Xueji L et al (2023) ADVANCEMENTS IN THERAPEUTIC STRATEGIES: THE ROLE OF BMAL1 IN ENHANCING CEREBRAL GLIOMA ANGIOGENESIS IN ATHLETES THROUGH THE MODULATION OF VEGF AND ANG2. Revista Internacional de Medicina y Ciencias de la Actividad Física y el Deporte vol. 23 (93) pp. 294-309.
Doi: https://doi.org/10.15366/rimcafd2023.93.020

ORIGINAL

ADVANCEMENTS IN THERAPEUTIC STRATEGIES: THE ROLE OF BMAL1 IN ENHANCING CEREBRAL GLIOMA ANGIOGENESIS IN ATHLETES THROUGH THE MODULATION OF VEGF AND ANG2

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Recibido 20 de agosto de 2022 **Received** August 20, 2022
Aceptado 24 de octubre de 2023 **Accepted** October 24, 2023

ABSTRACT

Objective: This study aims to investigate the role of BMAL1 in promoting angiogenesis in cerebral glioma among athletes by regulating vascular endothelial growth factor (VEGF) and angiopoietin-2 (ANG2). **Methods:** Human brain tumor cells, representative of gliomas often observed in athletes, were categorized into three groups: blank, control, and BMAL1-treated research group. Cell growth and apoptosis were analyzed using the MTT assay and flow cytometry, respectively. VEGF and ANG2 expressions, along with microvessel density (MVD), were assessed via immunohistochemistry. The Spearman rank test evaluated the correlation between VEGF and ANG2 levels. Further, groups A, B, and C (representing different pathological grades of human glioma tissues) and BMAL1-treated groups (D, E, and F) were compared to analyze BMAL1's role in modulating VEGF and ANG2. **Results:** The control group showed significantly lower cell survival and higher apoptosis rates compared to the blank group ($P<0.05$). Higher glioma grades correlated positively with increased apoptosis rates ($P<0.05$). VEGF, ANG2, and MVD expressions were significantly higher in the control group than in the blank group ($P<0.05$), with notable differences observed between the study and control groups ($P<0.05$). A positive correlation was found between glioma grade, MVD count, and the expressions of VEGF and ANG2 ($P<0.05$). Groups D, E, and F demonstrated significant differences in VEGF and ANG2 expressions, with group F exhibiting the highest levels ($P<0.05$), indicating BMAL1's

regulatory effect on these angiogenic factors. **Conclusion:** The study confirms that BMAL1 regulates VEGF and ANG2 expression, promoting angiogenesis in cerebral glioma, a condition relevant to athletes due to their unique physiological stresses. This highlights the potential of targeting BMAL1 in developing therapeutic strategies for glioma angiogenesis in athletic populations, providing a novel approach to manage this challenging aspect of cerebral glioma treatment.

KEYWORDS: Glioma, Emerging therapeutics, BMAL1, VEGF, ANG2

1. INTRODUCTION

Cerebral glioma, a form of brain tumor, poses a significant health challenge globally. Its complexity and aggressive nature make it a formidable foe in the realm of neuro-oncology. Athletes, particularly those in contact sports, represent a unique subset of the population at an increased risk for brain injuries and subsequent conditions like gliomas. This study focuses on understanding the role of BMAL1 (Brain and Muscle ARNT-Like 1) in promoting angiogenesis in cerebral glioma, specifically in athletes, by regulating critical angiogenic factors Vascular Endothelial Growth Factor (VEGF) and Angiopoietin-2 (ANG2) (Eichberg et al., 2020).

The relationship between physical trauma, such as that frequently encountered in sports, and the incidence of cerebral gliomas is an area of growing concern(Talos et al., 2006). Athletes are often subjected to repeated head trauma, which has been linked to various neurological conditions. The exploration of this link is crucial, as it may provide insights into targeted therapeutic approaches for athletes suffering from gliomas(Monteiro et al., 2019).

BMAL1, a core component of the circadian clock, has emerged as a significant factor in cancer biology. Its role in the regulation of angiogenesis, a critical process for tumor growth and metastasis, is particularly noteworthy. Angiogenesis, the formation of new blood vessels, is essential for providing tumors with the necessary nutrients and oxygen for growth (ERGÜDEN, KABASAKAL, & KABAKLI, 2020). VEGF and ANG2 are key players in this process, with VEGF being a potent mediator of angiogenesis and ANG2 playing a role in blood vessel maturation and stabilization(Nagaraja et al., 2021; Piegu, Yakubu, Kudese, & Fokuoh). The interaction between BMAL1 and these angiogenic factors could reveal new therapeutic targets and strategies(Franz-Badur et al., 2019; Shahabaz & Afzal).

Given the unique physiological and lifestyle factors associated with athletes, including altered circadian rhythms due to training schedules and potential for increased oxidative stress and inflammation, understanding the specific mechanisms of glioma progression and angiogenesis in this group is

vital. This study aims to dissect the complex interactions between BMAL1, VEGF, and ANG2 in the context of cerebral glioma in athletes(Mesgar et al., 2022; Ye et al., 2020). By doing so, it seeks to pave the way for more effective, tailored therapeutic strategies that address the specific needs and challenges faced by athletes with this condition.

2. Materials and methods

2.1 Research materials

Bacterial strain: human glioma tissues from April 2019 to April 2021, 38 paraffin specimens which were pathologically confirmed as gliomas after surgical resection in our hospital and were archived and still well preserved were selected, the participants' ages ranged from 24 to 61, with a mean of (54.26 3.17 years). There were 23 men and 15 women in all. all of them met the diagnosis of gliomas in the classification criteria for tumors in the central nervous system by the world health organization, and they had not received surgery, chemoradiotherapy or treatment. The tumor diameter was (4.62±1.45)cm according to the Kermohan's classification method (Zi et al., 2019). Nineteen cases were of grade I–II (8, 6, 3, and 2 for star, mixed star, oligodendrocyte, and ependymal cell tumors, respectively), 13 cases were of grade III (8 and 5 for star and oligodendrocytoma, respectively), and 6 cases were of grade IV (4 and 2 for glioblastoma and medulloblastoma, respectively). In addition, 20 cases of normal brain tissue undergoing open craniotomy decompression after craniocerebral injury were taken as the control group.

