Research Article

Differential hepatic gene regulation in melanoma: Combined exercise and anti-inflammatory supplementation selectively lowers CXCL2 but not bFGF2

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Abstract

Hepatic gene expression of inflammatory and growth factors such as IL-8 and bFGF2 may be modulated in melanoma metastasis. Non-pharmacological interventions like exercise and anti-inflammatory supplements represent potential complementary strategies for modification. This study aimed to investigate the effects of aerobic exercise, pineapple extract supplementation, and their combination on the hepatic expression of CXCL2/IL-8 HOMOLOG and bFGF2 genes in a murine melanoma model. Melanoma-bearing mice were allocated into four groups (n=5 per group): Control, Aerobic Exercise, Pineapple Extract Supplement, and Aerobic Exercise+Pineapple Extract. After the intervention period, liver tissue was analyzed for CXCL2/IL-8 HOMOLOG and bFGF2 gene expression via one-way ANOVA and Tukey HSD test. Pearson correlation assessed the relationship between the two genes. A significant difference was observed in CXCL2/IL-8 HOMOLOG gene expression between groups ($F=4.211$, $p=0.0239$). Post hoc analysis revealed that only the combined Aerobic Exercise + Pineapple Extract group showed a significant decrease in hepatic CXCL2/IL-8 HOMOLOG compared to the Cancer Control group ($p=0.0251$). In contrast, no significant difference was found in bFGF2 gene expression across groups ($F=1.425$, $p=0.2745$). Correlation analysis indicated a significant negative relationship between CXCL2/IL-8 HOMOLOG and bFGF2 exclusively in the Cancer Control group ($r=-0.948$, $p=0.013$). The combination of aerobic exercise and pineapple extract supplementation significantly reduces hepatic CXCL2/IL-8 HOMOLOG expression in melanoma-bearing mice, suggesting a potential synergistic effect in modulating the hepatic inflammatory microenvironment. The distinct lack of effect on bFGF2 and the specific negative correlation in controls highlight pathway-selective responses.

Key Words: Melanoma, Liver, CXCL2/IL-8 HOMOLOG, bFGF2, Aerobic exercise, Pineapple extract, Gene expression, Murine model

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Introduction

Cutaneous melanoma, an aggressive malignancy arising from melanocytes, represents a significant clinical challenge due to its high propensity for metastatic dissemination (Joshi et al., 2025). The liver is a common site for melanoma metastasis, contributing substantially to patient morbidity and mortality (O'Neill et al., 2024). The establishment of metastatic foci is not a passive process but is critically influenced by the distant organ's microenvironment, which is primed or altered by tumor-derived factors in a process termed pre-metastatic niche formation. Within this context, the hepatic microenvironment undergoes significant molecular reprogramming, characterized by the upregulation of various pro-inflammatory and pro-angiogenic cytokines that facilitate the recruitment and survival of circulating tumor cells (Su et al., 2025). Investigating modifiable factors within this hepatic niche is therefore paramount for understanding how the liver responds to distant melanoma and identifying potential targets for interventions aimed at disrupting the pre-metastatic microenvironment. It is important to note that while hepatic gene expression changes can reflect pre-metastatic niche formation, direct assessment of metastatic burden requires spontaneous metastasis models.

Central mediators of the metastatic niche are specific chemokines and growth factors. Interleukin-8 (IL-8/CXCL8), a potent pro-inflammatory CXC chemokine, plays a multifaceted role in cancer progression (Mir et al., 2023; Shkundin & Halaris, 2024). It is a key recruiter of neutrophils and other myeloid-derived suppressor cells to the tumor site, promotes angiogenesis, and directly enhances tumor cell survival, proliferation, and invasiveness. Elevated systemic and tumoral IL-8 is associated with poor prognosis in various cancers, including melanoma (Zou et al., 2023). Concurrently, basic fibroblast growth factor 2 (bFGF2 or FGF-2) is a fundamental angiogenic factor that stimulates endothelial cell proliferation and new blood vessel formation, providing essential nutrients and oxygen to growing metastases (Ardizzone et al., 2023). The interplay between inflammatory signals like CXCL2/IL-8 HOMOLOG and angiogenic drivers like bFGF2 in the hepatic

soil prior to overt metastasis remains an area of active investigation, as their co-regulation could unveil critical pathways for therapeutic intervention.

Given the limitations and toxicities associated with conventional cancer therapies, there is growing interest in safe, non-pharmacological adjuvant strategies. Among these, structured aerobic exercise has emerged as a potent systemic modulator with demonstrated anti-cancer effects (Spanoudaki et al., 2023). Regular physical activity is known to reduce chronic inflammation, improve immune surveillance, normalize angiogenic pathways, and enhance metabolic health, collectively creating a less permissive environment for tumor growth and spread (Buzaglo et al., 2024). Parallel to this, dietary interventions with anti-inflammatory and antioxidant supplements have garnered attention. Natural compounds, such as bromelain from pineapple extract, possess documented anti-inflammatory, immunomodulatory, and anti-angiogenic properties, offering a promising nutritional approach to complement standard care (Kumar et al., 2023).

