Research Article

The relationship between wnt/β-catenin pathway and muscle-enriched myokine musclin following combined exercise and curcumin consumption in rats with glioblastoma multiforme

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Abstract

This study aims to investigate the effect of resistance-aerobic exercise and curcumin consumption on brain wnt/β-catenin expression and their correlation with muscle-enriched myokine musclin in rats with glioblastoma multiforme. Fourty male Wistar rats were used in this study. After getting familiarized with the research environment, the rats were randomly divided into five groups of eight: healthy control, tumor control, tumor + resistance-aerobic exercise, tumor + Nano-curcumin supplement, and tumor + aerobic-resistance exercise + Nano-curcumin supplement. For tumor induction, glioblastoma stereotaxic injection was utilized in the frontal cortex. The exercise training group did aerobic training and resistance training 3 days a week for 4 weeks. Nano curcumin supplement was gavage with a dose of 80 mg/kg for 4 weeks, 5 days a week. The brain histology changes of the groups with GBM injection confirmed brain tumor induction compared to the healthy control group ($p<0.05$). The expression of wnt/β-catenin genes in all tumor groups showed a significant increase compared to the healthy control group. Treatment with resistance-aerobic exercise and curcumin (especially the combination of both) revealed the greatest decrease in the expression of wnt/β-catenin genes compared to the tumor group. Wnt mRNA had a negative correlation with musclin mRNA in Tumor+N. cur group ($r=-0.905$, $p=0.001$). It seems that the use of nano supplements along with combined resistance-aerobic exercise can control the wnt/β-catenin signaling pathway in brain tumor tissue. In addition, the cross talk between muscle and brain should be more studied with considering different myokine.

Key Words: **Brain cancer, Musclin, Resistance-aerobic exercise, Curcumin, Gene expression**

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Introduction

Brain cancer is a devastating and very debilitating form of cancer. In 2014, there were an estimated 23,000 new cases of brain and nervous system cancer in the United States (Siegel et al., 2014). But over time this number increased. Although this statistic is relatively low compared to the incidence of breast or prostate cancer, brain tumors are challenging due to the poor prognosis and high mortality of this type of cancer. The approximate five-year survival rate is less than 30% (Deorah et al., 2006) and the average survival time is approximately 15 months for glioblastoma which is the most common malignant brain tumor. The impact of brain cancer and related treatments often results in impaired physical abilities, mild, or major cognitive dysfunction, and compromised quality of life. Therefore, adjuvant and supportive care that improves quality of life and reduces these adverse effects should be considered for brain cancer survivors (Wang et al., 2024). There is a large body of evidence to support the prescription of exercise as a form of therapy and support for cancer survivors (Chen et al., 2024). In fact, the American College of Sports Medicine (ACSM) and the American Cancer Society (ACS) are among the international organizations that have provided specific exercise guidelines for survivors of prostate, colon, and hematologic cancers. Previous research has pointed out the relationship between exercise involvement and survival time in patients with glioma (Ruden et al., 2011). Additionally, Cormie et al. provided a theoretical perspective on the potential effects of exercise as a form of supportive care for neuro-oncology survivors (Cormie et al., 2015). In addition, concerning the effect of exercise on the expressed tumor tissue, skeletal muscles are considered as endocrine organs that perform their biological functions through endocrine, autocrine, and paracrine systems by secreting different types of myokines. The amount of secreted myokines varies depending on the intensity, type, and duration of exercise. Recent studies have shown that muscle-derived myokines are strongly involved in the effect of exercise on cancer (Huang et al., 2022). Several myokines, such as interleukin-6 (IL-6), oncostatin M (OSM), secreted protein acidic

and rich in cysteine (SPARC), and irisin, are directly involved by influencing proliferation, apoptosis, drug resistance, and controlling cancer progression (Park et al., 2023). In addition, some myokines can regulate the tumor microenvironment such as angiogenesis and immunity (Huang et al., 2022). Cancer cachexia occurs in 80% of cancer patients is responsible for 22-30% of patient deaths and is characterized by systemic inflammation and muscle mass reduction (Huang et al., 2022). Exercise-induced myokin production is significant in the regulation of cancer cachexia. One of the most important myokines is musclin, and most of its role in controlling cancer cachexia has been evaluated, but its relationship with the tumor tissue itself is limited. Meanwhile, the potentially beneficial effect of exercise, particularly at the cellular level, for brain cancer remains unknown.