GIBCO provided the fetal bovine serum and the RPMI-1640 medium, the United States, for the experiments. The National Standard Solution Customization Center provided phosphate buffered saline (PBS), which was used in the experiment. Lipfertamine 3000 transfection reagent was purchased from Invitrogen Co., Ltd., the US. Methylthiazolyldiphenyl-Tetrazolium (MTT) was purchased from Sigma Company of the USA. Coomassie brilliant blue G-250 was purchased from Beijing Solaebo Biological Technology Co., Ltd: Skimmed milk powder purchased from BD Company in the USA; TBST solution was provided by Shanghai Kanglang Biotechnology Co., Ltd.; Polyvinylidene fluoride (PVDF) membrane was purchased from Merck, Germany. BMAL1 primary antibody is purchased from Sigma Company in the US and secondary antibody is purchased from Santa Cruz Biotechnology Company in the US. Anti-VEGF antibody from Santa Cruz Biotechnology; China's Beijing Boson Biotechnology purchased the ANG2 antibody; It was decided to use Shanghai Biyun Biotechnology Co., Ltd.'s RIPA lysis buffer, ECL enhanced luminescent agent, and fluorescent chemiluminescence kit; Tiangen Biochemical Technology (Beijing) Co., Ltd. provided the protein quantitative kit; Fuzhou Maixin Biotechnology Development Co., Ltd. provided the secondary anti-VEGF mouse anti-human Ig-type antibody; DAB color reagent kits were

purchased from Beijing Zhongshan Jinqiao Co., Ltd., 10% formaldehyde, 3% hydrogen peroxide, 95% ethanol, paraformaldehyde, Dimethyl sulfoxide (DMSO) and sodium dodecyl sulfate-poly acrylamide gel (SDS-PACE) were all of analytical grade and provided by China Pharmaceutical Group. Multifunctional microplate reader was purchased from BioTek, the US; Constant temperature incubator was purchased from Thermo Fisher Scientific Shile Technology (China) Co., Ltd.

2.2 Research methods

2.2.1 Cell culture and transfection

Slices of 3–4 μm thick were cut from all specimens that had been fixed in 10% formaldehyde, dehydrated with alcohol, imbedded in paraffin and sliced. Immunohistochemistry revealed the presence of the instructions. In RPMI-1640 media (containing 12 percent FBS, 2 percent glutamine, 1 percent penicillin and streptomycin) in 10 percent FBS, the slices were cultured after dewaxing and hydration in 3 percent hydrogen peroxide and microwave treatment. The incubator's culture environment was set to 37 degrees Celsius, 5% CO2 volume fraction, and 97% humidity. Every two days, the culture media was re-used. The cells were cleaned with PBS solution, 0.25 percent trypsin was added for digestion, and subcultured after they were fused to 80% to 90% of their original size.

The cells of 3–4 generations were selected and washed for 3 times by PBS solution. After centrifugation, the lower layer solution was selected and placed in a 6-well plate with 3x10 cells /ml. 1.5-2 mL culture medium was added for transfection when the cell density was fused to 50%–60%. μL/ well Lipfertamine 3000 transfection reagent was added with si-BMAL1 and out-of-order negative control for transfection into the culture medium cells. It took 5–6 hours to cultivate the cells after they were combined and allowed to stand for 15 minutes at room temperature in RPMI-1640 media without 12 percent fetal bovine serum. The cell microscopic images were collected by a microscope to calculate the total protein of the cells.

2.2.2 Research Group

They were separated into three groups based on the experiment's requirements: ① An 8-hour culture period was used for the blank group, which consisted of normal brain tissue that had undergone a craniocerebral lesion;21 ② Human glioma cells in the control group were cultured for 8h. ③ The study group consisted of human glioma cells with different pathological degrees after BMAL1 treatment, which were classified as grade I ~ II, grade III and grade IV, and cultured normally for 8h.To explore the mechanism of VEGF and ANG2 expression in BMAL1 promoting angiogenesis of glioma, the control group consisted of a variety of human glioma tissues with varying degrees of

pathology (Group A, Group B and Group C), and BMAL1 was applied to the control group as the research group (Group A, Group B and Group C).

2.3 Observation indicators

2.3.1 Western blotting confirmed the presence of BMAL1

Cells in the logarithmic phase after transfection were washed for 3 times by adding PBS buffer, mixed on ice, and then centrifuged for 15min under the condition of 4℃ and 10000 r/min. After the cells were allowed to stand for 10min, the supernatant was discarded, added into an EP tube, and added with an appropriate amount of cell lysate. After the cells were shaken well and lysed for 30min, the supernatant was taken and placed into an EP tube to obtain the total protein of each tissue, which was stored at low temperature for subsequent use. Using the Coomassie brilliant blue G-250 technique and 6 loading buffer, the protein concentration was measured; For 5 minutes, the mixture was cooked in water at 100 C; 5% concentration gel and 10% separation gel were prepared and added into the buffer; Electrophoresis at 80 V and 9 percent SDS-PACE was carried out for 30 minutes; The protein was transferred to a PVDF membrane for transfer printing at 25 V for 30 minutes after a 120 V 10 percent separation gel electrophoresis. Dilution 1:2000 rabbit anti-GFP anti-body and GAPDH Antibody were sealed overnight at 4 C after being blocked for 2 hours with 5% defatted milk powder. In order to wash the membrane a third time with the horseradish peroxidase-labeled secondary antibody, the TBST was replaced with a dilution of 1:50000 and gentle shaking for three hours. The newly prepared ECL enhanced luminescent agent in a ratio of 1:1 was spread on the membrane for color development, and Bio-Rad gel was used for imaging, fixation and quantitative brightness scanning analysis.