Notably, while the independent effects of exercise or specific anti-inflammatory supplements on systemic inflammation are increasingly recognized, their potential combinatorial impact on the hepatic pre-metastatic landscape in melanoma is poorly understood. It is hypothesized that their mechanisms of action may act synergistically to more effectively downregulate key metastatic facilitators like CXCL2/IL-8 HOMOLOG and bFGF2 within the liver. However, the differential regulation of inflammatory versus angiogenic genes by such a combined modality remains unexamined. Furthermore, elucidating whether these interventions alter the potential correlation between these critical pathways could provide deeper insight into their mechanistic interplay.

Therefore, this study was designed to investigate the hypothesis that a combined intervention of aerobic exercise and an anti-inflammatory dietary supplement (pineapple extract) would more effectively attenuate the expression of pro-metastatic genes in the liver of melanoma-bearing mice than either intervention alone. We specifically focused on the hepatic gene expression of CXCL2/IL-8 HOMOLOG, a master inflammatory regulator, and bFGF2, a central angiogenic factor. A secondary aim was to explore the relationship between these two genes across different treatment groups to discern if the interventions decouple their potential interaction within the hepatic microenvironment.

Material and methods

Animals

Twenty male C57BL/6 mice (six to eight weeks old, 20–25 g body weight) were obtained from the Pasteur Institute of Iran. Animals were housed in the controlled-environment animal facility at Islamic Azad University, Yazd Laboratory. Following a one-week acclimatization period, mice were maintained under standard conditions (22±2°C, 55% humidity, 12-hour light/dark cycle) in polycarbonate cages (four mice per cage) with ad libitum access to standard rodent chow and water. All animal procedures were conducted in strict accordance with ethical guidelines for laboratory animal research and were approved by the institutional ethics committee of Islamic Azad University of Tehran (Iran). Mice were then randomly assigned to one of four experimental groups (n=5 per group): Melanoma Control (C), Aerobic Exercise (AE), Pineapple Extract Supplement (S), and Aerobic Exercise + Pineapple Extract (AE+S).

Melanoma induction

The B16F10 murine melanoma cell line was acquired from the Pasteur Institute of Iran Cell Bank. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in a humidified 5% CO_2 incubator. For tumor induction, donor mice bearing subcutaneous B16F10 tumors were euthanized, and tumor tissue was aseptically excised. The tissue was minced into 2–3 mm^3 fragments in phosphate-buffered saline (PBS) and cleared of necrotic material and connective tissue. Recipient mice were anesthetized via intraperitoneal injection of a ketamine/xylazine mixture. A small incision was made on the shaved right flank, and a single tumor fragment was implanted subcutaneously into a small pocket. The wound was closed with surgical adhesive. Tumor development was monitored weekly. This subcutaneous implantation model establishes a primary tumor and allows investigation of the hepatic molecular response to a distant melanoma (a key component of pre-metastatic niche formation). However, as tumor fragments are implanted subcutaneously rather than disseminating spontaneously from an orthotopic site, this model does not directly assess metastatic colonization of the liver.

Aerobic exercise protocol

The aerobic training program consisted of six weeks of treadmill running, designed as a moderate-intensity continuous training (MICT) protocol based on established guidelines for murine exercise studies (Ferreira et al., 2007; Fernando et al., 2019). Exercise sessions were performed five days per week with a progressive overload design to ensure physiological adaptation while minimizing injury risk.

The protocol was structured in three two-week phases:

Weeks 1-2 (Familiarization): Mice were acclimated to treadmill running at 6-8 m/min for 20 minutes/session. This low-intensity phase (approximately 40-50% of maximal running capacity) serves to habituate animals to the apparatus and handling stress

(Amani-Shalamzari et al., 2014).

Weeks 3-4 (Moderate intensity): Speed was increased to 10-12 m/min for 25 minutes/session, corresponding to approximately 60-65% of maximal oxygen consumption (VO2max) in C57BL/6 mice (Schefer & Talan, 1996).

Weeks 5-6 (Established moderate intensity): Speed reached 14-16 m/min for 30 minutes/session, representing approximately 70-75% of VO2max, which is classified as moderate-to-vigorous intensity and has been shown to induce anti-inflammatory adaptations in tumor-bearing mice (Pedersen et al., 2016).

This progressive protocol was selected to: (1) ensure consistent training stimulus throughout the six-week intervention despite increasing tumor burden, (2) match intensities previously shown to modulate hepatic inflammatory gene expression, and (3) maintain adherence to ethical guidelines for exercise training in tumor-bearing animals. To control for handling and treadmill stress, control group mice were placed on stationary treadmills for matched durations (20-30 minutes) throughout the intervention period (Amani-Shalamzari et al., 2014).