Curcumin is a polyphenol extracted from the rhizome of the turmeric plant, Curcuma longa, which has anti-inflammatory and anti-cancer properties (Moon, 2024). Chronic inflammation is linked to the development of cancer. Curcumin regulates various immune modulators including cytokines, cyclooxygenase-2 (COX-2) and reactive oxygen species (ROS), which partially confirms its anticancer effects. Also, it is involved in the down regulation of growth factors, protein kinases, oncogene molecules, and different signaling pathways, such as nuclear factor kappa light chain enhancer of activated B lymphocytes (NF-κB), C-Jun N-terminal kinase (JNK) and signal transducer and activator of signaling transcription (STAT3) (Zoi et al., 2021). Curcumin can also be effective in controlling cancer cells through its polyphenols. In this regard, Pashirzad et al. (2021) confirmed the therapeutic effects of curcumin polyphenols on the treatment of colorectal cancer by regulating the wnt/β-catenin signaling pathway (Pashirzad et al., 2021). The results of Shao et al. (2020) also showed that curcumin suppresses cell proliferation through downregulating lincROR and inactivating Wnt/β-catenin signaling, which confirms that curcumin may be a potential anticancer candidate for patients with carcinoma liver cells (Shao et al., 2020). Hao et al. (2021) stated that curcumin suppresses colorectal tumorigenesis through the Wnt/β-catenin signaling pathway by reducing Axin2 (Shao et al., 2020). However, the Nano effects of this supplement on this pathway, especially in brain cancer, have not been investigated. Nanocurcumin has been found to have a targeted cytotoxic effect on leukemic stem cells that function as cell clones by inducing apoptosis (Beylerli et al., 2022). This effect is because nanocurcumin improves bioavailability and helps to deliver nanocurcumin directly to the tumor site. Wnt signaling is the most common pathway involved in cell cycle regulation and cancer (Zhan et al., 2017). In colon cancer, it has been found that Wnt/β-catenin signaling is one of the key signaling cascades. In vitro, it has been confirmed that activation of Wnt/β-catenin signaling increases the motility of glioblastoma cells by activating ZEB1 and other activators of epithelial-to-mesenchymal transition (Kahlert et al., 2012). Glioma is one of the most resistant intracranial tumors that are resistant to treatment, and the Wnt/β-catenin pathway is closely related to glioma malignancy. In this regard, Wnt/β-catenin signaling plays an essential role in cell proliferation, migration, invasion, and angiogenesis, thus contributing to the development of glioma (He et al., 2019). On the other hand, controlling this pathway can reduce the progression of glioma. There is a strong relationship between the inhibition of this pathway and the exposure of cancer cells to curcumin (Leow et al., 2010). Since exercise training has been able to regulate cellular pathways in preclinical studies, and considering the role of curcumin in the negative regulation of the Wnt/β-catenin signaling pathway, in this study we investigated the antitumor effect of curcumin and resistance-aerobic exercise through controlling Wnt/β-catenin signaling pathway in brain tumor. In the meantime, the correlation between these factors and FHL muscle musclin will be investigated.

Materials and Methods

Animals

Forty-eight-week-old Wistar male rats (223 ± 16.99 g) were purchased from Pasteur Institute, Tehran, Iran. At first, in order to get familiar with the new laboratory environment, the rats were kept individually in transparent polycarbonate cages for one week (all five rats in one cage) with a temperature of 22 ± 2 degrees Celsius and a relative humidity of 55% and were exposed to 12 hours' light and 12 hours' dark period cycle. Food and water were freely available to the rats. After getting acquainted with the laboratory environment and training on the treadmill, the rats were divided into five healthy control groups, tumor group (induction with GBM), tumor + resistance-aerobic exercise (Tumor + Ex), tumor + nanocurcumin supplement (Tumor + N.cur) and tumor+aerobic resistance training+nanocurcumin supplement (Tumor+Ex+N.cur) in such a way that there were eight male rats in each group. Cancer induction was established using glioblastoma cells. After confirmation of cancer induction by the pathologist, the animals entered the training period (3 days of endurance training per week, 3 days of resistance training program per week). All stages of this study were under the supervision of the Ethics Committee of Islamic Azad University, Tehran, Iran (ethics code: IR.IAU.VARAMIN.REC.1402.018).

Brain cancer induction

In the next step, C6 mouse glioblastoma cells (National Center for Genetic Resources) were prepared. C6 cells were cultured in flasks in RPMI medium (Roswell Park Memorial Institute), 300 mg

/ml penicillin, 720 mg/ml streptomycin (Jabarban Hayan Pharmaceuticals) and 2 g/L sodium bicarbonate 10%. The final volume of the cell culture medium was 1000 ml; its pH was adjusted to 1.7. After washing, the supernatant was neutralized with PBS (buffered saline Pho) and 0.025% trypsin-EDTA solution and with 10% FBS medium. Then, the solution was centrifuged at 1200 rpm for 5m and the cells were separated. The initial density for cell culture was considered to be 100,000 cells/cm2. Finally, 10 microliters of trypan blue dye (0.4% by weight and volume) and 90 microliters of cell suspension and neobar slide were used for cell counting and survival. The percentage of stained cells (blue) was determined as the percentage of dead cells.

