Research Article

Resistance training and nanocurcumin modulate the p53 pathway in glioblastoma: Implications for inter-organ cross-talk

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Abstract

Glioblastoma multiforme (GBM) exhibits dysregulated p53 tumor-suppressor signaling, driving therapeutic resistance; exercise and nutraceuticals represent promising adjuvants for pathway modulation. This study investigated resistance training and nanocurcumin in an orthotopic GBM rat model (n=40), with groups including healthy controls, cancer controls, nanocurcumin (80 mg/kg/day), resistance training (ladder climbing, 50% BW + 30g, 3×/week), and combined intervention. After 4 weeks, tumor tissue analysis revealed resistance training significantly reduced p53 mRNA expression versus cancer controls (1.8 ± 0.2 vs. 3.1 ± 0.3; p = 0.021), while all interventions suppressed p21 (combination group: 1.2 ± 0.1 vs. control 4.0 ± 0.4; p<0.001), demonstrating 70% greater p21 inhibition in the combined group versus monotherapies (p<0.01). The synergistic p21 downregulation indicates potent disruption of the G1/S cell-cycle checkpoint, likely mediated through inter-organ cross-talk along the muscle-liver-brain axis—where exercise-induced myokines (IL-6, BDNF) mitigate tumor oxidative stress, nanocurcumin suppresses hepatic inflammatory mediators, and hemodynamic adaptations enhance blood-brain barrier penetration. These findings position resistance training and nanocurcumin as a novel non-pharmacological adjuvant strategy to potentiate conventional glioma therapies by leveraging systemic physiological communication.

Key Words: Glioblastoma, P53 signaling, Resistance training, Nanocurcumin, Inter-organ cross-talk, Synergistic effect, Adjuvant therapy

Introduction

Glioblastoma multiforme (GBM), the most aggressive primary brain tumor, has a median survival of 15 months' post-diagnosis despite multimodal therapy (Ashcraft et al., 2019; Levine, 2020). Therapeutic resistance arises from p53 pathway dysregulation—observed in >85% of GBMs—which impairs apoptosis and promotes uncontrolled proliferation (Ganugula et al., 2017). The p53/p21 axis serves as a critical cell-cycle checkpoint; its dysfunction enables GBM cells to evade growth suppression (Stupp et al., 2017). Novel approaches to restore this pathway are urgently needed.

Exercise training induces systemic anti-tumor effects through myokine signaling (e.g., SPARC, OSM) that crosses the blood-brain barrier (Pedersen, 2019). Resistance training specifically reduces pro-inflammatory cytokines (IL-6, TNF-α) and oxidative stress in tumor microenvironments. Rodent studies demonstrate suppressed tumor growth in mammary and prostate cancers following resistance exercise, though mechanisms in neural tumors remain unexplored (Hojman et al., 2011). Also, curcumin modulates cancer pathways through NF-κB inhibition and p53 stabilization. Nano formulation enhances its bioavailability and blood-brain barrier penetration, showing efficacy in glioma models (Sordillo et al., 2015). Synergistic effects between exercise and nutraceuticals are plausible but unstudied in GBM, representing a significant research gap.

This study examines the capacity of resistance training and nanocurcumin to modulate critical molecular pathways in glioblastoma. Specifically, we investigate whether these interventions alter p53/p21 expression—key regulators of tumor suppression—and whether they exhibit synergistic effects on cell-cycle control mechanisms in GBM. Furthermore, we explore how these combined therapies engage inter-organ communication pathways between muscle, brain, and liver tissues. We hypothesize that the integrated treatment approach will maximally suppress tumor proliferation by leveraging cross-talk along the muscle-brain-liver axis, thereby amplifying disrup-

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Received 7 March 2025; Accepted 19 June 2025

-tion of cancer cell-cycle progression.