Pineapple extract supplementation

A hydroethanolic extract was prepared from the inner flesh of fresh pineapple (Ananas comosus). Briefly, the fruit was peeled, sliced, and dried at room temperature in a shaded, ventilated area for 72 hours. The dried material was ground to a powder, and 7 g of powder was subjected to cold maceration in 50 mL of 85% ethanol for 24 hours at 4°C. The mixture was filtered, and the solvent was evaporated at 37°C to yield a dry extract. Total bromelain activity was standardized and expressed in casein digestion units (CDU) per mg. For administration, the extract was dissolved in normal saline. Mice in the supplement groups (S and AE+S) received a daily oral gavage of pineapple extract at a dose of 300 mg dry extract/kg body weight. This dosage was selected based on the following rationale:

Previous literature: The 300 mg/kg dose falls within the range of bromelain-containing extracts (200-500 mg/kg) that have demonstrated anti-inflammatory and immunomodulatory effects in murine models without observed toxicity (Hale et al., 2005; Secor Jr et al., 2012). Specifically, bromelain at 10-30 mg/kg (pure enzyme) has shown efficacy, and our extract's standardized bromelain activity (approximately 10-15% of dry extract weight) yields a delivered bromelain dose of 30-45 mg/kg, which aligns with this therapeutic window.

Allometric scaling: Using standard body surface area conversion (Reagan-Shaw et al., 2008), the murine dose of 300 mg/kg corresponds to approximately 24 mg/kg in humans, which is consistent with recommended human supplementation doses of 500-2000 mg/day for average adult (equivalent to 8-30 mg/kg).

Pilot data: In preliminary experiments (unpublished data, n=3 per group), we observed that doses below 200 mg/kg failed to consistently reduce systemic inflammatory markers (serum TNF-α and IL-6) in melanoma-bearing mice, while doses above 400 mg/kg were associated with mild gastrointestinal distress (loose stools) in some animals. The 300 mg/kg dose provided optimal tolerability with measurable biological activity.

Feasibility: The 300 mg/kg dose allowed for reasonable gavage volumes (0.2-0.3 mL per mouse) using a soluble extract concentration that maintained stability throughout the six-week intervention

Sample collection and gene expression analysis

Forty-eight hours after the final exercise session, all mice were euthanized. Liver tissue was rapidly dissected, rinsed in PBS, and a section was preserved in RNAlater solution at -20°C for subsequent RNA extraction. Total RNA was isolated using a commercial RiboEx Total RNA isolation kit (GeneAll). RNA concentration and purity were assessed via NanoDrop spectrophotometry. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a reverse transcription kit (Solis BioDyne). Quantitative real-time PCR (qPCR) was performed on a Rotor-Gene Q system (QIAGEN) using a SYBR Green master mix. Reaction mixtures (20 µL) contained 10 µL master mix, 1 µL each of forward and reverse primer (10 pmol/µL), 2 µL cDNA template, and 6 µL nuclease-free water. The thermal cycling protocol was: 95°C for 15 min, followed by 40 cycles of 94°C for 10 sec and 60°C for 30 sec. A melt curve analysis (60–95°C) was performed to confirm amplification specificity. Primer sequences were as follows:

• IL-8 (CXCL2, functional murine homolog):

Forward: 5' CCAACCACCAGGCTACAGG-3',

Reverse: 5'-GCGTCACACTCAAGCTCTG-3'

• bFGF2 (Fgf2):

Forward: 5'-CTGCTGGCTTCTAAGTGTGT-3',

Reverse: 5'-CTGCCCAGTTCGTTTCAGTG-3'

•GAPDH (reference gene):

Forward: 5'-AAGTTCAACGGCACAGTCAAGG-3',

Reverse: 5'-CATACTCAGCACCAGCATCACC-3'

Relative gene expression was calculated using the 2^–ΔΔCT method, with GAPDH as the endogenous control and the Melanoma Control group as the calibrator.

Statistical analysis

All data are presented as mean ± standard deviation (SD). The normality of data distribution and homogeneity of variances were confirmed using Shapiro-Wilk and Levene's tests, respectively. Differences in hepatic CXCL2/IL-8 HOMOLOG and bFGF2 gene expression across the four experimental groups were analyzed by one-way analysis of variance (ANOVA). When ANOVA indicated a significant main effect ($p<0.05$), pairwise comparisons were conducted using Tukey HSD test. The relationship between CXCL2/IL-8 HOMOLOG and bFGF2 gene expression within each group was assessed using Pearson's correlation coefficient. Statistical analyses were performed using GraphPad Prism software (version 9.0), and statistical significance was set at $p< 0.05$.

Results

Gene expressions at liver tissue

The expression of CXCL2/IL-8 HOMOLOG and bFGF2 genes is shown in Figures 1 and 2 (respectively). The results of one-way ANOVA showed that there was a significant difference in CXCL2/IL-8 HOMOLOG gene expression between different groups ($F=4.211$, $p=0.0239$). The results of Tukey HSD test showed that compared to the melanoma cancer control group, the aerobic exercise and supplement (pineapple extract) groups showed a non-significant decrease, and the combined aerobic exercise + pineapple extract supplement treatment group showed a significant decrease in CXCL2/IL-8 HOMOLOG gene expression in the liver tissue of mice ($p=0.0251$) (Figure 1).