For tumor induction, animals were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (20 mg/kg). First, the hair of rat's heads were shaved. Then, the animal was fixed by inserting the rod into the ears and fixing the upper teeth to the stereotaxic device (Stoelting1, model 200195504). After making a skin incision in the back of the skull and removing the periosteum, the bone cap was opened using a dental drill. According to the instructions of Swanson's Stereotaxic Atlas (Swanson, 2004), the implant position was determined in the following coordinates and marked on the bone:

2.0 mm anteroposterior, 2.0 mm laterolateral, and a depth of 2.5 mm.

The cells were diluted with a concentration of 5x105 cells/30 µL. A Hamilton syringe was used to implant 10 microliters of culture medium cells in the right frontal cortex. The cells were slowly injected for 10min. The syringe was left in position for an additional 2 min before the removal. To avoid drawing the injected solution into the needle, the syringe was slowly lifted until it was completely out of the brain. Then the bone was closed using wax and the skin was sutured using cotton thread.

Exercise training protochol

After the induction of cancer cells in the animals and a period of one-week familiarization training (rehabilitation), the animals entered the main training body. The main resistance-aerobic training program was designed for four weeks by adjusting the previous programs and the initial pilot on rats; in this way, the aerobic training phase was performed for 20-35 min at a speed of 18 m/min on the treadmill, and the resistance training phase was performed at the same time in the range of 30 to 100 percent of body weight by tying weights to the rats' tails in 3 sets with 4 repetitions. It was performed by climbing a ladder (Shamsi et al., 2016).

Gene expression

To prepare Nano curcumin, 500 mg of chitosan, 50 ml of 2% acetic acid solution, curcumin, ethanol (1 mg/ml) and 15 ml of 1% TPP solution were used. The prepared solution was stirred for 1 h and centrifuged for 30 min at a speed of 10,000 rpm, and chitosan nanoparticles encapsulated in curcumin were obtained. Finally, after preparation of the product for each animal, 80 mg/kg of N-CUR was gavage for 4 weeks, 5 days per week (24). Forty-eight h after the last training session, the rats were anesthetized with ketamine/xylazine (ratio 1.3) and blood sampling was collected. After making sure that the animal was sacrificed, the brain tissue (half of the tumor area) and the FHL muscle were removed and transferred to a -80°C refrigerator using a nitrogen tank. Real-time PCR technique was used to investigate the expression of Wnt and β-catenin genes in brain glioblastoma and

Table 1. Concurrent training program for study training groups.

Frequency	Duration	Intensity	Sets & Repetitions	Week	Training program	
3days/week	20min/day	18meters/min		1	Phase Training Aerobics	Coccurrent Training
3days/week	25min/day	18meters/min		2		
3days/week	30min/day	18meters/min		3		
3days/week	35min/day	18 meters/min		4		
3days/week	30-60 breakتRepetition 3min between sets	30 percent of body weight	3 sets of 4	1	Resistance Training Phase	
3days/week	3-60 break 3 min between sets	50 percent of body weight	3sets of 4 Repetitive exercise	2		
3days/week	3-60 break Repition 3 min between sets	80 percent of body weight	3 sets of 4 Repetitive exercise	3		
3days/week	3-60 break 3 min between sets	100 percent of body weight	3 sets of 4 Repetitive exercise	4		

muscle musclin. Distilled water containing lyophilized primer 10 microliters, forward primer and reverse primer 0.5 microliters, cDNA 1 and DEPC water 8 microliters were used to prepare the primers. To check gene expression via qRT-PCR method, whole cell RNA was extracted using Kyazol solution and according to Cinagen company protocol. The quality of the extracted RNAs was evaluated by spectrophotometry. To prepare single-stranded cDNA, Oligo dt primer and reverse transcription enzyme were employed according to the relevant protocol. Each PCR reaction was performed in an ABI Step One machine according to the manufacturer's protocol. Real-time PCR reaction cycles for Wnt and β-catenin genes of the brain and muscle musclin were performed at three temperatures of 94, 60 and 72 degrees Celsius. A melting chart was conducted to check the accuracy of PCR reactions. GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) was used as reference gene for Wnt, β-catenin and musclin. The expression levels of control and experimental genes were measured together. The primers used are displayed in Table 2.

Table 2. Primer sequences.