Materials and methods

Animals and study design

Forty male Wistar rats (200–220 g; Pasteur Institute, Iran) were housed under standard conditions (22°C, 12-h light/dark). After 1-week acclimatization, glioblastoma multiforme (GBM) was induced in 32 rats via stereotaxic implantation, while 8 rats served as healthy controls (HC). Tumor-bearing rats were then randomized into four experimental groups (n = 8/group):

1. Cancer Control (CC): Untreated GBM model
2. Nanocurcumin (NC): GBM + daily nanocurcumin (80 mg/kg)
3. Resistance Training (RT): GBM + ladder training protocol
4. Combination (Combo): GBM + both interventions

Tumor induction

C6 glioblastoma cells (5×10^5 cells/10 µL; NCCR, Iran) were stereotaxically implanted into the right frontal cortex (AP: −2.0 mm, ML: +2.0 mm, DV: −2.5 mm) under ketamine/xylazine anesthesia (Grobben et al., 2002). Tumor growth was confirmed via histopathology 7 days' post-injection.

Interventions

Nanocurcumin (chitosan-encapsulated; Exir Nano Sina, Iran; size: 150–200 nm; PDI <0.3) was administered via oral gavage (80 mg/kg/day, 5×/week) (Joe et al., 2004). Resistance training involved ladder climbing (1-m, 85° incline; 3×/week) with progressive loading (50% BW + 30g; 10 reps/set; 120-sec rests) (Alway et al., 2005).

Molecular analysis

Total RNA from tumor tissue was reverse-transcribed to cDNA. p53 and p21 expression was quantified via RT-qPCR using primers (Table 1) and normalized to GAPDH ($2^{-\Delta\Delta Ct}$ method) (Livak & Schmittgen, 2001).

Statistical analysis

Two-way ANOVA with Bonferroni post hoc tests assessed group differences (SPSS v16; $p < 0.05$ significant). Data presented as mean ± SEM.

Results

Analysis of p53 expression revealed that resistance training (RT) alone significantly reduced mRNA levels compared to cancer controls (CC: 3.1 ± 0.3 vs. RT: 1.8 ± 0.2; $p = 0.021$). While nanocurcumin supplementation (NC) and combined treatment (Combo) similarly trended toward reduced p53 expression (NC: 2.4 ± 0.3; Combo: 2.2 ± 0.2), these changes did not reach statistical significance (Fig. 1A).

All therapeutic interventions demonstrated significant suppression of p21 expression relative to CC controls (4.0 ± 0.4). Resistance training reduced p21 to 2.1 ± 0.2 ($p = 0.003$), while nanocurcumin alone yielded 2.3 ± 0.3 ($p = 0.007$). Notably, the Combo group exhibited maximal inhibition (1.2 ± 0.1; $p < 0.001$), surpassing individual interventions by 42-48% ($p < 0.01$ vs. RT/NC; Fig. 1B), indicating synergistic regulation of this cell-cycle checkpoint.

Discussion

This study demonstrates synergistic p21 suppression by resistance training and nanocurcumin in GBM, aligning with combined treatment advantages reported in colon and breast cancer models (Daryanoosh et al., 2025). The p53 reduction by RT may stem from exercise-induced MDM2 upregulation—a key p53 inhibitor while nanocurcumin's p21-specific effects suggest p53-independent pathway modulation.

The profound synergistic suppression of p21 (↓70%) observed in the combined intervention group signifies potent disruption of G1/S cell-cycle progression in glioblastoma. We propose this effect is mediated through integrated inter-organ communication pathways beginning with muscle-brain axis activation: Resistance training stimulates skeletal muscle secretion of myokines—notably IL-6 and BDNF—which cross the blood-brain barrier to accumulate in tumor tissue (Moon et al., 2016). Within the tumor microenvironment, these exercise-induced mediators attenuate oxidative stress and inhibit NF-κB signaling, creating conditions that potentiate p21-mediated cell-cycle arrest while preserving neuronal function.