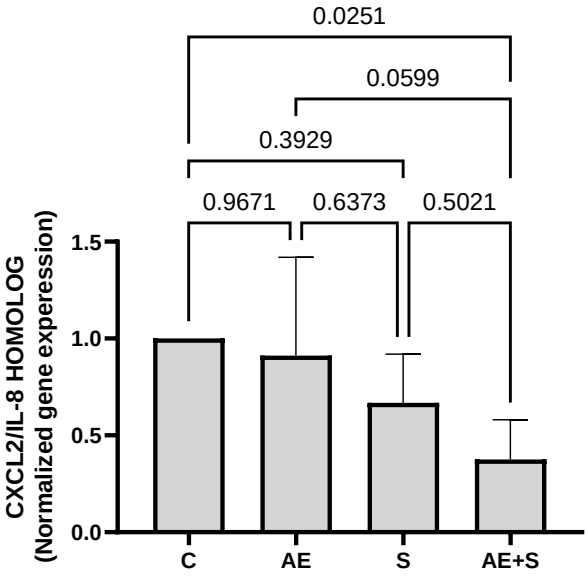

Figure 1. Expression of CXCL2/IL-8 HOMOLOG gene at liver of mice with melanoma cancer. Data are shown as means ± standard deviation (SD) (n= 5 in each group). Sign of significant: *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001. Abbreviations: C: Control, AE: Aerobic Exercise, S: Supplement (Pineapple extract), AE+S: Aerobic Exercise+ Supplement

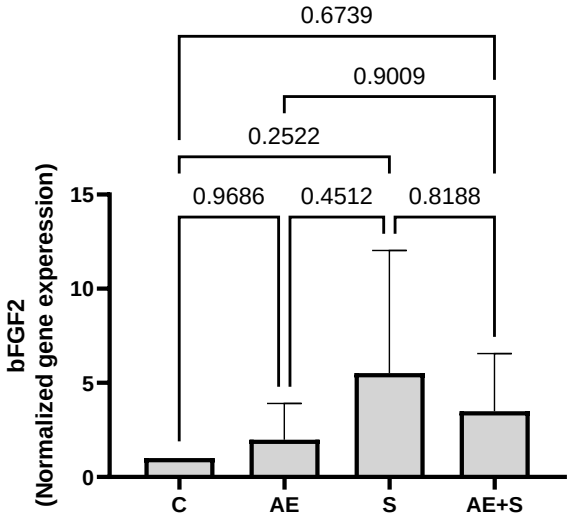

Figure 2. Expression of bFGF2 gene at liver of mice with melanoma cancer. Data are shown as means ± standard deviation (SD) (n= 5 in each group). Sign of significant: *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001. Abbreviations: C: Control, AE: Aerobic Exercise, S: Supplement (Pineapple extract), AE+S: Aerobic Exercise+ Supplement

Unlike the CXCL2/IL-8 HOMOLOG results, the results of one-way ANOVA showed that there was no significant difference in hepatic bFGF2 gene expression between different groups of animals with melanoma cancer ($F=1.425$, $p=0.2745$). Therefore, the results of Tukey HSD test also did not show significant changes in the expression of this hepatic gene (bFGF2) in different groups with melanoma cancer (Control, Aerobic, Supplement, and Aerobic+Supplement) (Figure 2).

Correlation between CXCL2/IL-8 HOMOLOG and hepatic bFGF2 in mice with melanoma cancer

Pearson correlation analysis was performed to explore the relationship between hepatic CXCL2 and bFGF2 gene expression within each experimental group (Figure 3a-d; scatter plots with regression lines). Given the small sample size per group (n=5), these correlational findings must be interpreted with extreme caution as they may be heavily influenced by individual data points.

The analysis revealed a strong negative correlation between CXCL2 and bFGF2 expression exclusively in the Cancer Control group ($r=-0.948$, $p=0.013$; Figure 3a). Visual inspection of the scatter plot (Figure 3a) indicates that this correlation is heavily driven by a single animal with low CXCL2 and high bFGF2 expression, and another with high CXCL2 and low bFGF2. With only five data points, the correlation coefficient is unstable and highly sensitive to individual values.