Gene	Primer
GAPDH	Forward: CAAGTTCAAGGGCACAGTCA
	Reverse: CCCCATTTGATGTTAGCGGG
Wnt	Forward: CCACATGAAGCCCGTAGACA
	Reverse: TTCTGCTCCCACCCAAATCC
β-catenin	Forward: CTTCCAGACACGCCATCATG
	Reverse: CGACCCAAGCATTTTCACCA
Musclin	Forward: CCACCCACAACCAGAGAAGA
	Reverse: TATGCTCTACAGACCCAGCC

Hematoxylin and Eosin (H&E) staining

The brain tissue (half of the tumor area) was also fixed in 4% formalin. Formalin-fixed, paraffin-embedded tissue was sectioned at 5 µm thickness and stained with hematoxylin and eosin. Histological analysis was evaluated based on scoring criteria.

Statistical analysis

The Shapiro-Wilk test was used to determine the normality of the data. To determine the significance of the difference between the variables in the groups, one-way Anova and Tukey's post hoc test were used. Data analysis was performed using SPSS version 26 software at a significance level of $p \leq 0.05$ and Graph pad prism software was used to draw the graph. Correlations were analyzed using Pearson's Product Moment formula and 95% confidence.

Results

Brain tumor histology

The histological changes of the tumor tissue are shown in Figure 1. Using the quantitative data obtained from the Image J software, the results of the one-way ANOVA test showed that there is a significant difference between the different research groups (F=11.21, p=0.0010). As can be observed, in the histological images, the proliferation values of glioblastoma multi-

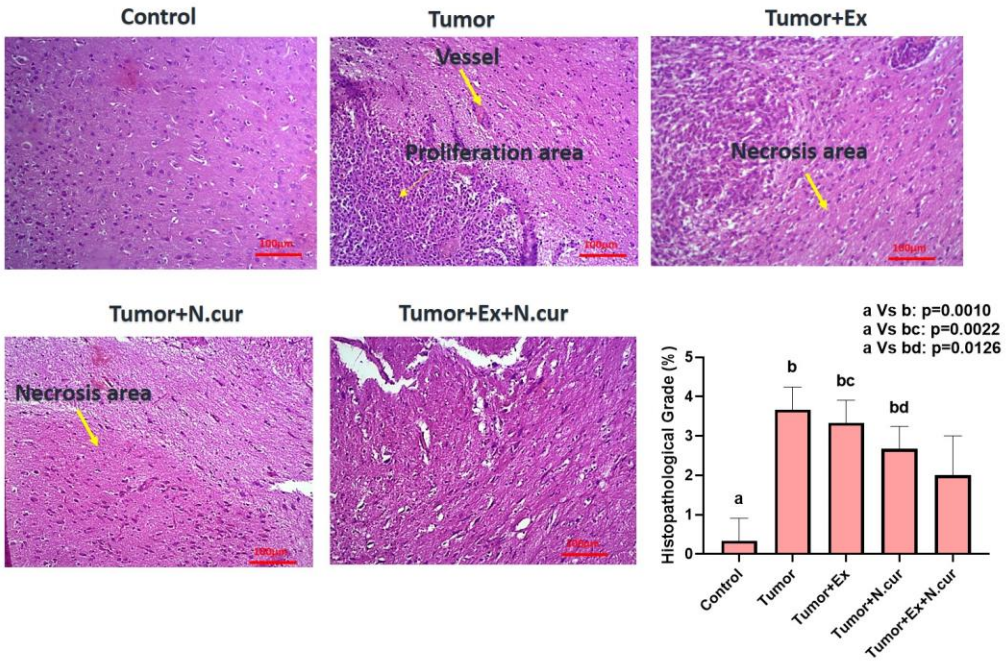

Figure 1. Brain tumor histological changes in different study groups using hematoxylin and eosin (H&E) staining, 100um magnification. Data are shown as mean ± standard deviation. Different letters above each column indicate significance (p<0.05). Control: Tumor: tumor (glioblastoma multiforme), Ex: exercise (resistance-aerobic exercise), N.cur: nanocurcumin

multiforme in the tumor groups have increased significantly compared to the healthy control group (p<0.05). However, exercise training and nanocurcumin supplementation could not cause a significant decrease in the proliferation of tumor cells compared to the tumor control group (p>0.05).

Wnt mRNA expression

Changes in Wnt mRNA expression in different research groups are illusterated in Figure 2. The results of the one-way ANOVA test showed that there is a significant difference between different research groups in the expression of brain tumor Wnt mRNA (F=72.45, p<0.0001). Tukey's post hoc test results showed that GBM injection and brain tumor induction caused a significant increase in all tumor groups compared to the healthy control group (p<0.0001). However, compared to the tumor group, Tumor+Ex (p=0.0039), Tumor+N.cur (p<0.0001) and Tumor+Ex+N.cur (p<0.0001) groups showed a significant decrease in mRNA expression. Wnt showed the brain tumor, the greatest decrease was related to the combined treatment group (Tumor+Ex+N.cur).