2.3.2 MTT assay measuring cell proliferation

Cultured cells in the logarithmic phase were seeded onto 96-well plates, each with 5 × 104 cells, washed with PBS solution and set with five duplicate wells. After culture for 8h, 10μL5 mg/ mL MTT was added for culture for 4h to remove the supernatant, and 100 μL DMSO was added for uniform shaking for 10min. A multipurpose microplate reader assessed the absorbance (A value) of each well at 490 nm. The cells' viability was determined by averaging the findings of three separate experiments.

2.3.3 Flow cytometry to determine apoptosis

A total of 2.5 x 104 cells per well was used to inoculate 24-well plates with the logarithmic phase cells. After adhering to the wall for 8 hours and being cultured in PBS solution, the cells were washed and put to Binding Buffer for suspension. Then, for 15 minutes in the dark, 5 μL of Annexin V-FITC was added and mixed. After 5μL PI was added for staining and incubation for 5min,

the cells were mixed with Binding Buffer again to monitor the apoptosis rate. To calculate the apoptosis rate, the experiment had to be done three times.

2.3.4 Determination of expression levels of VEGF and ANG2 and staining results by immunohistochemistry

96-well plates were inoculated with 5x104 cells/well from the logarithmic phase cells, and the cells were allowed to grow. After being operated by the instructions of UltrasensitiveTMs- pKit, SP, antigen repair and 3% hydrogen peroxide treatment were performed before transfection, then 50μL VEGF and ANG2 primary antibodies were added and cultured at 25 C for 2h, for 1 hour at 37 degrees Celsius, 50 μL murine anti-rabbit Ig secondary antibody was then added, followed by DAB staining and hematoxylin counterstaining. PBS buffer was used as the blank control, and the known positive tissues were the positive controls. Determination of staining results of VEGF and ANG2: In a semi-quantitative manner, the brown or brownish-yellow particles in the tumor cell cytoplasm and cell membrane were regarded as positive, the fields with high positive density and non-repetitive areas were randomly selected from each section in a 400-fold field for positive expression, each field had between 500 and 1000 tumor cells counted. It appears that there is a ratio of positive cells in the area that is equal to (the number of positive cells/the total number of cells) x 100%. There are four levels of the negative degree, with a 0 point for a ratio of 5% or less of positive cells (negative,-), the ratio 5%–10% being 1 point (weak positive,+), the ratio 11%–30% being 2 points (positive,++), and the ratio > 30% being 3 points (strong positive,++++); Staining intensity score: 0 point means colorless, 1 point means weak and pale yellow staining, 2 points means medium and dark yellow staining, and 3 points means obvious and dark brown staining. The positive intensity of the case was determined according to the proportion of the two scores: 0–2 as negative (-), 3–5 as weak positive (+), 6–9 as positive (++) 10–12 as strong positive (+++). To sum up, the total number of indicators ≥6 was high expression, and the total number < 6 was low expression.

2.3.5 Determination of microvascular density (MVD)

Vascular endothelial cells or cell clusters that were brownish or tan-positive in semi-quantitative form, which did not contain cells in the hemorrhagic and marginal reaction zones. For positive expression, fields of view with three positive density and non-repetitive areas were randomly selected under a field 400 times larger than that of the microscope, and the micro vessels per field were counted. Multiple tightly connected endothelial cells or isolated endothelial cells that were only significantly different from nearby tissues or cells were counted as one counting vessel, regardless of the existence of lumen or red blood cells, and the average value of the maximum microvessels counts in the three fields of view was taken as the MVD of the specimen in units/x400.

2.4 Statistical methods

Data processing was carried out by SPSS20.0 statistical software. Normality and homogeneity of variance were checked on all data. For group comparisons, one-way ANOVA was employed, followed by the SNK-q test. The chi-square test was used to compare measurement data reported as n or %. The significance level is set at P<0.05.

3. Outcome

3.1 Effect of BMAL1 on angiogenesis of glioma cells

3.1.1 Proliferation and apoptosis of different groups of cells

The results revealed that the control group's cell viability was much lower than the blank group's, and that the apoptosis rate was significantly higher than the blank group's. P<0.05 indicated that the difference was statistically significant. The research group's cell viability in grades I–II, III, and IV was lower than the control group's, and the cell apoptosis rate was higher. As indicated in Table 1 and Figure 1, the research group's grades I–II, III, and IV were negatively connected with cell viability (P<0.05) and positively correlated with apoptosis rate (P<0.05).

Table 1: Proliferation and apoptosis of different groups of cells

GROUP	BLANK GROUP	CONTROL GROUP	GRADE I~II	GRADE III	GRADE IV
Cell survival rate	102.92±11.72	52.81±8.96a	66.82±10.72b	49.32±7.32bc	41.82±4.01bc
Apoptosis rate	8.45±1.13	12.96±1.27a	15.99±1.30b	20.19±1.48bc	24.87±1.52bc