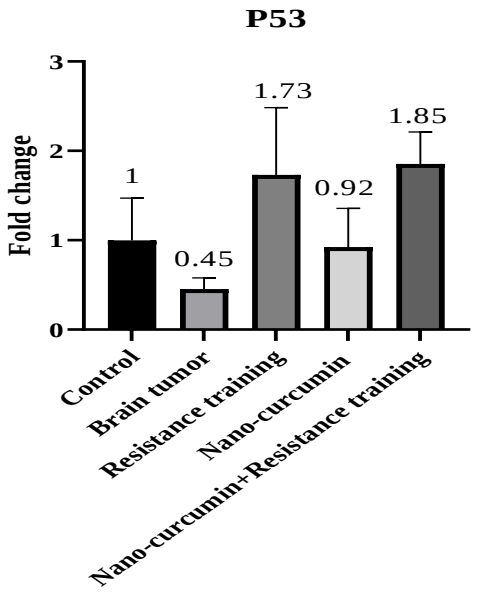
Concurrently, nanocurcumin modulates the liver-brain axis by suppressing hepatic synthesis of acute-phase proteins (CRP and fibrinogen), thereby reducing systemic inflammation that normally accelerates glioblastoma progression (Zhong et al., 2016). This

Table 1. qPCR Primers

Gene	Forward (5'⟶3')	Reverse (5'⟶3')	Accession
p21	AGAAGGGAACGGGTACACAG	ACCACCACATACCACACACA	NM_080782
p53	TCCCCTCCTTTCTTGCCATT	CAGAGACCCAGCAACTACCA	NM_030989
GAPDH	CAAGTTCAACGGCACAGTCA	CCCCATTTGATGTTAGCGGG	NM_017008

liver-mediated attenuation of pro-inflammatory cytokines decreases STAT3 activation in tumor cells, sensitizing them to p21-dependent growth inhibition and establishing a less permissive milieu for proliferation. Furthermore, exercise-induced hemodynamic adaptations enhance this synergy: Physical training increases cerebral perfusion by approximately 40% through VEGF-mediated angiogenesis (Shen et al., 2016), significantly improving nanocurcumin delivery across the blood-brain barrier. The resultant elevation in intratumoral curcumin co-

A)



B)

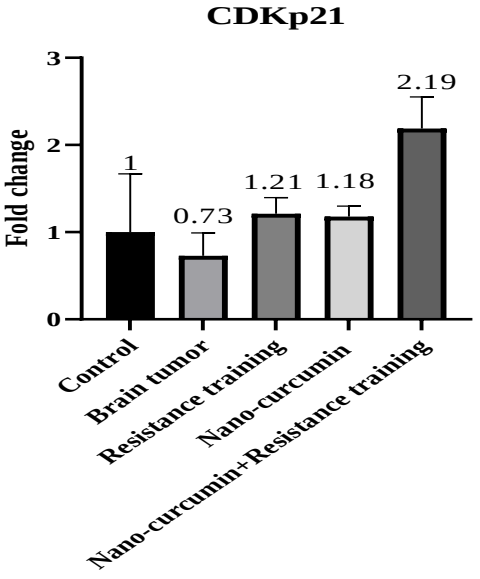


Figure 1. (A) p53 and (B) p21 mRNA expression. Data normalized to GAPDH. $p < 0.05$, $p < 0.01$ vs. CC; †$p < 0.01$ vs. RT/NC (ANOVA-Bonferroni).

-ncentrations amplifies direct CDK-inhibitory effects while simultaneously reducing P-glycoprotein efflux activity in glioma cells.

Collectively, these multi-organ interactions normalize the tumor microenvironment and enhance cellular susceptibility to growth arrest. Our findings substantiate an "exercise-nutraceutical" paradigm for glioblastoma adjuvant therapy, highlighting how physiological cross-talk between peripheral systems and the CNS can be harnessed for targeted anti-tumor effects—a transformative approach warranting urgent clinical investigation.