β-catenin mRNA expression

Changes in β-catenin mRNA expression in different research groups are shown in Figure 3. The results of the one-way ANOVA test showed that there is a significant difference between the different research groups in the expression of β-catenin mRNA in brain tumors (F=33.46, p<0.0001). Tukey's post hoc test results showed that GBM injection and brain tumor induction caused a significant increase in β-catenin mRNA in all tumor groups compared to the healthy control group (p<0.05).

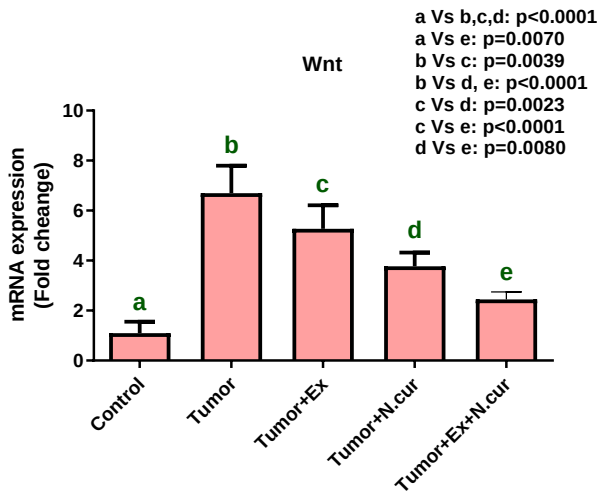

Figure 2. Expression of Wnt mRNA in brain tumor tissue in different study groups using Real time PCR method. Data are shown as mean ± standard deviation. Different letters above each column indicate significance (p<0.05). Control: Tumor: tumor (glioblastoma multiforme), Ex: exercise (resistance-aerobic exercise), N.cur: nanocurcumin.

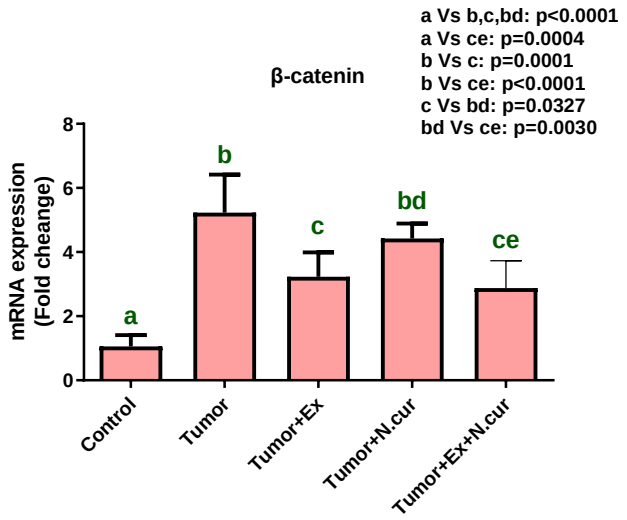

Figure 2. Expression of β-catenin mRNA in brain tumor tissue in different study groups using Real time PCR method. Data are shown as mean ± standard deviation. Different letters above each column indicate significance (p<0.05). Control: Tumor: tumor (glioblastoma multiforme), Ex: exercise (resistance-aerobic exercise), N.cur: nanocurcumin.

However, compared to the tumor group, the Tumor+Ex (p=0.0001) and Tumor+Ex+N.cur (p<0.0001) groups showed a significant decrease in β-catenin mRNA expression in the brain tumor, in which is the most amount of decrease was related to the combined treatment group (Tumor+Ex+N.cur).

Correlation of musclin with Wnt and β-catenin

The correlation between FHL musclin with Wnt and β-catenin in glioblastoma rats is shown in Figures 4 and 5, respectively. In the investigation of the relationship between FHL muscle and Wnt in different research groups, it was found that all groups except the Tumor+N.cur group (r=-0.905, p=0.001) did not have a correlation (Figure 4). Also, in the investigation of the relationship between FHL musclin and β-catenin in different research groups, it was found that the correlation was not confirmed in all groups (Figure 5).

