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The Role of Mir-34a and Mir-145 as Potential Biomarkers of Meningioma Recurrence

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Abstract

The expression of miRNAs is associated with a variety of diseases, including neoplasms. In recent years, a large number of abnormally expressed miRNAs have been shown to be effective in understanding the oncogenesis, development, progression and prognosis of meningiomas. Furthermore, it is known that miRNAs act as oncogenes or tumor suppressors and that they regulate essential molecular pathways such as transcription factors involved in the pluripotency phenotype of stem cells. Therefore, the aim of this study was to analyze the expression of microRNAs miR-34a, miR-145 and miR-221 that regulate the pluripotency pathway of stem cells and correlate with tumor recurrence in grade I meningiomas. We used 30 samples, belonging to 15 patients who presented recurrences of grade I meningiomas. We observed low expression levels of miR-34a in the group of tumor recurrences when compared to control individuals and primary tumors, which may be associated with the tumor suppressor role of this miR. The miR-145 also showed decreased expression levels between the control group and the group of tumor recurrences. We also observed decreased expression levels in miR-145 between the control group and the primary tumors group. MiR-221 did not differ between the studied groups. MiR-34a and miR-145 microRNAs that regulate the stem cell pluripotency pathway are shown to be hypo expressed in tumor recurrences of grade I meningiomas and are shown to be good candidates for prognosis and recurrence biomarkers in meningiomas.

Keywords

Meningiomas, Tumor Recurrence, microRNA, Cell Pluripotency

1. Introduction

Meningiomas are the most common intracranial tumors [1]-[5], slow-growing, believed to arise from arachnoid meningothelial cells (MECs), whose occurrence increases with age and is prevalent in females, with a frequency of two to three women for every man [6]-[9]. These comprise 37.6% of all primary tumors of the Central Nervous System (CNS) and 53.3% of all benign tumors of the CNS. Pediatric meningiomas typically present as higher grade, with a higher risk of recurrence and overall mortality [10].

These tumors are categorized according to the World Health Organization (WHO) classification of Grade I, Grade II or Grade III tumors [11]-[19]. Grade I meningiomas, considered benign tumors, represent 80.5% of all meningiomas of the neuroaxis, whose treatment is complete resection [9] [20]. Grade II meningiomas represent 17.7% of meningiomas and have an approximate recurrence rate of 40% in five years [8] [20]. Grade III meningiomas represent 2% to 1.7% of meningiomas. They are considered malignant and have a worse prognosis and survival rate under two years due to recurrence rates up to 80% [20] [21]. The Ki-67 proliferation index of >4% and >20% describes the increased risk of recurrence and mortality in grade II and III meningiomas, respectively. Unlike brain neoplasms, the 2016 WHO classification system incorporates no genomic or molecular features [10].

In recent years, studies have shown that the histopathological classification of meningiomas is not associated with the occurrence of recurrences and suggest the existence of key regulators, whose identification would allow the understanding of the behavior of tumor initiating and maintenance cells [22]-[34]. The origin of tumor initiating and maintenance cells is still under investigation and current hypotheses propose that tumor stem cells derived from a population of resident or dedifferentiated stem cells, with pluripotency and self-renewal being the most prominent attributes of these embryonic or dedifferentiated adult stem cells [35] [36].

Transcription factors regulate pluripotency and self-renewal of embryonic stem cells and reprogram differentiated somatic cells into induced pluripotent stem cells. The transcription factor OCT4 is a pluripotency-associated transcription factor important in locoregional tumor recurrence and metastasis. The transcription factor NANOG plays a role in the regulation of self-renewal and pluripotency transcription. It is believed to coordinate the self-renewal and pluripotency of embryonic stem cells, in addition to contributing to carcinogenesis and metastasis. The SOX2 transcription factor promotes stem cell maintenance and tumor stem cell pluripotency. The expression of these transcription factors is associated with the formation of metastases in many types of tumors, including meningiomas, where cells that express these markers demonstrate phenotypes of greater aggressiveness and invasiveness [37]. In 2017, Freitag *et al.* analyzed the expression of NANOG in 33 surgical specimens of human meningiomas by immunofluorescence and correlated the expression of this marker with the clinicopathological

variables. Positive expression of NANOG was observed in all specimens studied, however subgroup analysis revealed differences in its expression. That is, a positive expression of NANOG of 1% was observed in low-grade meningiomas and 2% in grade II/III meningiomas that co-express OCT4 and SOX2. NANOG + cells, which express SOX2 and OCT4, were successfully identified (26% low grade versus 20% high grade). The authors suggest that the overexpression of OCT4, NANOG and SOX2 may have an impact on tumorigenesis and progression of human meningiomas [36].

In this context, studies reveal that microRNAs (miRNAs) act as tumor suppressors or oncogenes, demonstrating that, in addition to the action of transcription factors on the potentiality of tumor stem cells (CSCs: Cancer Stem Cells) of meningiomas, microRNAs can also act in the regulation of molecular mechanisms of these CSCs [37] [38].

MiRNAs are small non-coding RNAs – consisting of 19 to 25 nucleotides – capable of regulating gene expression at the post-transcriptional level through degradation or repression of the translation of messenger RNA (mRNA) target molecules [39]-[41].

Several studies have observed that a large amount of abnormally expressed miRNAs is effective in understanding the oncogenesis, development, progression, and prognosis of meningiomas [42]-[53]. Studies demonstrate that miR-34a, miR-145 and miR-221 microRNAs act as a tumor suppressor in several types of cancer, being able to restrict cell growth and facilitate cell apoptosis [42]-[59].

In a study of 60 grade 1 and grade 2 intracranial human meningiomas plus 20 healthy meningeal tissues, the expressions of miR-16 and miR-519 were analyzed. Decreased expression of miR-16 and miR-519 was detected in tumor tissue when compared with healthy patients. *In vitro* functional assays showed the relationship of these microRNAs to biological processes such as regulation of the mitotic cell cycle, pre-replicative complex and brain development [60].

In a study with 15 patients and 5 controls, nineteen differentially expressed miRNAs were validated by RT-qPCR. MiR-218 and miR-34a were upregulated relative to normal controls, and miR-143, miR-193b, miR-451, and miR-21 were downregulated. This shows that grade I and II meningiomas appear to share biomarkers with malignant tumors [48].

Through in silico prediction, miR-34a-3p showed potential targeting of SMAD4, FRAT1 and BCL2. In meningioma cells, overexpression and inhibition of miR-34a-3p resulted in inverse protein levels of SMAD4, FRAT1 and BCL2. Furthermore, altered expression of miR-34a-3p modified cell proliferation and apoptosis of meningioma cells in vitro [61].

Increased expression of miR-145 in meningioma cells reduced proliferation and increased sensitivity to apoptosis in nude mice compared with control cells. Furthermore, meningioma cells overexpressing miR-145 had impaired migratory and invasive potential in vitro and in vivo. miR-145 expression was found to be reduced in atypical and anaplastic tumors when compared with benign tumors [62].