In contrast, no significant correlation was observed in the Aerobic

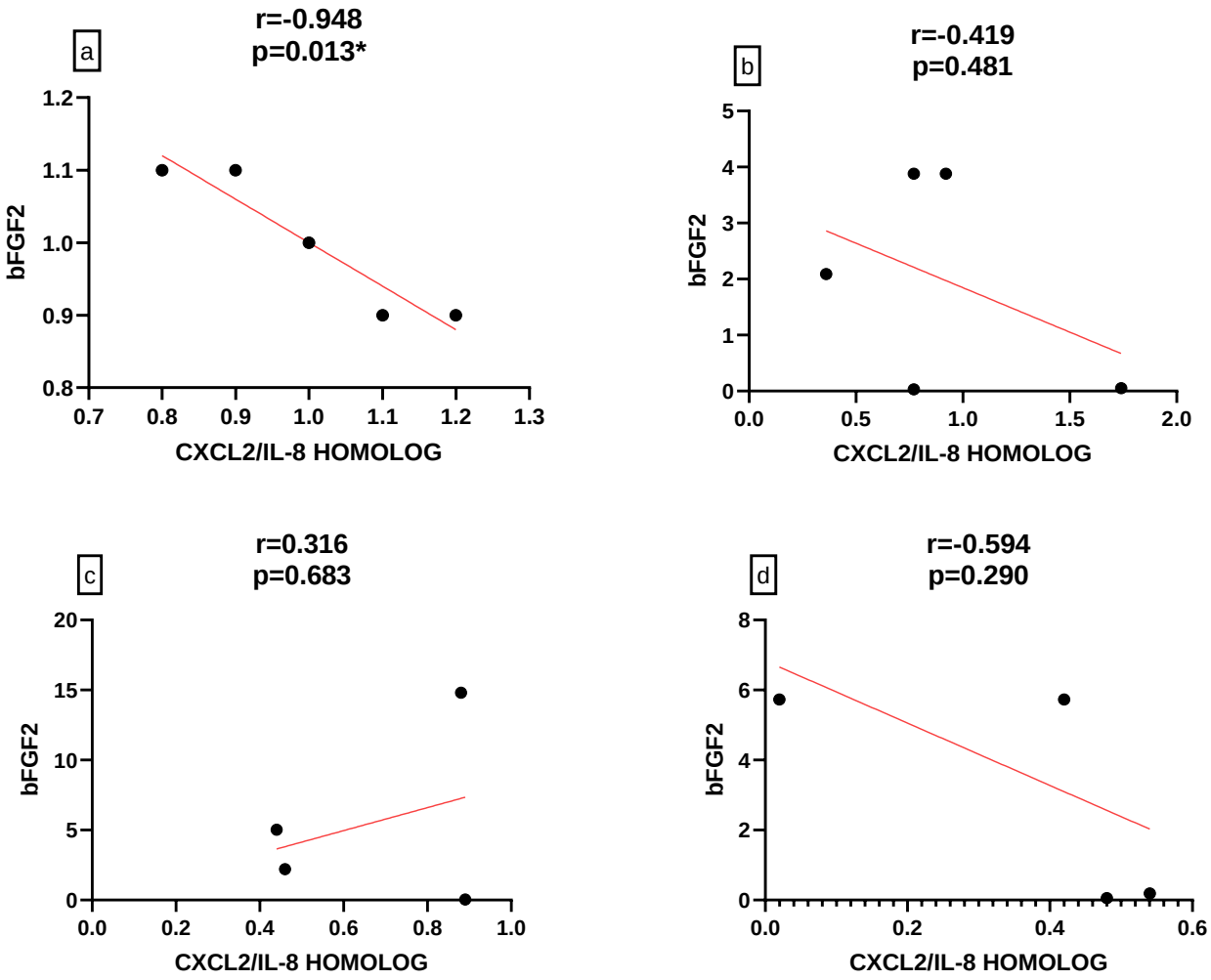

Figure 3. Pearson correlation between bFGF2 and CXCL2/IL-8 HOMOLOG genes at liver tissue of mice with melanoma cancer at control (a), Aerobic (b), Supplement (c) and Aerobic+Supplement (d) groups. Data were show as means ± standard division (n=5 in each group). * show the sign of significant ($p<0.05$). Supplement: Pineapple extract.

Exercise (r=- 0.419, p=0.481; Figure 3b), Supplement (r=0.316, p = 0.683; Figure 3c), or Combined (r=-0.594, p=0.290; Figure 3d) groups. The absence of correlation in the intervention groups should not be interpreted as evidence of pathway 'decoupling,' as the small sample size precludes definitive conclusions about the true relationship between these genes. These findings are best considered hypothesis-generating and require validation in larger cohorts.

Discussion

The present study demonstrates that a combined intervention of aerobic exercise and pineapple extract supplementation selectively downregulates hepatic CXCL2/IL-8 HOMOLOG gene expression in a murine melanoma model, while exerting no significant effect on bFGF2 expression. This differential gene regulation underscores the pathway-specific nature of non-pharmacological interventions in modulating the hepatic pre-metastatic niche. The significant reduction in CXCL2/IL-8 HOMOLOG was observed only in the combined treatment group, suggesting a potential synergistic effect not achieved by either intervention alone. This finding aligns with the growing body of evidence positioning the liver as a critical organ for pre-metastatic reprogramming, where systemic signals from primary tumors and host interventions converge to alter the local microenvironment (Ormseth et al., 2022). The selective suppression of a key inflammatory chemokine like CXCL2/IL-8 HOMOLOG, but not a core angiogenic factor like bFGF2, indicates distinct regulatory networks governing these pro-metastatic pathways within the liver, which may be differentially susceptible to combinatorial lifestyle interventions.