Discussion

The brain, the main organ of the central nervous system, controls and processes most of the body's activities. Therefore, the most aggressive brain tumor, glioblastoma and its metastasis, is fatal and patients have a very short survival time. Apart from surgery and chemotherapy, recently researchers are looking for lifestyle modification or the use of polyphenols to reduce mortality or control the growth of brain tumors, including glioblastoma (Beylerli et al., 2022). In the present study, the aim was to investigate the effect of simultaneous resistance-aerobic training and curcumin consumption on expression of the wnt/β-catenin p-

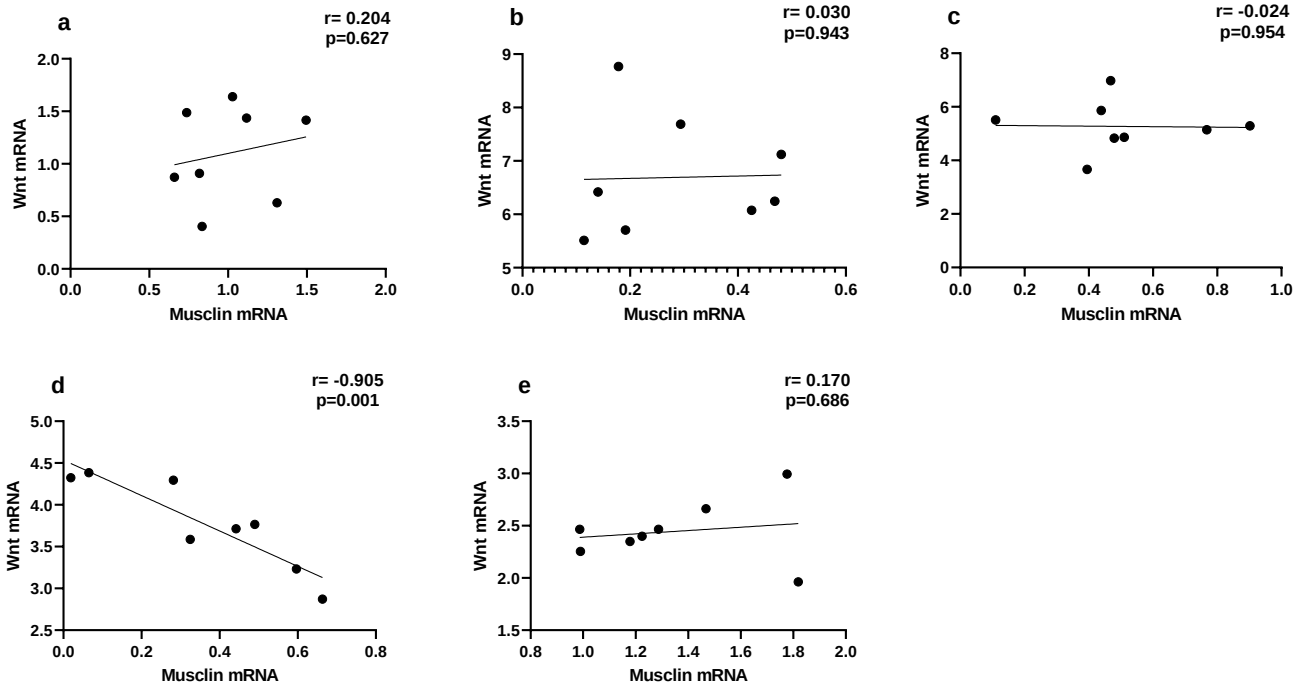

Figure 4. Pearson's correlation coefficients (r) between musclin mRNA and Wnt mRNA at different groups of study (a: control, b: Tumor, c: Tumor+Ex, d: Tumor+N.cur, e: Tumor+Ex+N.cur). The correlations are shown with 95% confidence intervals (dotted lines).