In another study, miR-221 was highly expressed in glioma cells, and miR-221 suppression was performed on cell replication, migration and invasion in glioma cells. Functional experiments showed that miR-221 participated in the regulation of glioma cell replication and invasion. The experiment further demonstrated that miR-221 knockdown caused a significant reversal of the phenotypes induced by it [63].

In this aspect, we analyzed the expression of miR-34a, miR-145 and miR-221 microRNAs, which are stem cell pluripotency pathway regulators and correlated with tumor recurrence in grade I meningiomas, since miRNAs that regulate targets involved in the pluripotency phenotype of stem cells may be associated with the appearance of recurrences in meningiomas.

2. Patients and Methods

Thirty samples were selected from 15 patients with a histopathological diagnosis of grade I meningiomas—confirmed according to WHO criteria—who evolved with grade I tumor recurrence. The collection was carried out during surgery performed by the medical team of the Neurosurgery Division of the Clinical Hospital, Ribeirão Preto School of Medicine, University of São Paulo (HCFMRP/SP), between 2002 and 2020. As a control for tissue analysis, five samples of arachnoids obtained from previously healthy patients, without a history of chronic diseases and who died of sudden death were used.

MiRNAs miR-34a, miR-145 and miR-221 were selected from resources of platforms available for public use (Gene Cards, TatgetScan and Mirdb databases) where microRNAs that regulate targets involved in the pluripotency phenotype of stem cells were identified: OCT4, SOX2 and NANOG.

RNA Isolation and Real-Time Polymerase Chain Reaction

Total RNA was extracted using Trizol reagent (Applied Biosystems, Foster City, United States) in accordance with the manufacturer's instructions. In preparation for the real-time polymerase chain reaction (PCR), reverse transcription of RNA samples was performed using the High-Capacity cDNA kit (Applied Biosystems).

The cDNA was amplified with quantitative real time polymerase chain reaction (q-PCR) using TaqMan Master Mix (Applied Biosystems) for the reaction of microRNAs. The U6 gene was used as an endogenous control (housekeeping) for the reaction of the microRNA. The PCR conditions included pre-heating at 50˚C for two minutes, denaturation at 95˚C for ten minutes, and 50 cycles of amplification and quantification (15 seconds at 95˚C, and one minute at 60˚C). All reactions were performed in duplicate and analyzed with the 7500 Sequence Detection System apparatus (Applied Biosystems). The data were analyzed using ABI-7500 SDS software. Dissociation curves were performed (melting curves) after amplification by RQ-PCR. The samples that showed dissociation curves with different temperatures or more than one point of dissociation in the same sample were discarded and repeated.

For the evaluation of miRNA expression, statistical analysis was performed

using the Kruskal-Wallis tests and Dunns and Mann-Whitney multiple comparison post-test. The GraphPad Prism version 9.2 for Windows program (GraphPad Software, San Diego—California USA) was used, with p values < 0.05 being considered statistically significant.

3. Results

In this study, 33% of the sample were male and 67% were female – 5 and 10 patients respectively. The entire case series, according to WHO criteria, was diagnosed with grade I meningioma and evolved with a recurrence of the same tumor grade. The meningothelial histological type was the most incident in this series of recurrence (47%), the transitional type was the second most incident (27%), the fibrous type was the third most incident (12%), the angiomatous and microcystic types, the fewer incidents, had the same percentage (7%). The mean age at diagnosis was 47 years, with a median of 49 years, with the youngest patients being 25 years old and the oldest patients being 75 years old. The mean time to recurrence in the sample was 5.4 years. None of the patients died until the last follow-up.

MiRNA-34a analysis showed that there was a significant difference between the Control Group and the Tumor Recurrence group (p = 0.0134, Kurskall Wallis test and p = 0.0100 post-test Dunn's multiple comparisons test) (Figure 1).

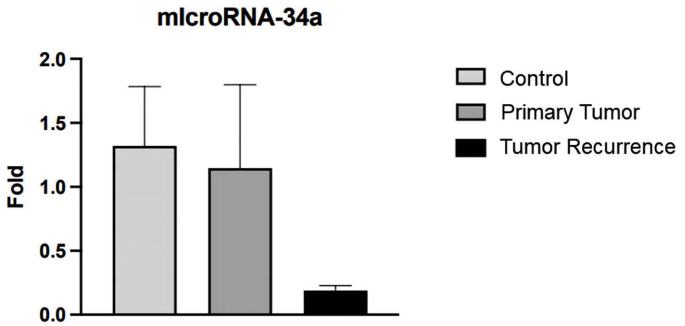

Figure 1. Representation of the mean values (±standard error) of microRNA-34a expression between the studied groups. Source: Own preparation (GraphPad Prism software version 9.2).

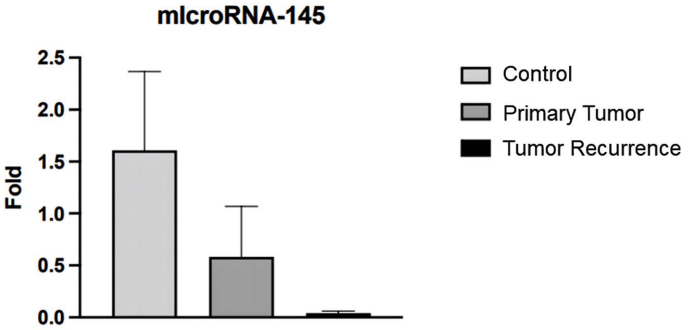

Figure 2. Representation of the mean values (±standard error) of microRNA-145 expression between the studied groups. Source: Own preparation (GraphPad Prism software version 9.2).

MiRNA-145 analysis showed that there was a significant difference (p= 0.0059, Kurskall Wallis test) between the Control Group and the Primary Tumor Group (p= 0.0088 post-test Dunn's multiple comparisons test) and between the Control Group and the Tumor recurrence group (p = 0.0075 post-test Dunn's multiple comparisons test) (Figure 2).

The analysis of miRNA-221 showed that there was no significant difference between the studied groups (p = 0.0826, Kurskall Wallis test) (Figure 3).

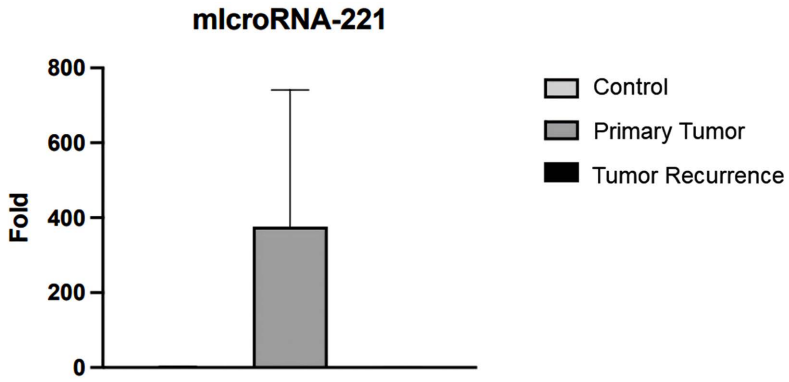

Figure 3. Representation of the mean values (±standard error) of microRNA-221 expression between the studied groups. Source: Own preparation (GraphPad Prism software version 9.2).