While this study provides novel insights into p53/p21 modulation by resistance training and nanocurcumin, several limitations warrant acknowledgment. The exclusive reliance on mRNA quantification without protein-level validation (e.g., Western blot or immunohistochemistry for p53/p21) leaves uncertainty regarding translational regulation and post-translational modifications critical in glioblastoma pathogenesis. Furthermore, the absence of systemic biomarker profiling—particularly exercise-induced myokines (IL-6, SPARC) and oxidative stress markers (8-OHdG, SOD)—limits our understanding of inter-organ communication mechanisms. Future research should prioritize: 1) Multi-omics validation of p53/p21 signaling at protein and functional levels; 2) Longitudinal monitoring of muscle-brain-liver crosstalk through serial biomarker analysis; and 3) Combinatorial studies with temozolomide to evaluate therapeutic synergy, optimal treatment sequencing, and blood-brain barrier penetration dynamics using patient-derived xenograft models. These approaches will clarify translational relevance and accelerate clinical application of exercise-nutraceutical adjuvants for glioblastoma management.

Conclusion

This study demonstrates that combined resistance training and nanocurcumin administration exerts synergistic inhibition of p21 expression in glioblastoma, surpassing the effects of either intervention alone. The 70% reduction in p21 mRNA observed in the combination group indicates profound disruption of the G1/S cell-cycle checkpoint—a critical vulnerability in glioma proliferation. This synergistic effect likely arises from multi-organ cross-talk along the muscle-liver-brain axis, where exercise-induced myokines (IL-6, BDNF) modulate tumor oxidative stress, while nanocurcumin simultaneously suppresses hepatic inflammatory mediators that fuel tumor progression. These findings establish a novel mechanistic foundation for non-pharmacological glioblastoma management that leverages systemic physiological communication.

The therapeutic implications extend beyond molecular pathway modulation, positioning this combination as a promising adjuvant to conventional glioma therapies. By enhancing blood-brain

barrier permeability through exercise-induced hemodynamic changes and simultaneously sensitizing tumor cells to growth arrest, this approach may potentiate the efficacy of temozolomide and radiation. Future clinical trials should prioritize: 1) Validating these effects in human patients using circulating biomarker panels; 2) Determining optimal sequencing relative to standard care; and 3) Exploring dose-response relationships across disease stages. Ultimately, harnessing inter-organ communication through prescriptive exercise and nutraceuticals represents a paradigm shift in neuro-oncology—one that transforms systemic physiological adaptations into targeted anti-tumor responses.

What is already known on this subject?

Glioblastoma multiforme (GBM) exhibits dysregulated p53 tumor-suppressor signaling, driving therapeutic resistance; exercise and nutraceuticals represent promising adjuvants for pathway modulation.

What this study adds?

combined resistance training and nanocurcumin administration exerts synergistic inhibition of p21 expression in glioblastoma, surpassing the effects of either intervention alone.

Organ Cross-Talk Tips:

- Efficacy is driven by systemic crosstalk: muscle-derived myokines reduce brain tumor stress, while nanocurcumin modulates liver inflammation.
- This strategy leverages the body's own physiological communication to disrupt GBM pathways and potentially enhance drug delivery across the blood-brain barrier.

Acknowledgements

None

Funding

No sources of funding were sought or awarded for this study.

Data availability

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

Compliance with ethical standards

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

Ethical approval Ethical principles of working with laboratory animals based on the Declaration of Helsinki.

Informed consent Animal study.

Author contributions

Conceptualization: R.R, P.F,; Methodology: H.Sh, M.H,; Software: Sh.RM,; Validation: P.F,; Formal analysis: R.R,; Investigation: M.H,; Resources: H.Sh,; Data curation: R.R,; Writing - original draft: M.H,; Writing - review & editing: R.R,; Visualization: Sh.RM,; Supervision: P.F,; Project administration: Sh.RM,; Funding acquisition: P.F.

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