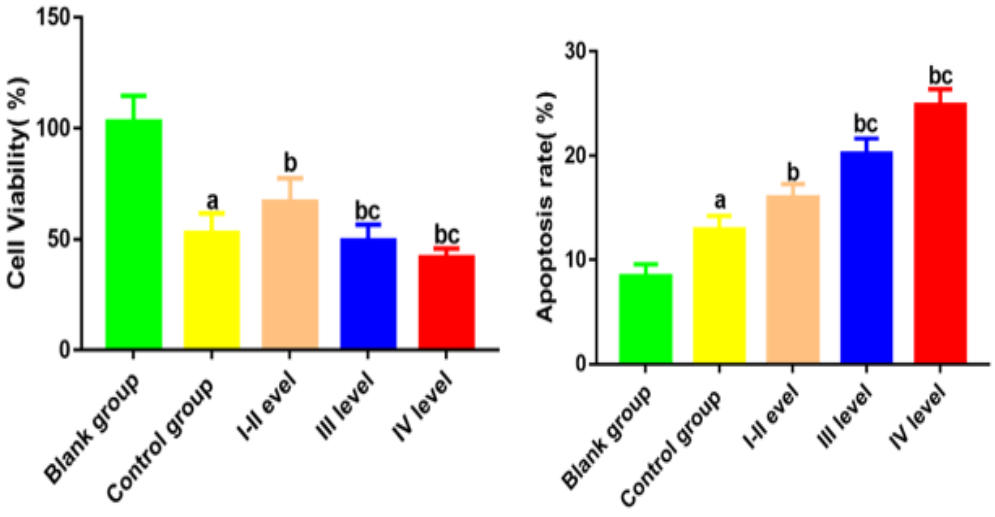

Figure 1: Proliferation and apoptosis of different groups of cells

3.1.2 VEGF and ANG2 expression levels, as well as MVD, were measured in separate groups of cells

The results showed that the control group's VEGF and ANG2 expression levels, as well as MVD count, were much greater than the blank group's, with a statistically significant difference (P<0.05). A statistically significant (P<0.05) difference was found between the research group and the control group in the expression levels of VEGF and ANG2 as well as the number of MVDs. Table 2 and Figure 2 show a significant positive correlation between the VEGF and ANG2 expression levels and the number of MVDs in the research group's grades I–IV (P<0.05).

Table 2: Expression levels of VEGF and ANG2 and MVD count in different groups of cells

GROUP	BLANK GROUP	CONTROL GROUP	GRADE I ~II	GRADE III	GRADE IV
VEGF	50.12±14.36	76.59±17.27a	93.01±19.57b	146.47±24.67bc	189.45±28.53bc
ANG2	218.92±20.11	248.91±26.84a	279.54±31.02b	322.32±38.74bc	389.34±42.23bc
MVD	22.17±4.02	37.91±13.62a	40.32±16.34b	54.82±17.94bc	62.39±20.18bc

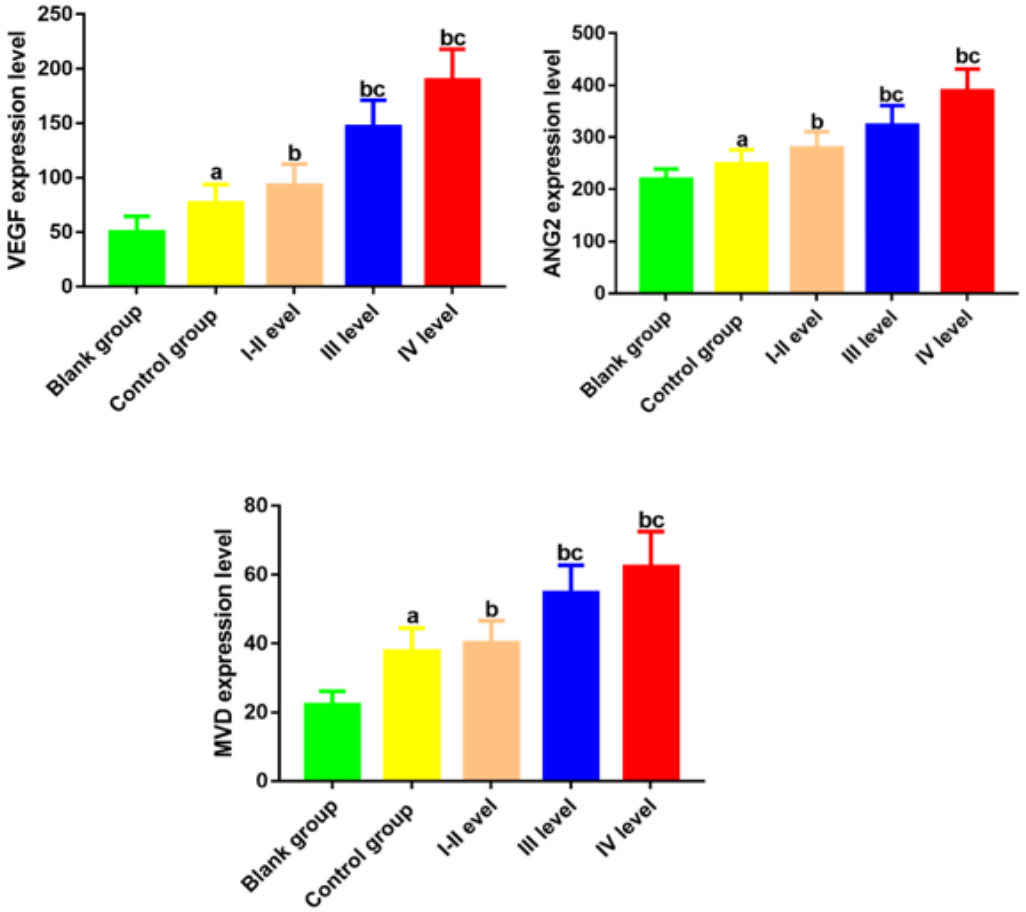

Figure 2: Expression levels of VEGF and ANG2 and MVD count in different groups of cells

3.2 The correlation between VEGF, ANG2 expression, and MVD in glioma

There was a statistically significant correlation between VEGF and ANG2 positive expressions (P<0.05). Glioma angiogenesis can be facilitated by the production of VEGF and ANG2.

As indicated in Table 3 and Figure 3, the MVD values of the VEGF and ANG2 high-expression group were significantly greater than those of the low-expression group, and the differences were statistically significant (P<0.05).