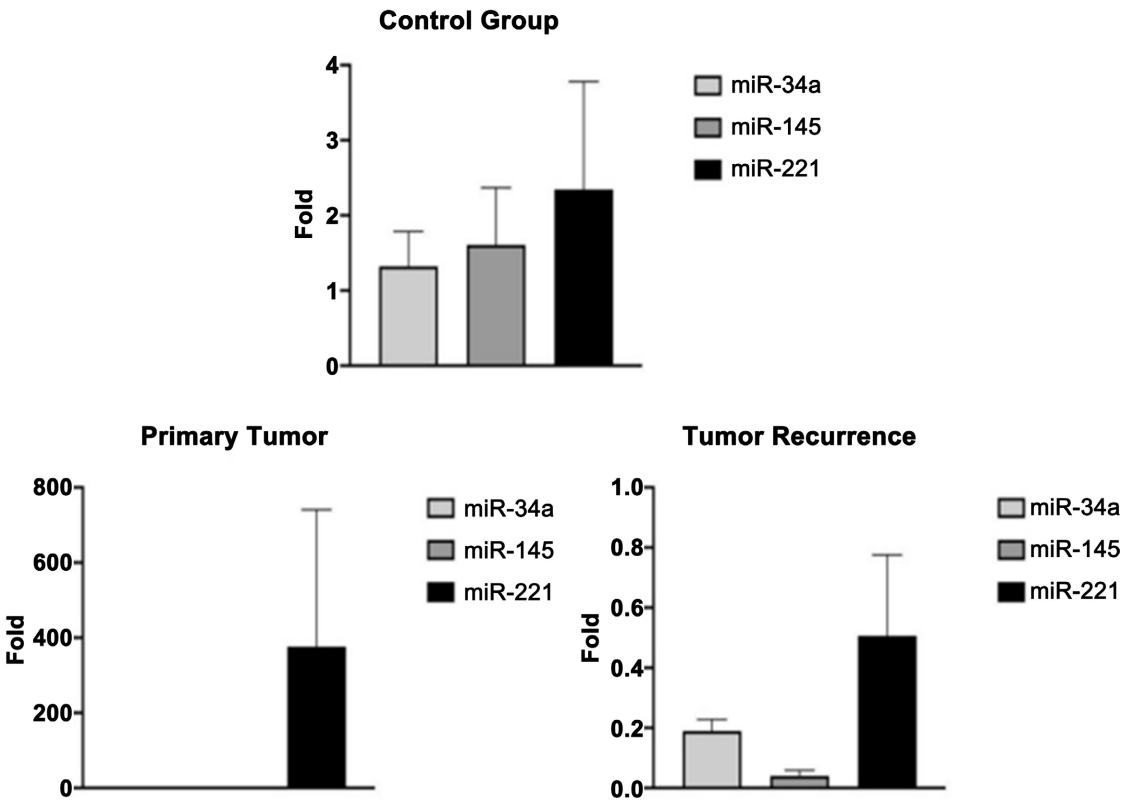

Figure 4. Representation of the mean values (±standard error) of the expression of microRNA-34a, microRNA-145 and miRNA-221 in the studied groups. Source: Own preparation (GraphPad Prism software version 9.2).

The analysis of microRNA expression by group showed that in the Control Group there was no significant difference between the studied microRNAs (p = 0.9976, Kurskall Wallis test), in the Primary Tumors group there was a significant difference (p = 0.0025, Kurskall Wallis test) between miR-34a and miR-145 microRNAs (p = 0.0018 post-test Dunn's multiple comparisons test) and in the Tumor Relapses group there was a significant difference (p = 0.0051, Kurskall Wallis test) between miR-34a and miR-145 (p = 0.0048 post-test Dunn's multiple comparisons test) (Figure 4). When analyzing the expression of microRNAs in the Primary Tumor versus Tumor Recurrence groups, we observed that there was no significant difference: miR-34a (p = 0.4124, Kurskall Wallis test), miR-145 (p = 0.9674, Kurskall Wallis test) and miR-221 (p = 0.6236, Kurskall Wallis test) (Figure 5).

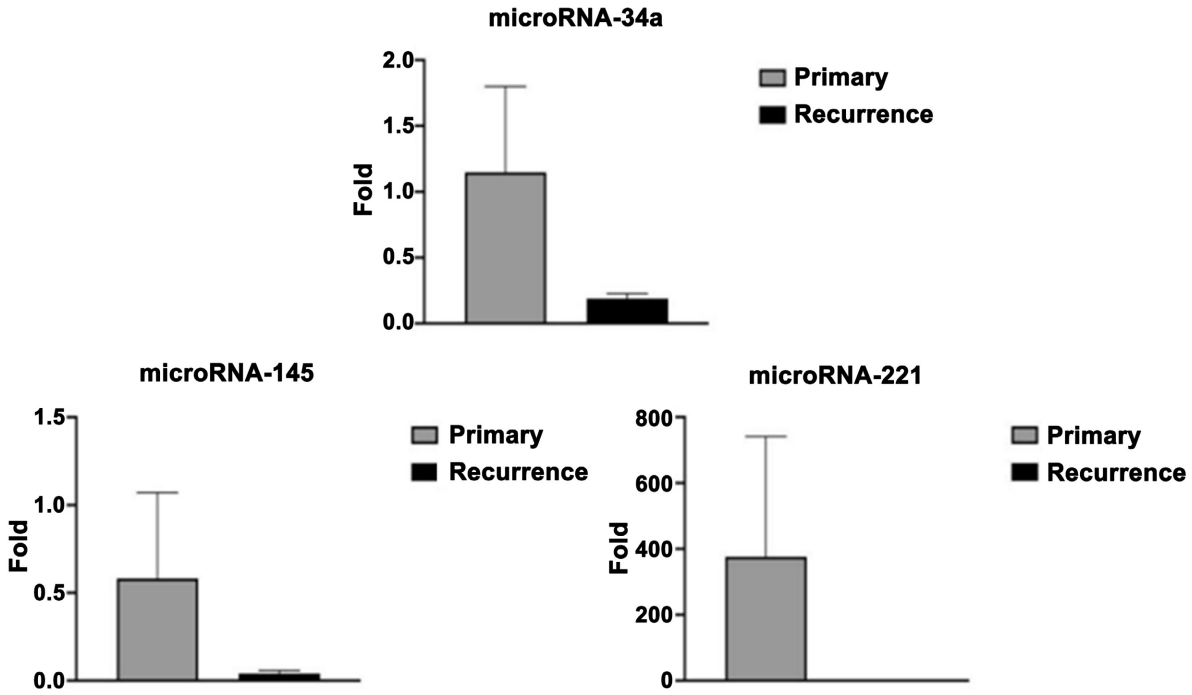

Figure 5. Representation of the mean values (±standard error) of the expression of microRNA-34a, microRNA-145 and miRNA-221 in the comparison between the Primary Tumor and Tumor Recurrence groups. Source: Own preparation (GraphPad Prism software version 9.2).

