycle, proliferation, invasion, migration and inhibition of apoptosis and genes that participate in antioxidant defence. To date, the expression of these genes has not been evaluated after P4 treatment, however, when verifying their expression in the GEPIA database and in published papers, the present study noted that these genes are key for GBM biology and characterized as oncogenes. As aforementioned, P4 promotes the malignancy of GBMs and according to the results of the present study, it upregulated miRNAs whose targets are these oncogenes. This was unexpected and therefore further research in this field is required.

The JUN gene encodes the transcription factor AP-1, which is constitutively activated in gliomas, contributing to their malignancy (64-66) and RHOA has been implicated in invasion and migration of human GBM cells through its signalization with YAP, MRTF-A, Daam1 and Wnt5a, also contributing to the malignancy of GBM (67,68). In the case of SOD2, its expression has been observed to be that significantly increased, at the mRNA and protein levels, in LN-239 and U87 cell lines (both derived from GBMs) exposed to oxidative stress (69-71). Although JUN, RHOA and SOD2 have been described as oncogenes, their regulation by some type of non-coding RNA has not been studied, in contrast to what has been observed in AUKRA. It can be hypothesized that the regulatory miRNAs of these hub genes, could have another level of regulation by including circular (circ)RNAs (69) or long non-coding RNAs, which also have response elements to miRNAs (70) For example AUKRA is a target gene of circMMP9 and hsa-miR-124 axis (72).

Finally, the interaction network between hub genes and their miRNAs allows the visualization of the complexity of the gene regulation by miRNAs; the value of the network is the demonstration of the interaction between miRNAs regulated by P4 and their target genes, which could aid in understanding the epigenetic alterations induced by P4 in GBM.

The present study described the global changes in the expression profile of miRNAs induced by P4 in cells derived from human GBM. Microarray expression analysis identified 8 miRNAs downregulated by P4 and 8 miRNAs upregulated by P4. As a result of bioinformatic analyses, it was found that P4 could regulate processes such as proliferation, cell cycle progression and cell migration of GBMs through the regulation of a network of miRNAs-mRNAs.

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Availability of data and materials

The miRNA expression data used to support the findings of the present study have been deposited in the NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE144204 (www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE144204).

Authors' contributions

All authors contributed to the study conception and design. The material preparation, data collection and analysis were performed by DEVV and ADMM. The verification of the raw data was performed by ICA, MRD and EMM. The first draft of the manuscript was written by DEVV. All authors provided technical and experimental advice and reviewed and approved the final 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.

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