Table 3: The relationship between VEGF, ANG2 expression and MVD in glioma

GROUP	PROTEIN EXPRESSION	MVD	P VALUE
VEGF	Low expression	35.88±13.78	0.042
	High expression	58.92±18.95a	
ANG2	Low expression	41.02±12.63	0.023
	High expression	60.75±17.82a	

***Note*:** *Compared with low expression, a P<0.05.*

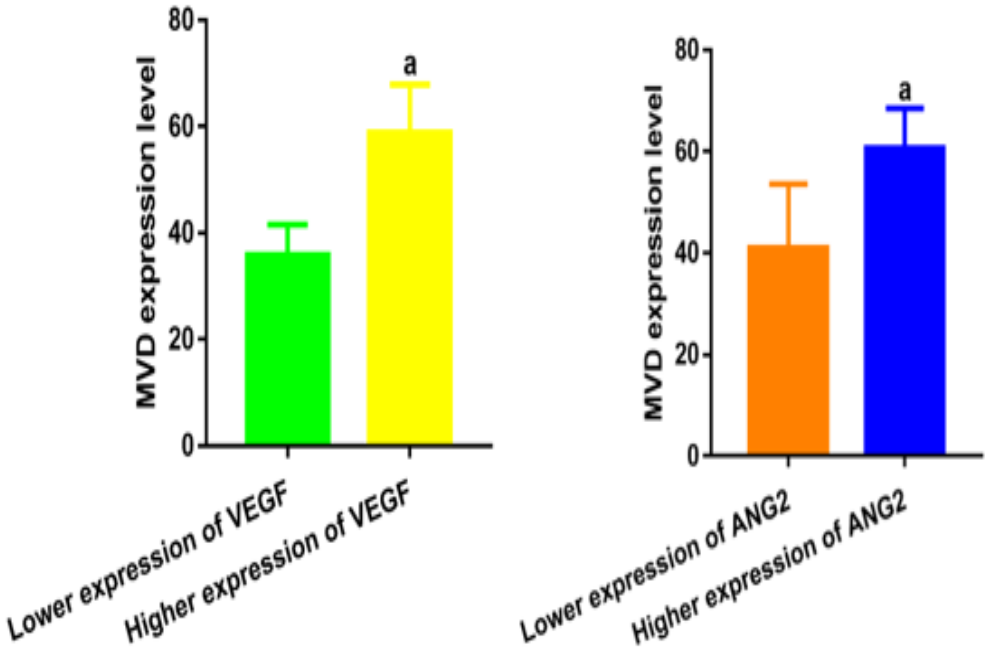

Figure 3: MVD expression level of VEGF and ANG2

3.3 BMAL1 regulates VEGF and ANG2 and promotes glioma angiogenesis

Groups D, E, and F had significantly higher levels of VEGF, ANG2, and MVD expression than did groups A, B, and C (P<0.05), according to the findings. There was a statistically significant difference (P<0.05) between group F and the other two groups when it came to VEGF and ANG2 and MVD expression levels. It is proved that BMAL1 can regulate VEGF and ANG2 to promote angiogenesis of glioma, as shown in Table 4 and Figure 4.

Table 4: BMAL1 regulates VEGF and ANG2 and promotes glioma angiogenesis

GROUP	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	GROUP F
VEGF	93.01±19.57	146.47±24.67	189.45±28.53	108.91±22.45a	159.03±28.78a	240.76±31.99abc
ANG2	279.54±31.02	322.32±38.74	389.34±42.23	283.90±34.21aa	341.93±40.98a	409.91±47.81abc
MVD	40.32±16.34	54.82±17.94	62.39±20.18	43.86±17.53a	59.03±18.72a	70.30±24.45abc