4. Discussion

MicroRNAs are non-coding, single-stranded RNAs that play regulatory roles in important biological processes, such as cell cycle, proliferation, differentiation, migration, and apoptosis [42]-[49] [60] [61]. They promote the silencing of post-transcriptional gene expression by binding to complementary sites on their target mRNAs and initiating degradation or inhibition of translation. The dysregulated expression of miRNAs has been described in several human diseases, including cardiovascular, autoimmune, inflammatory, neurodevelopmental diseases, and cancer [38]-[40] [42]-[45] [56] [58]-[64]. Altered miRNA expression is associated

with genetic and epigenetic mechanisms. MiRNAs act as oncogenes or tumor suppressors according to their targets [65].

There are three miR-34 homologs (hsa-miRNA34a, hsa-miRNA34b, and hsa-miRNA34c) in humans [60]-[62]. The human miR-34a precursor is transcribed from chromosome 1. It maps to the distal region of chromosome 1p, which is a fragile chromosomal region commonly deleted in some pathologies [66]. It has been shown that miR-34a exerts a tumor suppressor effect in breast, lung and colorectal cancer, involving cell cycle arrest, senescence and apoptosis and that miR-34a-induced apoptosis is at least partially dependent on the presence of wild-type p53 genes, indicating that miR-34a can refeed p53 [60]-[64] [66]. In 2013, Gao *et al.*, when evaluating miR-34a expression in glioma tissues of various grades, found that high-grade gliomas (grades III and IV) had much lower miR-34a expression compared to normal brain tissues. When following up for 72 months, 146 glioma patients also observed that miR-34a expression levels were positively correlated with glioma grades, according to WHO criteria, and that in patients with grade III and IV gliomas, the lowest miR-34a expression correlated with worse progression-free survival and overall survival, thus demonstrating that miR-34a, being downregulated in glioma samples compared to normal brain tissue samples, is associated with grade and prognosis of glioma being an independent prognostic indicator for glioma [65].

In 2015, Ludwig *et al.* analyzed the expression of 1205 miRNAs in different grades of meningioma and histological subtypes using microarrays and quantitative real-time PCR. The study demonstrated the downregulation of miR-34a in high-grade meningiomas, but also in meningothelial meningioma, and that this same miRNA is significantly increased in grade I and II meningiomas, but expression levels significantly decreased in grade III meningiomas compared to normal dural tissue [50].

In 2016, Duan *et al.* investigated the function of miR-34a in U87 human glioma cells by exogenous transfection of cells with a miR-34a mimic, and observed that overexpression of miR-34a inhibited proliferation and induced apoptosis of U87 cells, further demonstrating that the Bcl2 gene was a target of miR-34a, with restoration of Bcl2 expression indicated to partially block miR-34a-induced apoptosis, and concluded that miR-34a was a tumor suppressor in glioma cells, suppressing cell proliferation and inducing cell apoptosis, targeting Bcl2 [61].

In 2017, Werner *et al.*, and in 2019, Hu *et al.*, through their in vitro studies, demonstrated that, in meningioma cells, overexpression of miR-34a-3p resulted in decreased protein levels of SMAD4, FRAT1 and BCL2, inhibiting meningioma cell proliferation, and inducing cellular apoptosis in vitro, while miR-34a-3p inhibition led to increased levels of these proteins [57] [67].

SOX2, NANOG, and OCT4 synergistically regulate other self-renewal genes, thus repressing differentiation genes. In turn, they were amplified from mesenchymal stem cells (MSCs) with miR-34 transfection, demonstrating that this miRNA participates in regulating MSCs and maintaining their undifferentiated

state [68].

Furthermore, miR-34 affects the reprogramming of embryonic fibroblasts into induced pluripotent stem cells (iPS) by regulating genes such as OCT4, SOX2, KLF4, and MYC [69].

In our study, we observed low levels of miR-34a expression in the tumor recurrence group when compared with control and primary tumor groups, which may be associated with the tumor suppressor role of this miRNA as described previously in several studies in the literature, since its hypo expression does not promote the negative regulation of its target oncogenes, contributing to the formation of tumor recurrences [60]-[64] [69].

We can also suggest, according to the miR-34a targets on which we based our hypothesis associated with stem cell pluripotency: OCT4, SOX2 and NANOG, that this miRNA may have an important role in tumor malignancy signature by the regulation of these transcription factors in meningiomas correlated both with progression of the histopathological grade as observed by Ludwig *et al.* (2015) and the emergence of recurrences in our study.

In recent years, studies have reported that miR-145, located on chromosome 5, plays an important role in the regulation of normal cellular functions, while dysregulated expression of this miRNA can result in tumor initiation and malignant progression [58]. Most studies describe it. that miR-145 regulates the expression of different genes and regulates molecular pathways such as cancer cell invasion, migration and metastasis acting as a tumor suppressor consistently with low levels of expression in most cancers, suggesting that miR-145 plays a potential role in the diagnosis, prognosis, and therapy of cancer, since overexpression can increase the sensitivity of cancer cells to chemotherapy, being a promising molecular target in drug resistance [58] [59] [70] [71].

In 2013, Kliese *et al.* studied the role of miR-145 in meningiomas and detected a significant reduction in miR-145 expression in grade II and III meningiomas compared with grade I meningiomas. The authors report an association of miR-145 with high-grade meningiomas and the invasive growth of these tumors [72].

Recently, Zheng *et al.* (2020) demonstrated that miR-145 was related to high-grade meningiomas and played an important role in the growth of brain invasive meningioma and although the molecular mechanism underlying the decreased level of miR-145 in grade II and III meningiomas is still unknown, miR-145 low expression is related to high-grade meningiomas and associated with brain invasion [73].

In a recent literature review, it was observed that by up-regulating miR-145 expression, cyclin-D1 protein and SOX2 transcription factor are downregulated, leading to G0/G1 arrest in patient-derived glioblastoma neurospheres, resulting in growth inhibition. Furthermore, it was observed that miR-145 expression is significantly downregulated in primary non-small cell lung cancer and brain metastases by up-regulating the expression of transcription factors OCT4, MUC-1, EGFR, c-MYC and TPD52 [74]. miR-145, an important regulator of SOX2, KLF4,

and OCT4, is downregulated in Ewing sarcoma cells. Therefore, when overexpressed, it inhibits the epithelial-mesenchymal transition (EMT) process, which leads to reduced tumor growth and metastasis [75].

The human testicular nuclear receptor 4 (TR4) is a gene involved in the progression of prostate cancer, contributing to chemoresistance through the activation of OCT4. Studies have shown that TR4 promotes the downregulation of miR-145 by directly binding to its promoter, thereby inhibiting miR-145's ability to target OCT4. This interaction helps sustain chemoresistance in prostate cancer [76].

The administration of Adiantum pedatum extract (AP), piceatannol (P), or a combination of AP + P, as either therapy or prophylaxis for colon cancer, may exert anticancer effects by increasing the expression of miR-145, P53 mRNA, and PDCD-4, while reducing the mRNA expression of PI3K, AKT, C-MYC, CK-20, SOX-2, OCT-4, and NANOG [77].