The synergistic reduction of hepatic CXCL2/IL-8 HOMOLOG by combined exercise and pineapple extract likely stems from conv-

-erging anti-inflammatory and immunomodulatory mechanisms. Aerobic exercise is a potent systemic modulator that reduces chronic, low-grade inflammation through multiple pathways, including the downregulation of nuclear factor kappa B (NF-κB) signaling, a master regulator of IL-8 transcription (Cavalcante et al., 2017). Exercise also enhances antioxidant defenses and promotes a shift in immune cell profiles towards a less inflammatory state (Ji, 2002). Bromelain, the primary proteolytic enzyme complex in pineapple extract, exhibits documented anti-inflammatory properties by inhibiting NF-κB activation, reducing prostaglandin E2 synthesis, and modulating leukocyte migration and adhesion (Varilla et al., 2021). The combination may thus create a more robust suppression of the NF-κB/IL-8 axis in hepatic tissue than either intervention alone. This is critical in melanoma, as IL-8 is a pivotal chemoattractant for neutrophils and myeloid-derived suppressor cells (MDSCs) that foster an immunosuppressive and pro-metastatic niche in distant organs like the liver (Tobin et al., 2019).

In contrast, the lack of a significant effect on hepatic bFGF2 gene expression across all intervention groups reveals the complexity of angiogenic regulation within the pre-metastatic liver. While both exercise and bromelain have been reported to possess anti-angiogenic properties in certain contexts, their failure to modulate bFGF2 in this model suggests several possibilities (Ausaj et al., 2024; Wang et al., 2025). First, bFGF2 regulation may be governed by signaling pathways (e.g., specific receptor tyrosine kinase cascades) that are less responsive to the systemic metabolic and inflammatory changes induced by these interventions compared to the IL-8 pathway (Katoh & Katoh, 2006). Second, the timing of the intervention may be crucial; bFGF2 upregulation might occur during later stages of metastatic colonization rather than in the pre-metastatic phase captured here. Third, the hepatic expression of bFGF2 in this model may be driven by stronger, more direct tumor-derived signals that are not easily counteracted by the chosen non-pharmacological strategies.

The discovery of a significant negative correlation between CXCL2/IL-8 HOMOLOG and bFGF2 expression exclusively in the Cancer Control group provides further mechanistic insight. This inverse relationship in untreated melanoma-bearing mice suggests a potential compensatory or regulatory interplay between inflammatory and angiogenic pathways in the baseline pre-metastatic liver. The loss of this correlation in all intervention groups, including those where CXCL2/IL-8 HOMOLOG was not significantly reduced (AE and S alone), indicates that even sub-threshold modulatory effects were sufficient to disrupt this baseline statistical coupling. This decoupling implies that exercise and supplementation, alone or combined, alter the coordinated molecular dialogue within the hepatic microenvironment. The interventions may independently affect upstream regulators, thereby breaking the observed correlative link without necessarily changing bFGF2 expression itself, highlighting a more nuanced effect on pathway crosstalk.

The translational implications of these findings are twofold. First, the efficacy of the combined regimen in lowering hepatic CXCL2/IL-8 HOMOLOG supports the exploration of multimodal lifestyle interventions as adjuvants to standard cancer care to potentially mitigate pro-metastatic systemic inflammation. Targeting the pre-metastatic niche, particularly in a common metastatic site like the liver, represents a promising preventive strategy (Tsilimigras et al., 2021). Second, the selective effect warns against assuming broad-spectrum benefits. Interventions must be tailored and their molecular impacts precisely characterized. The data argue for the combined approach specifically for modulating certain inflammatory components of the metastatic microenvironment, while alternative or additional strategies may be required to co-target angiogenic factors like bFGF2 effectively.

Several important limitations must be acknowledged. First, and most critically, the subcutaneous tumor fragment implantation model used in this study, while valuable for investigating the hepatic response to a distant melanoma, does not model spontaneous metastasis. The gene expression changes observed in the liver reflect the establishment of a 'pre-metastatic' molecular landscape in response to primary tumor-derived factors, but we did not directly assess whether these changes translate to altered metastatic burden. Therefore, while our findings suggest that combined exercise and supplementation modulates the hepatic inflammatory microenvironment, we cannot conclude that this intervention would reduce actual metastatic colonization. Future studies employing spontaneous metastasis models (e.g., intravenous injection or orthotopic implantation with subsequent metastasis quantification) are essential to determine functional consequences.