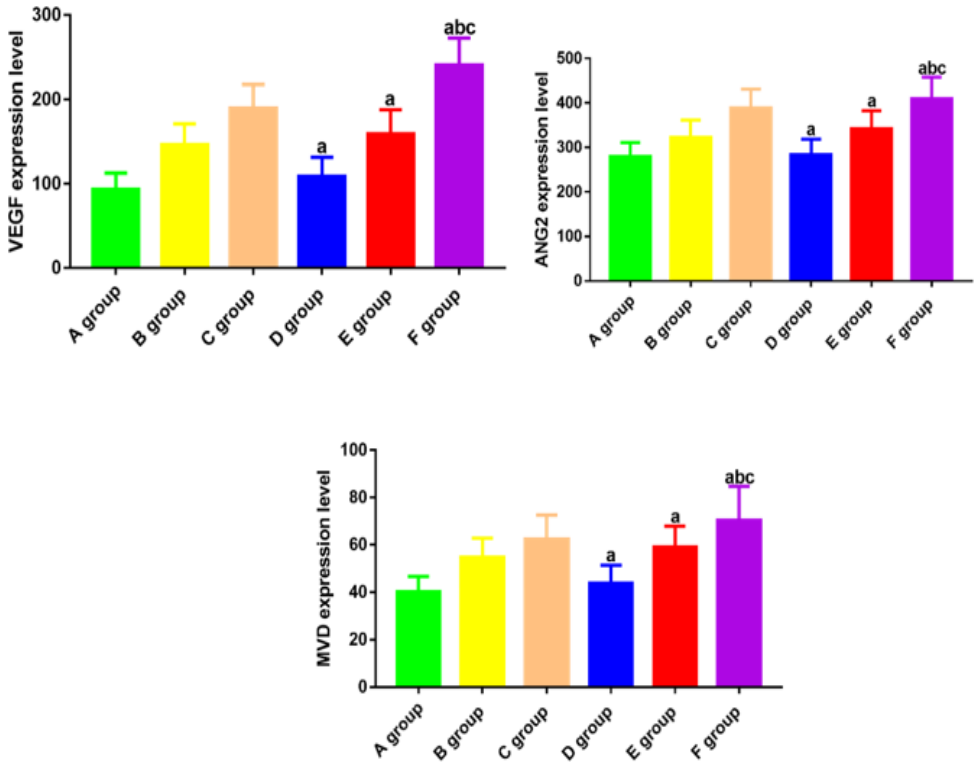

Figure 4: Expression level of VEGF, ANG2 and MVD

4. Discussion

Glioma, as a clinical neuroepithelial malignant tumor, has many causes, including congenital inheritance, carcinogenic factors, electromagnetic radiation and other causes, which leads to the mutation of neuroectodermal glial stem cells and infiltration of neurovessels, with rapid cell differentiation and metastasis, and is easy to compress brain tissue, and finally damages the central nervous system, causing language, vision and limb dysfunction. If the tumor cells continue to progress, it can lead to systemic multiple organ failure and even death (Cote et al., 2021; Suh, Kim, Jung, Choi, & Kim, 2019). At present, the main carcinogenic factors and pathogenesis of the disease are still unclear, and the symptoms and signs are non-specific, resulting in the lack of key breakthroughs in the diagnosis and treatment of the disease (Sun et al., 2020). As a result of their development conditions, invasion and metastatic

spread, some researchers (Ji, Wang, Chen, Zhou, & Liu, 2019) believe that the expression of VEGF and ANG2 can reflect glioma angiogenesis and is associated with glioma cell levels of these proteins. When it comes to angiogenesis, it's still not understood how to regulate levels of VEGF and ANG2. According to research, the TTFL loop plays an important role in maintaining healthy organ function and preventing tumor cell growth. However, clinical studies mostly focus on the free cells in blood vessels, and the cell link between relevant factors in the physiological process of circadian rhythm regulation and tumor blood vessels still needs to be verified (Chiou, Li, Yang, & Sancar, 2020; Knoedler, Ávila-Mendoza, Subramani, & Denver, 2020). In this work, it was found that Bmal1 was involved in the control of VEGF and ANG2 expression levels and enhanced angiogenesis in glioma, which may serve as a possible therapeutic target for glioma treatment.

Eukaryotic cells have a unique process called autophagy. Autophagy-related genes control the activity of the enzyme, cells are involved in the degradation of damaged organelles and macromolecular substances. There are two sides to this process, including cell survival and apoptosis (Edward & Joshua, 2019). When there is significant difference between the internal and external environments of cells, the damaged cytoplasm and organelles can be removed or degraded to provide intracellular energy cycle for protecting the damaged cells, promote the exchange of cytoplasm and organelles, generate new cells, and maintain the water and electrolyte homeostasis. Autophagy is an essential component in the survival, growth, and progression of malignant tumors (Pang et al., 2019; Yang, Wang, Liu, & Wu, 2020). Long Y et al (Long et al., 2020) glutamic acid transport inhibits the VEGF level in glioblastoma by enhancing the expression of regulatory T cell genes, thereby achieving the effects of improving tumor survival rate and intratumor immunosuppression. Quintanilha J et al. (Quintanilha et al., 2022) believed that VEGF and ANG2 could be used as vascular markers of the anti-angiogenic drug bevacizumab, with potential value for predicting vascular hypertension and vascular function. Researchers found that the control group had much less viable cells and a higher rate of apoptosis compared to its blank counterparts, based on this study's findings.

The research group's grade I–II, III–IV cell viability was lower than the control groups, and the apoptosis rate was higher. There was a negative link between the cell viability and apoptosis rate between grades I–II, III, and IV in the research group. All of the control groups had higher levels of the growth factor and angiotensin II (VEGF and ANG2), as well as larger numbers of MVDs. Grade I–II, grade III, and IV VEGF expression and MVD numbers were substantially greater in the study group than in the control group. The grade I–II, grade III and IV in the study group were positively correlated with the expression levels of VEGF and ANG2 and MVD counts. The positive expressions of VEGF and ANG2 were positively correlated. The expressions of

VEGF and ANG2 could promote vascular growth in gliomas. The MVD value of the high-expression group of VEGF and ANG2 was significantly higher than that of the low-expression group. The results were basically consistent with those of Long Y and Quintanilha J, indicating that the expression of VEGF and ANG2 regulated the angiogenesis process of glioma and was closely related to the survival and apoptosis of glioma cells.

Analysis of the causes: Tumor angiogenesis is a process in which tumor cells, vascular endothelial cell (VEC) and immune cells jointly release regulatory factors to inhibit and stimulate capillary angiogenesis. VEGF and ANG2, as important mediators of the above process, stimulate the proliferation and growth of endothelial cells by binding to specific receptors on endothelial cells, resulting in tumor angiogenesis. Among them, VEGF increase that vascular endothelial permeability by stimulating the vesicle vacuole on the tumor VEC, thereby promote the infiltration of stimulating factors and the outflow of plasma proteins, regulate the internal and external environment of the tumor VEC, and promoting cell migration, growth and proliferation, which is beneficial to vascular growth; As an angiogenic hormone, ANG2 protein can participate in angiogenesis and excitation stages, and specifically block the vascular stabilization effect, destroy the vascular basement membrane, increase the permeability of vascular VEC, and promote the sensitive proliferation of effector factors such as VEGF. In addition, ANG2 protein can regulate actin fibers, destroy the VEC structure, promote the angiogenic function of endothelial cells, and facilitate vascular growth. Therefore, controlling the expression levels of VEGF and ANG2 can increase glioma angiogenesis (Wolf & Langmann, 2019; Zhan, Lei, & Yang, 2021).