According to our analysis on bioinformatics platforms, miR-145 has the same targets (OCT4, SOX2 and NANOG) as miR-34a. It also has low expression levels associated with tumor progression in meningiomas as observed in studies by Kliese *et al.* (2013) and Zheng *et al.* (2020) as well as miR-34a [74]. However, our results showed that, despite a similar expression profile to miR-34a, that is, with a decrease in expression levels observed between the control group and the tumor recurrence group, we also observed a decrease in miR-145 expression between the control and primary tumor groups, which may be associated with their involvement with tumorigenesis described in the literature [58].

Pseudogenes are non-functional gene copies that cannot produce full-length proteins; however, they contribute to oncogenesis by competing with miRNAs for binding to parental genes, thus interfering with miRNA-mediated gene suppression. In this way, they act as "RNA sponges." The OCT4-pg5 pseudogene can promote oncogenesis by competing with miR-145-5p for binding to OCT4B mRNA, a variant of the OCT4 gene. This interaction disrupts the miR-145-5p-mediated negative regulation of OCT4B, ultimately enhancing cell proliferation and invasion in bladder cancer [78].

Balachandran *et al.* (2020) describe the regulation of OCT4 and SOX2 by miR-145 in neurospheres of glioblastoma and brain metastases, which reinforces the potentiality of this miRNA and the need for further studies [79].

MiR-221, located on the chromosome X, has been confirmed to be involved in radiosensitivity regulation of glioblastoma, gastric carcinoma, colorectal carcinoma, and nasopharyngeal carcinoma. A recent study that evaluated malignant meningioma cells of the IOMM-Lee cell line, transfected with mimetics or inhibitors of miR-221 and irradiated with different doses, observed that radiation inhibits proliferation and promotes apoptosis and invasiveness in IOMM-Lee cells and that downregulation of miR-221 expression can reverse this radiation-induced cell invasion, while enhancing the effects of promoting apoptosis and inhibiting radiation proliferation, promoting cellular radiosensitivity. Meanwhile,

the irradiation dose rate was also revealed to affect the cell cycle distribution and cell apoptosis of IOMM-Lee. A high dose rate irradiation induces G0/G1 cell cycle arrest and an apoptosis-promoting effect. These findings suggest that downregulation of miR-221 is a promising method to improve radiotherapy efficacy and prevent post-radiotherapy tumor recurrence. Future investigations of meningioma cells may focus on the mechanisms of interaction between miR-221 and IR-induced EMT and EMT-TFs, which may improve understanding of radiotherapeutic toxicities and achieve more effective prevention of toxicity [80]. Although we did not observe a significant difference, our results showed a significant increase in miR-221 expression levels in the primary tumor group when compared to the control group and the tumor recurrence group. However, two patients in our sample had much higher expression levels, not representing the expression profile of the case series. In our analysis of bioinformatics platforms, miR-221 presented OCT4 and NANOG as targets.

In the current literature, there are few studies that have characterized the role of miRNAs that regulate targets involved in the pluripotency phenotype of stem cells in brain tumors, and our study suggests the potential of miR-34a and miR-145 miRNAs that regulate these targets associated with the emergence of recurrences in meningiomas.

5. Conclusion

The miR-34a and miR-145 microRNAs that regulate the stem cell pluripotency pathway are shown to be hypo expressed in tumor recurrences of grade I meningiomas and miR-221 was not differentially expressed between the studied groups.

Conflicts of Interest

The authors have no conflict of interest.

References

[1] Saraf, S., McCarthy, B.J. and Villano, J.L. (2011) Update on Meningiomas. *The Oncologist*, **16**, 1604-1613. https://doi.org/10.1634/theoncologist.2011-0193

[2] Ortiz-Quintero, B. (2016) Cell-Free microRNAs in Blood and Other Body Fluids, as Cancer Biomarkers. *Cell Proliferation*, **49**, 281-303. https://doi.org/10.1111/cpr.12262

[3] Mayo Clinic (2020) Meningioma. https://www.mayoclinic.org/diseases-conditions/meningioma/symptoms-causes/syc-20355643#dialogId1941967

[4] Kishida, Y., Natsume, A., Kondo, Y., Takeuchi, I., An, B., Okamoto, Y., *et al.* (2011) Epigenetic Subclassification of Meningiomas Based on Genome-Wide DNA Methylation Analyses. *Carcinogenesis*, **33**, 436-441. https://doi.org/10.1093/carcin/bgr260

[5] McNeill, K.A. (2016) Epidemiology of Brain Tumors. *Neurologic Clinics*, **34**, 981-998. https://doi.org/10.1016/j.ncl.2016.06.014

[6] Baldi, I., Engelhardt, J., Bonnet, C., Bauchet, L., Berteaud, E., Grüber, A., *et al.* (2018) Epidemiology of Meningiomas. *Neurochirurgie*, **64**, 5-14.

https://doi.org/10.1016/j.neuchi.2014.05.006

[7] Porter, K.R., McCarthy, B.J., Freels, S., Kim, Y. and Davis, F.G. (2010) Prevalence Estimates for Primary Brain Tumors in the United States by Age, Gender, Behavior, and Histology. *Neuro-Oncology*, **12**, 520-527. https://doi.org/10.1093/neuonc/nop066

[8] Louis, D.N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W.K., *et al.* (2016) The 2016 World Health Organization Classification of Tumors of the Central Nervous System: A Summary. *Acta Neuropathologica*, **131**, 803-820. https://doi.org/10.1007/s00401-016-1545-1

[9] Barresi, V., Caffo, M. and Tuccari, G. (2016) Classification of Human Meningiomas: Lights, Shadows, and Future Perspectives. *Journal of Neuroscience Research*, **94**, 1604-1612. https://doi.org/10.1002/jnr.23801

[10] Ogasawara, C., Philbrick, B.D. and Adamson, D.C. (2021) Meningioma: A Review of Epidemiology, Pathology, Diagnosis, Treatment, and Future Directions. *Biomedicines*, **9**, Article No. 319. https://doi.org/10.3390/biomedicines9030319

[11] Greenberg, M.S. (2010) Handbook of Neurosurgery. 6th Edition, Thieme Publishers.