Second, the small sample size (n=5 per group) necessitates cautious interpretation and requires validation in larger cohorts. Third, gene expression was measured at the mRNA level; confirmation at the protein level and assessment of functional outcomes (e.g., neutrophil infiltration, microvessel density, MDSC accumulation) are needed. Fourth, while we interpret CXCL2 reduction as potentially beneficial, direct evidence linking reduced hepatic CXCL2 to impaired metastasis in this model is lacking. Fifth, pineapple extract contains multiple bioactive compounds beyond bromelain; the specific contribution of bromelain versus other constituents requires further isolation studies. Finally, the six-week intervention period, while appropriate for exercise adaptation, may not capture dynamic changes in the pre-metastatic niche across different stages of tumor progression.

Conclusion

In conclusion, this study demonstrates that the combination of aerobic exercise and pineapple extract supplementation exerts a selective, synergistic effect in reducing the hepatic expression of CXCL2 (the murine IL-8 homolog) in mice bearing subcutaneous melanoma tumors, without concurrently affecting bFGF2 expression. These findings indicate that combined non-pharmacological interventions can modulate the inflammatory component of the hepatic pre-metastatic microenvironment. However, whether this molecular modulation translates to reduce metastatic colonization remains to be determined in spontaneous metastasis models. The disruption of the baseline negative correlation between CXCL2 and bFGF2 by all interventions further suggests they alter critical crosstalk within the hepatic microenvironment, though this observation requires validation with larger sample sizes. This study provides a preclinical rationale for investigating combined exercise and dietary anti-inflammatory strategies as complementary approaches to target specific facets of the pre-metastatic niche, while emphasizing the critical need for metastasis-directed studies to establish functional relevance.

What is already known on this subject?

It is well-established that the liver is a principal site for melanoma metastasis, and that the formation of a permissive pre-metastatic niche is driven by the upregulation of hepatic pro-inflammatory and pro-angiogenic factors, such as CXCL2/IL-8 HOMOLOG and bFGF2. Both structured aerobic exercise and anti-inflammatory dietary supplements, like bromelain from pineapple, have independently demonstrated potential to modulate systemic inflammation and cancer progression in various models.

What this study adds?

This study provides novel experimental evidence that a combined intervention of aerobic exercise and pineapple extract synergistically and selectively reduces the expression of hepatic CXCL2/IL-8 HOMOLOG, a master inflammatory chemokine, in melanoma-bearing mice, while having no significant effect on bFGF2. Furthermore, it reveals that this combined regimen, along with each individual intervention, disrupts the baseline negative correlation between CXCL2/IL-8 HOMOLOG and bFGF2 observed in untreated controls. These findings highlight the pathway-specific efficacy of non-pharmacological combinatorial strategies and advance our understanding of their potential to differentially rewire the molecular crosstalk within the hepatic pre-metastatic microenvironment.

Organ Cross-Talk Tips:

- Lifestyle and nutritional strategies can selectively tame hepatic pro-inflammatory pathways without broadly suppressing growth factors. For melanoma patients at risk of liver metastasis, combining aerobic exercise with anti-inflammatory supplementation (e.g., pineapple extract) might offer a targeted, low-toxicity adjunct to modulate the metastatic soil.

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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 there is no conflict of interest in the present research.

Ethical approval All animal procedures were conducted in strict accordance with ethical guidelines for laboratory animal research and were approved by the institutional ethics committee of Islamic Azad University of Tehran (Iran).

Informed consent Animal study.

Author contributions

Conceptualization: M.Z., Methodology: M.Z., Software: M.Gh., Validation: H.A.,; Formal analysis: M.Z.,; Investigation: M.Z.,; Resources: M.Z.,; Data curation: H.A.,; Writing - original draft: M.Gh.,; Writing–review & editing M.Z.,; Visualization: H.A.,; Supervision: H.A.; Project administration: H.A.,.; Funding acquisition: M.Z.

References

Amani-Shalamzari, S., Aghaalinejad, H., Alizadeh, S., Kazemi, A., Saei, M., Minayi, N., & Shokrolahi, F. (2014). The effect of endurance training on the level of tissue IL-6 and VEGF in mice with breast cancer. Journal of Shahrekord University of Medical Sciences, 16(2).

Ardizzone, A., Bova, V., Casili, G., Repici, A., Lanza, M., Giuffrida, R., . . . Esposito, E. (2023). Role of basic fibroblast growth factor in cancer: biological activity, targeted therapies, and prognostic value. Cells, 12(7), 1002. doi: https://doi.org/10.3390/cells12071002

Ausaj, A. A., Al-Shmgani, H. S., Abid, W. B., Gadallah, A. A., Mashlawi, A. M., Khormi, M. A., . . . Abada, E. (2024). Immobilization of Bromelain on Gold Nanoparticles for Comprehensive Detection of Their Antioxidant, Anti- Angiog-

-enic, and Wound-Healing Potentials. Inorganics, 12(12), 325. doi: https://doi.org/10.3390/inorganics12120325

Buzaglo, G. B., Telles, G. D., Araújo, R. B., Junior, G. D., Ruberti, O. M., Ferreira, M. L., . . . Conceição, M. S. (2024). The therapeutic potential of physical exercise in cancer: the role of chemokines. International journal of molecular sciences, 25(24), 13740. doi: https://doi.org/10.3390/ijms252413740