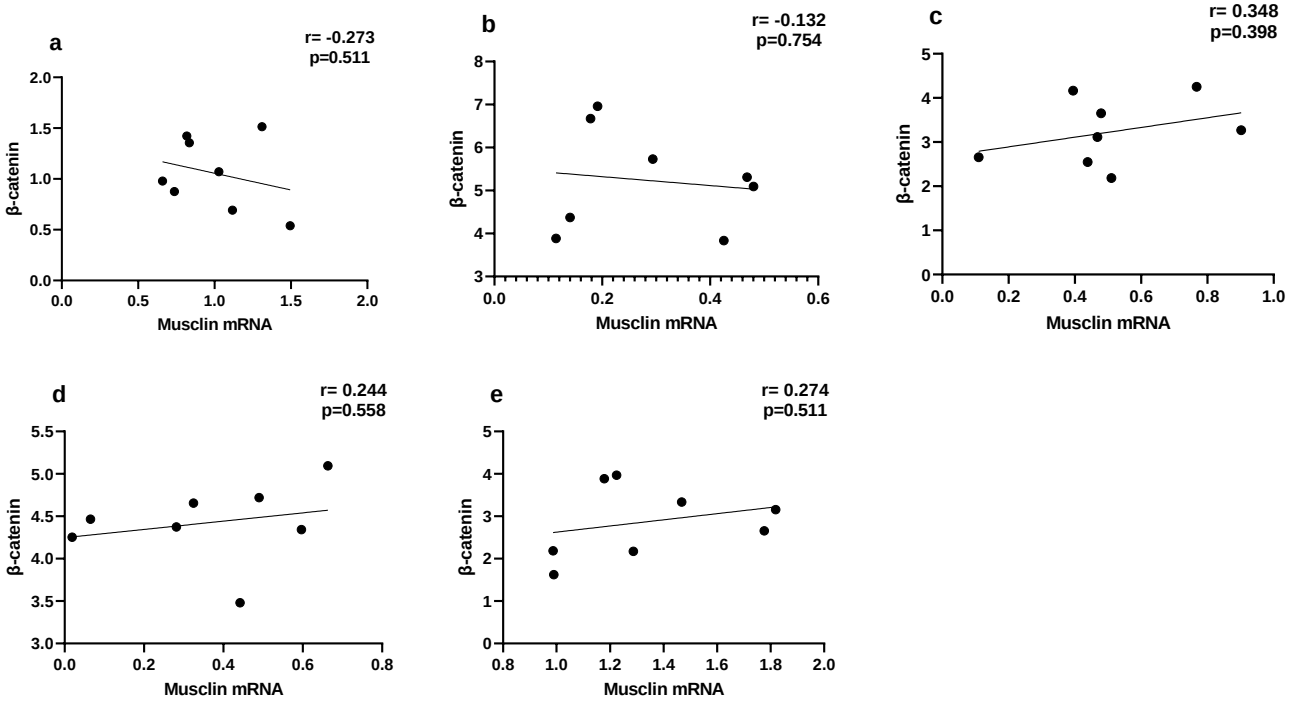

Figure 5. Pearson's correlation coefficients (r) between musclin mRNA and β-catenin mRNA at different groups of study (a: control, b: Tumor, c: Tumor+Ex, d: Tumor+N.cur, e: Tumor+Ex+N.cur). The correlations are shown with 95% confidence intervals (dotted lines).

-athway in the brain of mice affected by glioblastoma multiforme. The correlation between these factors and FHL muscle musclin was also investigated.

In the examination of the histology images of the current study groups, it was found that tumor induction by glioblastoma multiforme was able to increase these cells among nerve cells, which confirms brain cancer. However, treatment modalities did not cause significant histological changes. In relation to tumor induction by GBM, it should be stated that these cells are one of the most malignant types of tumors that have a very high proliferation ability (Sharma et al., 2023), which has been confirmed in many preclinical studies in line with the results of the present study (Dzhalilova et al., 2023). The current standard for the diagnosis and evaluation of GBM is histopathological analysis of tumor samples. The diagnostic criteria of GBM of the World Health Organization are based on the presence of microvascular hyperplasia, cell proliferation, nuclear atypia, architectural disorder and necrosis, and in the present study, the presence of necrosis in brain tissue was confirmed by histological images. Since the histological changes of the treatment groups were not statistically confirmed, we examined the changes at the cellular level, i.e. the Wnt/β catenin pathway.