[12] Perry, A. (2018) Meningiomas. In: Perry, A. and Brat, D.J., Eds., *Practical Surgical Neuropathology: A Diagnostic Approach*, 2nd Edition, Elsevier, 259-298. https://doi.org/10.1016/b978-0-323-44941-0.00013-8

[13] Goldbrunner, R., Minniti, G., Preusser, M., Jenkinson, M.D., Sallabanda, K., Houdart, E., *et al.* (2016) EANO Guidelines for the Diagnosis and Treatment of Meningiomas. *The Lancet Oncology*, **17**, e383-e391. https://doi.org/10.1016/s1470-2045(16)30321-7

[14] Riemenschneider, M.J., Perry, A. and Reifenberger, G. (2006) Histological Classification and Molecular Genetics of Meningiomas. *The Lancet Neurology*, **5**, 1045-1054. https://doi.org/10.1016/s1474-4422(06)70625-1

[15] Stawicki, S. and Guarnaschelli, J. (2017) Recurrent Brain Meningiomas. *International Journal of Academic Medicine*, **3**, 115-118. https://doi.org/10.4103/ijam.ijam_100_16

[16] Fathi, A. and Roelcke, U. (2013) Meningioma. *Current Neurology and Neuroscience Reports*, **13**, Article No. 337. https://doi.org/10.1007/s11910-013-0337-4

[17] Och, W., Szmuda, T., Sikorska, B., Springer, J., Jaskólski, D., Zakrzewska, M., *et al.* (2016) Recurrence-Associated Chromosomal Anomalies in Meningiomas: Single-Institution Study and a Systematic Review with Meta-Analysis. *Neurologia i Neurochirurgia Polska*, **50**, 439-448. https://doi.org/10.1016/j.pjnns.2016.08.003

[18] Lee, Y., Liu, J., Patel, S., Cloughesy, T., Lai, A., Farooqi, H., *et al.* (2010) Genomic Landscape of Meningiomas. *Brain Pathology*, **20**, 751-762. https://doi.org/10.1111/j.1750-3639.2009.00356.x

[19] Gallagher, M.J., Jenkinson, M.D., Brodbelt, A.R., Mills, S.J. and Chavredakis, E. (2016) WHO Grade 1 Meningioma Recurrence: Are Location and Simpson Grade Still Relevant? *Clinical Neurology and Neurosurgery*, **141**, 117-121. https://doi.org/10.1016/j.clineuro.2016.01.006

[20] Mawrin, C. and Perry, A. (2010) Pathological Classification and Molecular Genetics of Meningiomas. *Journal of Neuro-Oncology*, **99**, 379-391. https://doi.org/10.1007/s11060-010-0342-2

[21] Cimino, P.J. (2015) Malignant Progression to Anaplastic Meningioma: Neuropathology, Molecular Pathology, and Experimental Models. *Experimental and Molecular Pathology*, **99**, 354-359. https://doi.org/10.1016/j.yexmp.2015.08.007

[22] Park, J. (2021) Epidemiology, Pathology, Clinical Features, and Diagnosis of

Meningioma. UpToDate.
http://maadi-clinical.byethost8.com/d/topic.htm?path=epidemiology-pathology-clinical-features-and-diagnosis-of-meningioma&i=1

[23] Slavik, H., Balik, V., Vrbkova, J., Rehulkova, A., Vaverka, M., Hrabalek, L., *et al.* (2020) Identification of Meningioma Patients at High Risk of Tumor Recurrence Using MicroRNA Profiling. *Neurosurgery*, **87**, 1055-1063. https://doi.org/10.1093/neuros/nyaa009

[24] Haddad, A.F., Young, J.S., Kanungo, I., Sudhir, S., Chen, J., Raleigh, D.R., *et al.* (2020) WHO Grade I Meningioma Recurrence: Identifying High Risk Patients Using Histopathological Features and the MIB-1 Index. *Frontiers in Oncology*, **10**, Article No. 1522. https://doi.org/10.3389/fonc.2020.01522

[25] Perry, A., Gutmann, D.H. and Reifenberger, G. (2004) Molecular Pathogenesis of Meningiomas. *Journal of Neuro-Oncology*, **70**, 183-202. https://doi.org/10.1007/s11060-004-2749-0

[26] Youngblood, M.W., Miyagishima, D.F., Jin, L., Gupte, T., Li, C., Duran, D., *et al.* (2020) Associations of Meningioma Molecular Subgroup and Tumor Recurrence. *Neuro-Oncology*, **23**, 783-794. https://doi.org/10.1093/neuonc/noaa226

[27] Pereira, B.J.A., Oba-Shinjo, S.M., de Almeida, A.N. and Marie, S.K.N. (2019) Molecular Alterations in Meningiomas: Literature Review. *Clinical Neurology and Neurosurgery*, **176**, 89-96. https://doi.org/10.1016/j.clineuro.2018.12.004

[28] Gao, F., Shi, L., Russin, J., Zeng, L., Chang, X., He, S., *et al.* (2013) DNA Methylation in the Malignant Transformation of Meningiomas. *PLOS ONE*, **8**, e54114. https://doi.org/10.1371/journal.pone.0054114

[29] Holland, H., Mocker, K., Ahnert, P., Kirsten, H., Hantmann, H., Koschny, R., *et al.* (2011) High Resolution Genomic Profiling and Classical Cytogenetics in a Group of Benign and Atypical Meningiomas. *Cancer Genetics*, **204**, 541-549. https://doi.org/10.1016/j.cancergen.2011.10.007

[30] Bi, W.L., Mei, Y., Agarwalla, P.K., Beroukhim, R. and Dunn, I.F. (2016) Genomic and Epigenomic Landscape in Meningioma. *Neurosurgery Clinics of North America*, **27**, 167-179. https://doi.org/10.1016/j.nec.2015.11.009

[31] Petrilli, A.M. and Fernández-Valle, C. (2015) Role of Merlin/NF2 Inactivation in Tumor Biology. *Oncogene*, **35**, 537-548. https://doi.org/10.1038/onc.2015.125

[32] Domingues, P., González-Tablas, M., Otero, Á., Pascual, D., Ruiz, L., Miranda, D., *et al.* (2015) Genetic/Molecular Alterations of Meningiomas and the Signaling Pathways Targeted. *Oncotarget*, **6**, 10671-10688. https://doi.org/10.18632/oncotarget.3870

[33] Matthias, S., Andreas, D. and Jefrey, L. (1995) Allelic Losses on Chromosomes 14, 10 and 1 in Atypical and Malignant Meningiomas: A Genetic Model of Meningioma Progression. *Cancer Research*, **55**, 4696-4701.

[34] Simon, M., Boström, J.P. and Hartmann, C. (2007) Molecular Genetics of Meningiomas: From Basic Research to Potencial Clinical Applications. *Neurosurgery*, **60**, 787-798. https://doi.org/10.1227/01.neu.0000255421.78431.ae

[35] Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of Cancer: The Next Generation. *Cell*, **144**, 646-674. https://doi.org/10.1016/j.cell.2011.02.013

[36] Freitag, D., McLean, A.L., Simon, M., Koch, A., Grube, S., Walter, J., *et al.* (2017) NANOG Overexpression and Its Correlation with Stem Cell and Differentiation Markers in Meningiomas of Different WHO Grades. *Molecular Carcinogenesis*, **56**, 1953-1964. https://doi.org/10.1002/mc.22653

[37] Shivapathasundram, G., Wickremesekera, A.C., Brasch, H.D., Marsh, R., Tan, S.T.

and Itinteang, T. (2018) Expression of Embryonic Stem Cell Markers on the Microvessels of WHO Grade I Meningioma. *Frontiers in Surgery*, **5**, Article No. 65. https://doi.org/10.3389/fsurg.2018.00065

[38] Chakraborty, C., Chin, K. and Das, S. (2016) miRNA-Regulated Cancer Stem Cells: Understanding the Property and the Role of miRNA in Carcinogenesis. *Tumor Biology*, **37**, 13039-13048. https://doi.org/10.1007/s13277-016-5156-1

[39] Amaral, B.A., Nonaka, C.F.W., Freitas, R.A., Souza, L.B. and Pinto, L.P. (2010) MicroRNAs—Biogênese, Funções e seu papel potencial na carcinogênese oral. *Odontologia Clínico-Científica*, **9**, 105-109.