Cavalcante, P. A. M., Gregnani, M. F., Henrique, J. S., Ornellas, F. H., & Araújo, R. C. (2017). Aerobic but not resistance exercise can induce inflammatory pathways via toll-like 2 and 4: a systematic review. Sports medicine-open, 3(1), 42. doi: https://doi.org/10.1186/s40798-017-0111-2

Hale, L. P., Greer, P. K., Trinh, C. T., & James, C. L. (2005). Proteinase activity and stability of natural bromelain preparations. International immunopharmacology, 5(4), 783-793. doi: https://doi.org/10.1016/j.intimp.2004.12.007

Ji, L. L. (2002). Exercise-induced modulation of antioxidant defense. Annals of the New York Academy of Sciences, 959(1), 82-92.

Joshi, U. M., Kashani-Sabet, M., & Kirkwood, J. M. (2025). Cutaneous melanoma: a review. Jama, 334(23).

Katoh, M., & Katoh, M. (2006). Cross-talk of WNT and FGF signaling pathways at GSK3ß to regulate ß-catenin and SNAIL signaling cascades. Cancer biology & therapy, 5(9), 1059-1064. doi: https://doi.org/10.4161/cbt.5.9.3151

Kumar, V., Garg, V., & Dureja, H. (2023). Therapeutic Application of Pineapple: A Review. Recent Advances in Food Nutrition & Agriculture, 14(2), 107-125.

Mir, M. A., Rashid, M., & Jan, N. (2023). The interleukin-8 pathway in cancer. In Cytokine and Chemokine Networks in Cancer (pp. 165-190). Springer. doi: https://doi.org/10.1007/978-981-99-4657-0_6

O'Neill, R. S., Leaver, P., Ryan, C., Liang, S., Sanagapalli, S., & Cosman, R. (2024). Metastatic melanoma: an unexpected cause of acute liver failure. Clinical Journal of Gastroenterology, 17(6), 1125-1129. doi: https://doi.org/10.1007/s12328-024-02039-1

Ormseth, B., Onuma, A., Zhang, H., & Tsung, A. (2022). The hepatic pre-metastatic niche. Cancers, 14(15), 3731. doi: https://doi.org/10.3390/cancers14153731

Reagan-Shaw, S., Nihal, M., & Ahmad, N. (2008). Dose translation from animal to human studies revisited. The FASEB Journal, 22(3), 659-661.

Secor Jr, E. R., Shah, S. J., Guernsey, L. A., & Schramm, C. M. (2012). Bromelain limits airway inflammation in an ovalbumin-induced murine model of established asthma. Alternative therapies in the health and medicine, 18 (5), 9.

Shkundin, A., & Halaris, A. (2024). IL-8 (CXCL8) correlations with psychoneuroimmunological processes and neuropsychiatric conditions. Journal of personalized medicine, 14(5), 488. doi: https://doi.org/10.3390/jpm14050488

Spanoudaki, M., Giaginis, C., Karafyllaki, D., Papadopoulos, K., Solovos, E., Antasouras, G., . . . Papadopoulou, S. K. (2023). Exercise as a promising agent against cancer: evaluating its anti-cancer molecular mechanisms. Cancers, 15(21), 5135. doi: https://doi.org/10.3390/cancers15215135

Su, X., Yan, X., & Zhang, H. (2025). The tumor microenvironment in hepatocellular carcinoma: mechanistic insights and therapeutic potential of traditional Chinese medicine. Molecular cancer, 24(1), 173. doi: https://doi.org/10.1186/s12943-025-02378-8

Tobin, R. P., Jordan, K. R., Kapoor, P., Spongberg, E., Davis, D., Vorwald, V. M., . . . Borgers, J. S. (2019). IL-6 and IL-8 are linked with myeloid-derived suppressor cell accumulation and correlate with poor clinical outcomes in melanoma patients. Frontiers in oncology, 9, 1223.

Tsilimigras, D. I., Brodt, P., Clavien, P.-A., Muschel, R. J., D'Angelica, M. I., Endo, I., . . . Pawlik, T. M. (2021). Liver metastases. Nature Reviews Disease Primers, 7(1), 27. doi: https://doi.org/10.1038/s41572-021-00261-6

Varilla, C., Marcone, M., Paiva, L., & Baptista, J. (2021). Bromelain, a group of pineapple proteolytic complex enzymes (Ananas comosus) and their possible therapeutic and clinical effects. A summary. Foods, 10(10), 2249.

Wang, J., He, Y., Wang, Z., Wang, Z., Sun, Y., Lee, K.-H., & Choi, J.-Y. (2025). Effects of exercise on angiogenesis biomarkers in cancer patients: a systematic review and meta-analysis. Frontiers in immunology, 16, 1705472.

Zou, D., Song, A., & Yong, W. (2023). Prognostic role of IL-8 in cancer patients treated with immune checkpoint inhibitors: a system review and meta-analysis. Frontiers in oncology, 13, 1176574.