The results of the present study showed that the expression of Wnt and β catenin genes was significantly increased in brain tumor tissue (glioblastoma). The Wnt signaling pathway is classified into β-catenin-dependent (canonical) and β-catenin-independent (non-canonical) pathways (Rahmani et al., 2018). The canonical Wnt pathway was shown to have an important effect on tumor growth, angiogenesis, invasion, and metastasis (Yüksel et al., 2017). Therefore, the increase of this factor in glioblastoma seems reasonable. In the active form of conventional Wnt signaling, Wnt ligands bind to their receptors and lead to inactivation of β-catenin degradation. As a result, together with Wnt, the β-catenin factor is also activated, and in the present study, the increase of Wnt was also consistent with β-catenin. β-catenin proteins accumulated in the cytoplasm and then translocated to the nucleus, causing increased expression of downstream effectors of Wnt signaling, including cyclin D1, survivin, c-Myc, and matrix metalloproteinase enzymes, which are involved in cancer progression and development (Song et al., 2015). Therefore, controlling the Wnt/β catenin pathway can control the progress and development of cancer. In the present study, it was found that resistance-aerobic exercise and nanocurcumin supplementation, especially the combination of these two methods, significantly reduced Wnt and β-catenin genes. Consistent with the results of the present study, Chen et al. (2020) showed that exercise (running on a treadmill) reduces brain aging by negatively regulating Wnt/β catenin signals in aged mice (Chen et al., 2020). However, in the present study, this pathway was evaluated in the tumor sample. Contrary to the results of the present study, Fang et al. (2017) investigated the effect of aerobic exercise on the Wnt/β catenin pathway in the cerebral cortex and hippocampus of rats and showed that eight weeks of aerobic exercise on a treadmill can increase the activity of the Wnt/β pathway activate downstream gene transcription in the cerebral cortex and hippocampus of mice (Fang et al., 2017). It seems that the difference in the study design (healthy rats vs cancer rats in the present study), as well as the duration and type of training (8 weeks of aerobic vs. 4 weeks of resistance-aerobic in the present study), are among the reasons for the difference in the results of this study and that of Fang et al. Regarding the effect of curcumin on this pathway, in line with these results, Ghasemi et al. (2019) showed that curcumin can play a therapeutic role by inhibiting NF-κB and also the Wnt/β catenin pathway in cervical cancer cells (Ghasemi et al., 2019). Contrary to the results of the present study, Tiwari et al. (2014) showed that curcumin nanoparticles induced neurogenesis in adults through the activation of the conventional Wnt/β-catenin pathway, and it may be a therapeutic approach for the treatment of neurodegenerative diseases such as Alzheimer's disease by increasing the self-healing mechanism of the brain (Tiwari et al., 2014). The conventional pathway of Wnt/β-catenin can be effective in the proliferation of neurogenesis; however, in pathological conditions such as glioblastoma, this proliferation is considered a negative thing, which in the present study was observed with the negative regulation of Wnt/β-catenin in the exercise group and Nanocurcumin supplements have also reduced GBM cell proliferation. Bagharian et al. (2020) revealed that in the model of glioblastoma multiforme, treatment with nano curcumin significantly reduced the expression level of genes related to the Wnt pathway (β-catenin, cyclin D1, Twist and ZEB1) (Bagherian et al., 2020). According to the reviewed research, in pathological models, especially cancer, exercise and nano supplements can have significant effects on the Wnt/β-catenin pathway. Hence, the present study was able to control this pathway by combining these two treatment modalities.

In the present study, to investigate the relationship between muscle contraction and exercise on the tumor, muslin factor was also investigated. In this regard, it has been confirmed that exercise can continuously stimulate the contraction of skeletal muscles and, as a result, release a large amount of myokine. These myokines increase skeletal muscle hypertrophy autocrinely (Hoffmann & Weigert, 2017). Once released into the systemic circulation, myokines facilitate communication between muscle and other organs (Severinsen & Pedersen, 2020). The hypothesis of the present study was also based on the same basis, but it was not confirmed because the results of this study showed that the changes of musclin mRNA were negatively correlated with Wnt mRNA only in Tumor+N.cur group. However,

this correlation was not confirmed in sports groups. Nano supplements seem to be able to regulate tumor growth through myokine modulating pathway, but more detailed studies are needed in this field. On the other hand, exercise is one of the most important factors that play a role in the development of myokines with contraction. In the present study, only musclin mRNA levels were evaluated in FHL muscle tissue, but their protein levels were not evaluated in serum and tumor tissue itself, which could be effective in confirming this hypothesis. Therefore, future studies must pay enough attention to this issue. However, since in cancer, exercise through muslin prevents cachexia caused by the tumor, it can have an autocrine effect on the tumor tissue itself.

Conclusion

Considering the positive effects of exercise and herbal supplements, it seems that the combination of exercise and nano curcumin supplement can control the growth and proliferation of GBM cells by negatively regulating the Wnt/β-catenin pathway. It also seems that nanocurcumin supplementation plays a role in controlling tumor cell pathways by upregulating muslin. There is a need for more and more detailed studies, especially on human samples in this field.

What is already known on this subject?

Brain cancer is a devastating and very debilitating form of cancer. In 2014, there were an estimated 23,000 new cases of brain and nervous system cancer in the United States. However, over time this number increased.

What this study adds?

use of nano supplements along with combined resistance-aerobic exercise can control the wnt/β-catenin signaling pathway in brain tumor tissue. In addition, the cross talk between muscle and brain should be more studied with considering different myokine.

Organ Cross-Talk Tips:

- It seems that exercise training and muscle contraction in cancer patients can be effective in controlling tumor-signaling pathways by releasing myokines such as muslin.

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None.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All stages of this study were under the supervision of the Ethics Committee of Islamic Azad University, Tehran, Iran (ethics code: IR.IAU.VARAMIN.REC.1402.018).

Informed consent Animal study.

Author contributions

Conceptualization: S.A.; Methodology: Y.K., SMS.; Software: S.A., Y.K.; Validation: S.M.S.; Formal analysis: Y.K.; Investigation: S.A.; Resources: S.M.S.; Data curation: Y.K.; Writing - original draft: S.A.; Writing - review & editing: S.M.S.; Visualization: S.A.; Supervision: Y.K.; Project administration: S.M.S.; Funding acquisition: Y.K.

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