[40] Jorge, A.L., Pereira, E.R., Oliveira, C.S.D., Ferreira, E.D.S., Menon, E.T.N., Diniz, S.N., *et al.* (2021) MicroRNAs: Understanding Their Role in Gene Expression and Cancer. *Einstein* (*São Paulo*), **19**, eRB5996. https://doi.org/10.31744/einstein_journal/2021rb5996

[41] Xia, L., Zhang, D., Du, R., Pan, Y., Zhao, L., Sun, S., *et al.* (2008) miR-15b and miR-16 Modulate Multidrug Resistance by Targeting BCL2 in Human Gastric Cancer Cells. *International Journal of Cancer*, **123**, 372-379. https://doi.org/10.1002/ijc.23501

[42] Wang, L., Chen, S., Liu, Y., Zhang, H., Ren, N., Ma, R., *et al.* (2020) The Biological and Diagnostic Roles of MicroRNAs in Meningiomas. *Reviews in the Neurosciences*, **31**, 771-778. https://doi.org/10.1515/revneuro-2020-0023

[43] Pardo, O.E. (2015) Meningioma Dissemination and Growth: A Role for microRNAs. *Oncogene*, **34**, 1743-1744. https://doi.org/10.1038/onc.2014.263

[44] Gareev, I., Beylerli, O., Liang, Y., Xiang, H., Liu, C., Xu, X., *et al.* (2021) The Role of MicroRNAs in Therapeutic Resistance of Malignant Primary Brain Tumors. *Frontiers in Cell and Developmental Biology*, **9**, Article ID: 740303. https://doi.org/10.3389/fcell.2021.740303

[45] Mitha, R. and Shamim, M.S. (2020) Significance of Micro-RNA Expression in Patients with Meningioma. *Journal of the Pakistan Medical Association*, **70**, 1287-1288.

[46] Galani, V., Lampri, E., Varouktsi, A., Alexiou, G., Mitselou, A. and Kyritsis, A.P. (2017) Genetic and Epigenetic Alterations in Meningiomas. *Clinical Neurology and Neurosurgery*, **158**, 119-125. https://doi.org/10.1016/j.clineuro.2017.05.002

[47] El-Gewely, M., Andreassen, M., Walquist, M., Ursvik, A., Knutsen, E., Nystad, M., *et al.* (2016) Differentially Expressed MicroRNAs in Meningiomas Grades I and II Suggest Shared Biomarkers with Malignant Tumors. *Cancers*, **8**, Article No. 31. https://doi.org/10.3390/cancers8030031

[48] Galani, V., Alexiou, G., Miliaras, G., Dimitriadis, E., Triantafyllou, E., Galani, A., *et al.* (2015) Expression of Stem Cell Marker Nestin and MicroRNA-21 in Meningiomas. *Turkish Neurosurgery*, **25**, 574-577. https://doi.org/10.5137/1019-5149.jtn.10800-14.2

[49] Katar, S., Baran, O., Evran, S., Cevik, S., Akkaya, E., Baran, G., *et al.* (2017) Expression of miRNA-21, miRNA-107, miRNA-137 and miRNA-29b in Meningioma. *Clinical Neurology and Neurosurgery*, **156**, 66-70. https://doi.org/10.1016/j.clineuro.2017.03.016

[50] Ludwig, N., Kim, Y., Mueller, S.C., Backes, C., Werner, T.V., Galata, V., *et al.* (2015) Posttranscriptional Deregulation of Signaling Pathways in Meningioma Subtypes by Differential Expression of miRNAs. *Neuro-Oncology*, **17**, 1250-1260. https://doi.org/10.1093/neuonc/nov014

[51] Zhi, F., Shao, N., Li, B., Xue, L., Deng, D., Xu, Y., *et al.* (2016) A Serum 6-miRNA Panel as a Novel Non-Invasive Biomarker for Meningioma. *Scientific Reports*, **6**,

Article No. 32067. https://doi.org/10.1038/srep32067

[52] Zhi, F., Zhou, G., Wang, S., Shi, Y., Peng, Y., Shao, N., *et al.* (2012) A MicroRNA Expression Signature Predicts Meningioma Recurrence. *International Journal of Cancer*, **132**, 128-136. https://doi.org/10.1002/ijc.27658

[53] Kopkova, A., Sana, J., Machackova, T., Vecera, M., Radova, L., Trachtova, K., *et al.* (2019) Cerebrospinal Fluid MicroRNA Signatures as Diagnostic Biomarkers in Brain Tumors. *Cancers*, **11**, Article No. 1546. https://doi.org/10.3390/cancers11101546

[54] Chen, K.S., Stroup, E.K., Budhipramono, A., Rakheja, D., Nichols-Vinueza, D., Xu, L., *et al.* (2018) Mutations in microRNA Processing Genes in Wilms Tumors Derepress the IGF2 Regulator PLAG1. *Genes & Development*, **32**, 996-1007. https://doi.org/10.1101/gad.313783.118

[55] Hart, M., Walch-Rückheim, B., Krammes, L., Kehl, T., Rheinheimer, S., Tänzer, T., *et al.* (2019) miR-34a as Hub of T Cell Regulation Networks. *Journal for Immunotherapy of Cancer*, **7**, Article No. 187. https://doi.org/10.1186/s40425-019-0670-5

[56] Li, X.C., Hai, J.J., Tan, Y.J., Yue, Q.F. and Liu, L.J. (2019) MiR-218 Suppresses Metastasis and Invasion of Endometrial Cancer via Negatively Regulating ADD2. *European Review for Medical and Pharmacological Sciences*, **23**, 1408-1417.

[57] Hu, S., Wei, W., Yuan, J. and Cheng, J. (2019) Resveratrol Inhibits Proliferation in HBL-52 Meningioma Cells. *OncoTargets and Therapy*, **12**, 11579-11586. https://doi.org/10.2147/ott.s228513

[58] Wang, X., Wang, E., Cao, J., Xiong, F., Yang, Y. and Liu, H. (2017) Mir-145 Inhibits the Epithelial-to-Mesenchymal Transition via Targeting ADAM19 in Human Glioblastoma. *Oncotarget*, **8**, 92545-92554. https://doi.org/10.18632/oncotarget.21442

[59] Zeinali, T., Mansoori, B., Mohammadi, A. and Baradaran, B. (2019) Regulatory Mechanisms of miR-145 Expression and the Importance of Its Function in Cancer Metastasis. *Biomedicine & Pharmacotherapy*, **109**, 195-207. https://doi.org/10.1016/j.biopha.2018.10.037