Circadian rhythm, as an important endogenous mechanism in organisms, is the basic feature of the regular activities of all living organisms, and can guide the body to standardize the instructions through the signal transduction process, and regulate the rhythm of the signals in and around the body today. Relevant studies have confirmed that the circadian rhythm process is not only involved in the normal metabolism, proliferation, apoptosis and survival of organs and tissues in a coordinated and orderly manner, but also involved in the generation and progression of pathological processes in cells and tissues (Brancaccio et al., 2019). Takaguri A et al (Takaguri, Sasano, Akihiro, & Satoh, 2020) found that BMAL1 participated in vascular smooth muscle cells by regulating kinase part, thereby stimulating vascular growth factor to induce vascular proliferation. Compared to groups A, B, and C, the expression levels of VEGF, ANG2, and MVD in groups D, E, and F were found to be significantly higher. Compared with the groups D and E, the group F had the highest significant increase in the expression levels of VEGF and ANG2 and MVD, proving that BMAL1 could regulate VEGF and ANG2 to promote glioma angiogenesis. It was basically consistent with the results of Takaguri A study (Takaguri et al., 2020), indicating that BMAL1 could regulate the expression levels of VEGF and ANG2, leading

to the promotion of angiogenesis in glioma.

BMAL1, belonging to transcription factor family of bHLH-PAS structural domain, is able to participate in negative feedback transcription of biological clock genes, regulate circadian rhythm and maintain the stability of cells and tissues in vital activities. In the above-mentioned process, it mainly binds to E-box boxes of promoters such as Per, cry, and dbp, to initiate transcription of related genes, and stimulate and activate abnormal expressions of VEGF and ANG2 according to the original clock gene mechanism, leading to vascular germination and generation of tumor cells. In addition, promoters such as Per, cry, and dbp will negatively feedback the BMAL1 protein, resulting in its degradation from the heterodimer, further regulating the expression levels of VEGF and ANG2, promoting their vascular epithelial cells, and eventually promoting the angiogenesis of glioma. This study still has significant limitations. The number of glioma tissues available for study is restricted, and there may be deviations between the experimental data and clinical research, which has an impact on the reliability of research results. Angiogenesis was evaluated only by MVD count, without further evaluation of blood indexes and imaging data. There may be other action mechanisms and effects that BMAL1 is involved in the expression of VEGF and ANG2 in glioma cells, which have not been covered in this study yet and need to be further studied.

As a result, in order to improve clinical glioma prognosis and treatment, additional samples, animal experiments, and other sources of information are required. In summary, BMAL1 is able to bind to and activate the expressions of VEGF and ANG2 and increase the number of micro vessels by promoting the development and proliferation of vascular endothelial cells. Therefore, BMAL1 regulates VEGF and ANG2 to promote the angiogenesis process of glioma.

5.Conclusion

This study offers significant insights into the complex interplay between BMAL1 and key angiogenic factors, VEGF and ANG2, in the context of cerebral glioma, particularly within the athletic population. Our findings suggest that BMAL1 acts as a crucial regulatory element in the angiogenesis process of gliomas, evidenced by its influence on cell growth, apoptosis, and the expression levels of VEGF and ANG2. The increased expression of these angiogenic markers in BMAL1-treated groups indicates a direct correlation between BMAL1 activity and enhanced angiogenesis in cerebral glioma. For athletes, who may have an increased risk of cerebral glioma due to factors like repetitive head trauma or intense physical stress, these results are especially pertinent. The ability of BMAL1 to modulate angiogenesis presents a novel and promising therapeutic target for managing cerebral glioma in this specific group. By focusing on the molecular mechanisms driven by BMAL1, new therapeutic strategies can be developed that are tailored to the unique needs of athletes

facing this challenging condition.

In essence, our research shifts the paradigm in understanding and treating cerebral glioma in athletes. It underscores the potential of BMAL1 as a key therapeutic target, opening up possibilities for more effective and targeted interventions. These advancements could not only improve treatment outcomes but also enhance the overall well-being and health management of athletes dealing with cerebral glioma.

Declaration of conflict of interest

None.

Data availability Statement:

The data used to support the findings of this study are available from the corresponding author upon request.

Acknowledgement

This work was supported by Chinese Academy of Medical Sciences and Peking Union Medical College.

References

Brancaccio, M., Edwards, M. D., Patton, A. P., Smyllie, N. J., Chesham, J. E., Maywood, E. S., & Hastings, M. H. (2019). Cell-autonomous clock of astrocytes drives circadian behavior in mammals. *Science, 363*(6423), 187-192.

Chiou, Y.-Y., Li, T.-Y., Yang, Y., & Sancar, A. (2020). A sextuple knockout cell line system to study the differential roles of CRY, PER, and NR1D in the transcription-translation feedback loop of the circadian clock. *Frontiers in Neuroscience, 14*, 616802.

Cote, D. J., Samanic, C. M., Smith, T. R., Wang, M., Smith-Warner, S. A., Stampfer, M. J., & Egan, K. M. (2021). Alcohol intake and risk of glioma: results from three prospective cohort studies. *European journal of epidemiology, 36*, 965-974.

Edward, Y. W., & Joshua, D. (2019). Carotid Artery Stenosis. *Vascular & Endovascular Review, 2*(1).

Eichberg, D. G., Di, L., Morell, A. A., Shah, A. H., Semonche, A. M., Chin, C. N., . . . Komotar, R. J. (2020). Incidence of high grade gliomas presenting as radiographically non-enhancing lesions: experience in 111 surgically treated non-enhancing gliomas with tissue diagnosis. *Journal of neuro-oncology, 147*, 671-679.

ERGÜDEN, D., KABASAKAL, H., & KABAKLI, F. (2020). Young-of-the-year sandbar shark, Carcharhinus plumbeus (Nardo,

1827)(Carcharhiniformes: Carcharhinidae), caught in Iskenderun Bay. *FishTaxa, 18*, 18-22.