[60] Badr, E.A.E., Ali Assar, M.F., Gohar, S.F., Badr, M.H., Hathout, R.M. and El-kousy, S.M. (2018) The Clinical Impact of miRNA34a and P53 Gene Expression in Colon Cancer. *Biochemistry and Biophysics Reports*, **16**, 88-95. https://doi.org/10.1016/j.bbrep.2018.10.002

[61] Duan, J., Zhou, K., Tang, X., Duan, J. and Zhao, L. (2016) MicroRNA-34a Inhibits Cell Proliferation and Induces Cell Apoptosis of Glioma Cells via Targeting of Bcl-2. *Molecular Medicine Reports*, **14**, 432-438. https://doi.org/10.3892/mmr.2016.5255

[62] Wu, J., Wu, G., Lv, L., Ren, Y., Zhang, X., Xue, Y., *et al.* (2011) MicroRNA-34a Inhibits Migration and Invasion of Colon Cancer Cells via Targeting to Fra-1. *Carcinogenesis*, **33**, 519-528. https://doi.org/10.1093/carcin/bgr304

[63] Kato, M., Paranjape, T., Ullrich, R., Nallur, S., Gillespie, E., Keane, K., *et al.* (2009) The miR-34 MicroRNA Is Required for the DNA Damage Response *in Vivo* in *C. elegans* and *in Vitro* in Human Breast Cancer Cells. *Oncogene*, **28**, 2419-2424. https://doi.org/10.1038/onc.2009.106

[64] Stahlhut, C. and Slack, F.J. (2015) Combinatorial Action of MicroRNAs let-7 and miR-34 Effectively Synergizes with Erlotinib to Suppress Non-Small Cell Lung Cancer Cell Proliferation. *Cell Cycle*, **14**, 2171-2180. https://doi.org/10.1080/15384101.2014.1003008

[65] Gao, H., Zhao, H. and Xiang, W. (2013) Expression Level of Human miR-34a Correlates with Glioma Grade and Prognosis. *Journal of Neuro-Oncology*, **113**, 221-228. https://doi.org/10.1007/s11060-013-1119-1

[66] Ardekani, A.M. and Naeini, M.M. (2010) The Role of microRNAs in Human

Diseases. *Avicenna Journal of Medical Biotechnology*, **2**, 161-179.

[67] Arunachalam, G., Upadhyay, R., Ding, H. and Triggle, C.R. (2015) MicroRNA Signature and Cardiovascular Dysfunction. *Journal of Cardiovascular Pharmacology*, **65**, 419-429. https://doi.org/10.1097/fjc.0000000000000178

[68] Murnyák, B., Bognár, L., Klekner, Á. and Hortobágyi, T. (2015) Epigenetics of Meningiomas. *BioMed Research International*, **2015**, Article ID: 532451. https://doi.org/10.1155/2015/532451

[69] He, M., Gao, L., Zhang, S., Tao, L., Wang, J., Yang, J., *et al.* (2013) Prognostic Significance of miR-34a and Its Target Proteins of FOXP1, P53, and BCL2 in Gastric MALT Lymphoma and DLBCL. *Gastric Cancer*, **17**, 431-441. https://doi.org/10.1007/s10120-013-0313-3

[70] Werner, T.V., Hart, M., Nickels, R., Kim, Y., Menger, M.D., Bohle, R.M., *et al.* (2017) miR-34a-3p Alters Proliferation and Apoptosis of Meningioma Cells *in Vitro* and Is Directly Targeting SMAD4, FRAT1 and BCL2. *Aging*, **9**, 932-954. https://doi.org/10.18632/aging.101201

[71] Mohammadian, F. and Negahdari, B. (2017) Isolation and Characterization of Mesenchymal Stem Cells and Its Antitumor Application on Ovarian Cancer Cell Line. *Artificial Cells, Nanomedicine, and Biotechnology*, **46**, 1744-1753. https://doi.org/10.1080/21691401.2017.1391824

[72] Wang, C., Zhang, Z., Piao, S. and An, T. (2012) Role of MicroRNA in Induced Pluripotent Stem Cell. *Hereditas* (*Beijing*), **34**, 1545-1550. https://doi.org/10.3724/sp.j.1005.2012.01545

[73] Balachandran, A.A., Larcher, L.M., Chen, S. and Veedu, R.N. (2020) Therapeutically Significant MicroRNAs in Primary and Metastatic Brain Malignancies. *Cancers*, **12**, Article No. 2534. https://doi.org/10.3390/cancers12092534

[74] Kliese, N., Gobrecht, P., Pachow, D., Andrae, N., Wilisch-Neumann, A., Kirches, E., *et al.* (2012) miR-NA-145 Is Downregulated in Atypical and Anaplastic Meningiomas and Negatively Regulates Motility and Proliferation of Meningioma Cells. *Oncogene*, **32**, 4712-4720. https://doi.org/10.1038/onc.2012.468

[75] Zheng, J., Pang, C., Du, W., Wang, L., Sun, L. and Xing, Z. (2020) An Allele of rs619586 Polymorphism in MALAT1 Alters the Invasiveness of Meningioma via Modulating the Expression of Collagen Type V Alpha (COL5A1). *Journal of Cellular and Molecular Medicine*, **24**, 10223-10232. https://doi.org/10.1111/jcmm.15637

[76] Guzel Tanoglu, E. and Ozturk, S. (2021) Mir-145 Suppresses Epithelial-Mesenchymal Transition by Targeting Stem Cells in Ewing Sarcoma Cells. *Bratislava Medical Journal*, **122**, 71-77. https://doi.org/10.4149/bll_2021_009

[77] Zhu, J., Qin, P., Cao, C., Dai, G., Xu, L. and Yang, D. (2021) Use of miR-145 and Testicular Nuclear Receptor 4 Inhibition to Reduce Chemoresistance to Docetaxel in Prostate Cancer. *Oncology Reports*, **45**, 963-974. https://doi.org/10.3892/or.2021.7925

[78] Khamis, T., Diab, A.A.A., Zahra, M.H., El-Dahmy, S.E., Abd Al-Hameed, B.A., Abdelkhalek, A., *et al.* (2023) The Antiproliferative Activity of Adiantum Pedatum Extract and/or Piceatannol in Phenylhydrazine-Induced Colon Cancer in Male Albino Rats: The miR-145 Expression of the PI-3K/Akt/p53 and Oct4/Sox2/Nanog Pathways. *Molecules*, **28**, Article No. 5543. https://doi.org/10.3390/molecules28145543

[79] Zhou, W., Yang, Y., Wang, W., Yang, C., Cao, Z., Lin, X., *et al.* (2024) Pseudogene OCT4-pg5 Upregulates OCT4B Expression to Promote Bladder Cancer Progression by Competing with miR-145-5p. *Cell Cycle*, **23**, 645-661. https://doi.org/10.1080/15384101.2024.2353554

[80] Zhang, Q., Song, L., Huo, X., Wang, L., Zhang, G., Hao, S., *et al.* (2020) MicroRNA-221/222 Inhibits the Radiation-Induced Invasiveness and Promotes the Radiosensitivity of Malignant Meningioma Cells. *Frontiers in Oncology*, **10**, Article No. 1441. https://doi.org/10.3389/fonc.2020.01441