Franz-Badur, S., Penner, A., Straß, S., von Horsten, S., Linne, U., & Essen, L.-O. (2019). Structural changes within the bifunctional cryptochrome/photolyase Cr aCRY upon blue light excitation. *Scientific reports, 9*(1), 9896.

Ji, X., Wang, H., Chen, Y., Zhou, J., & Liu, Y. (2019). Recombinant expressing angiopep-2 fused anti-VEGF single chain Fab (scFab) could cross blood–brain barrier and target glioma. *AMB Express, 9*, 1-12.

Knoedler, J. R., Ávila-Mendoza, J., Subramani, A., & Denver, R. J. (2020). The paralogous krüppel-like factors 9 and 13 regulate the mammalian cellular circadian clock output gene Dbp. *Journal of biological rhythms, 35*(3), 257-274.

Long, Y., Tao, H., Karachi, A., Grippin, A. J., Jin, L., Chang, Y., . . . Na, M. (2020). Dysregulation of glutamate transport enhances treg function that promotes VEGF blockade resistance in glioblastoma. *Cancer research, 80*(3), 499-509.

Mesgar, S., Jameie, S. B., Aliaghaei, A., Parvardeh, S., Torabi, A., & Haghparast, A. (2022). Dopamine D1 Receptor-Mediated Regulation of Per1, Per2, CLOCK, and BMAL1 Expression in the Suprachiasmatic Nucleus in Adult Male Rats. *Journal of Molecular Neuroscience*, 1-8.

Monteiro, R. L., Damaceno, D. S., Kimura, L. M., Cirqueira, C. S., Guerra, J. M., & Araújo, L. J. T. (2019). Validation of chromogenic in situ hybridization reactions for DNA and RNA detection in formalin-fixed paraffin-embedded tissue. *Jornal Brasileiro de Patologia e Medicina Laboratorial, 55*, 57-68. doi:10.5935/1676-2444.20190008

Nagaraja, T. N., Elmghirbi, R., Brown, S. L., Rey, J. A., Schultz, L., Mukherjee, A., . . . Sarntinoranont, M. (2021). Imaging acute effects of bevacizumab on tumor vascular kinetics in a preclinical orthotopic model of U251 glioma. *NMR in Biomedicine, 34*(7), e4516.

Pang, H., Dang, X., Ren, Y., Zhuang, D., Qiu, T., Chen, H., . . . Zhang, J. (2019). 3D-ASL perfusion correlates with VEGF expression and overall survival in glioma patients: Comparison of quantitative perfusion and pathology on accurate spatial location-matched basis. *Journal of Magnetic Resonance Imaging, 50*(1), 209-220.

Piegu, M., Yakubu, F., Kudese, T., & Fokuoh, P. Effects of Nordox 75 WG on the Health Quality of Tomatoes, SPR, 2021, Volume 1, issue, 3, Page No.: 113-120. *DOI: https://doi.org/10, 52152*.

Quintanilha, J. C., Liu, Y., Etheridge, A. S., Yazdani, A., Kindler, H. L., Kelly, W. K., . . . Innocenti, F. (2022). Plasma levels of angiopoietin-2, VEGF-A, and VCAM-1 as markers of bevacizumab-induced hypertension: CALGB 80303 and 90401 (Alliance). *Angiogenesis, 25*(1), 47-55.

Shahabaz, A., & Afzal, M. Implementation of High Dose Rate Brachytherapy in Cancer Treatment, SPR, 2021, Volume 1, issue, 3, Page No.: 77-106.

DOI: https://doi.org/10, 52152.

Suh, C. H., Kim, H. S., Jung, S. C., Choi, C. G., & Kim, S. J. (2019). Imaging prediction of isocitrate dehydrogenase (IDH) mutation in patients with glioma: a systemic review and meta-analysis. *European radiology, 29*, 745-758.

Sun, H., Vachha, B., Laino, M. E., Jenabi, M., Flynn, J. R., Zhang, Z., . . . Peck, K. K. (2020). Decreased hand motor resting-state functional connectivity in patients with glioma: analysis of factors including neurovascular uncoupling. *Radiology*, 190089.

Takaguri, A., Sasano, J., Akihiro, O., & Satoh, K. (2020). The role of circadian clock gene BMAL1 in vascular proliferation. *European journal of pharmacology, 872*, 172924.

Talos, I.-F., Zou, K. H., Ohno-Machado, L., Bhagwat, J. G., Kikinis, R., Black, P. M., & Jolesz, F. A. (2006). Supratentorial low-grade glioma resectability: statistical predictive analysis based on anatomic MR features and tumor characteristics. *Radiology, 239*(2), 506-513.

Wolf, A., & Langmann, T. (2019). Anti-VEGF-A/ANG 2 combotherapy limits pathological angiogenesis in the eye: a replication study. *EMBO Molecular Medicine, 11*(5), e10362.

Yang, Z. Y., Wang, Y., Liu, Q., & Wu, M. (2020). microRNA cluster MC-let-7a-1~ let-7d promotes autophagy and apoptosis of glioma cells by down-regulating STAT3. *CNS Neuroscience & Therapeutics, 26*(3), 319-331.

Ye, J., Zhu, J., Chen, H., Qian, J., Zhang, L., Wan, Z., . . . Luo, C. (2020). A novel lncRNA-LINC01116 regulates tumorigenesis of glioma by targeting VEGFA. *International journal of cancer, 146*(1), 248-261.

Zhan, X., Lei, C., & Yang, L. (2021). Sevoflurane inhibits cell proliferation and migration of glioma by targeting the miR-27b/VEGF axis. *Molecular Medicine Reports, 23*(6), 1-9.

Zi, J., Wang, W., Sun, M., Mei, W., Li, S., Li, B., . . . Yu, M. (2019). A high expression of MTERF3 correlates with tumor progression and predicts poor outcomes in patients with brain glioma. *International Journal of Clinical and Experimental Pathology, 12*(5), 1909.