Research Article

A combined intervention of aerobic training and pineapple extract attenuates PD-1 expression in the melanoma tumor microenvironment, independent of systemic IL-10

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Abstract

This study aimed to evaluate the individual and combined effects of aerobic training (AT) and pineapple supplementation (extract) on programmed cell death protein 1 (PD-1) gene expression within the tumor microenvironment and on systemic interleukin-10 (IL-10) levels in a murine melanoma model. Twenty C57BL/6 mice were randomly assigned into four groups (n=5 per group) following melanoma tumor induction: Tumor control, AT, Pineapple Supplement (PS), and AT+PS. The AT group underwent a structured aerobic training program, while the PS group received pineapple extract (300 mg/kg/day) via oral gavage for six weeks. Serum IL-10 concentrations were quantified by ELISA, and PD-1 mRNA expression in tumor tissue was analyzed using quantitative RT-PCR. All intervention groups—AT, PS, and their combination—resulted in a significant downregulation of PD-1 gene expression within the tumor compared to the control group ($p<0.05$). In contrast, neither AT nor PS alone significantly altered systemic IL-10 levels. The combination therapy (AT+PS) produced the most pronounced suppression of PD-1 and was the only intervention to elicit a significant, though modest, reduction in serum IL-10. These findings indicate that the primary immunomodulatory effect of these interventions is localized to the tumor microenvironment and is largely independent of systemic IL-10 signaling. The synergistic combination of aerobic training and pineapple supplementation potently suppresses PD-1 gene expression, suggesting a promising, non-pharmacological strategy for enhancing anti-tumor immunity. Further investigation is required to confirm these effects at the protein level and to elucidate the underlying mechanisms.

Key Words: Tumor microenvironment; Cancer immunotherapy; PD-1/PD-L1 axis; Interleukin-10; Ananas comosus (Pineapple) supplementation; Aerobic exercise; Melanoma model

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Introduction

The programmed cell death protein 1 (PD-1) pathway is a pivotal immune checkpoint in the regulation of T cell activity within the tumor microenvironment, frequently hijacked by cancer cells such as melanoma to evade immune detection and destruction. PD-1, expressed on activated T cells, binds to its ligands PD-L1 and PD-L2, leading to suppression of T cell proliferation, cytokine production, and cytotoxic activity, ultimately promoting tumor immune escape (Zuazo et al., 2017; Mandalà, 2016). Therapeutic inhibition of PD-1 has transformed melanoma treatment by rejuvenating exhausted T cells and inducing durable anti-tumor immunity (Zhang et al., 2025). However, resistance to PD-1 blockade occurs, mediated by immune evasion mechanisms and insufficient tumor immunogenicity, underscoring the need for complementary adjunct interventions (Lei et al., 2020).

Exercise has emerged as a promising modulator of anti-tumor immunity, with aerobic training (AT) shown to enhance immunosurveillance by mobilizing immune cells and altering immune checkpoint expression, including PD-1 (Schenk et al., 2020; Yan et al., 2023). Physical activity can improve the tumor microenvironment by alleviating hypoxia and increasing infiltration and activation of cytotoxic T cells and natural killer (NK) cells, which may synergize with immunotherapies (Hojman et al., 2018; Jia et al., 2021). Aerobic exercise also enhances systemic inflammatory balance by modulating cytokines such as IL-6 and IL-10, which orchestrate immune responses in cancer (Shi et al., 2025).

Nutritional bioactive compounds like pineapple extract, rich in bromelain, have documented anti-inflammatory and immunomodulatory effects that may further influence tumor immunity (Hale et al., 2010; Pezzani et al., 2023). Bromelain modulates leukocyte activation, reduces pro-inflammatory cytokines, and may directly induce tumor cell apoptosis and enhance immune cell adhesion in the tumor microenvironment, potentially suppressing immune checkpoints like PD-1 (Khosravi etal.,2024;Rathnavelu etal.,2016). Such nutraceutical

interventions can complement exercise-induced immunomodulation to potentiate anti-cancer effects.

Interleukin-10 (IL-10) is an anti-inflammatory cytokine often overexpressed by melanoma cells and the tumor milieu, promoting immune suppression by inhibiting dendritic cell maturation, T cell activation, and enhancing regulatory T cell function, thereby facilitating tumor progression and metastasis (Itakura et al., 2011; Salkeni et al., 2023). However, IL-10 signaling is complex and context-dependent, sometimes promoting anti-tumor cytotoxic T cell function, necessitating deeper understanding of how lifestyle interventions influence systemic and tumor-associated IL-10 pathways (Wiguna et al., 2015; Salkeni et al., 2023). However, the potential synergistic effect of combining aerobic training with pineapple extract supplementation on the PD-1 pathway within the tumor microenvironment remains an unexplored research gap. Therefore, this study tests the hypothesis that this combined intervention will more effectively downregulate intra-tumoral PD-1 gene expression and modulate systemic IL-10 levels than either intervention alone.

This study investigates the integrative effects of aerobic training and pineapple extract supplementation on PD-1 gene expression in melanoma tumors and systemic IL-10 levels. Understanding their combined impact provides insights into potential non-pharmacological immunotherapeutic strategies that modulate tumor immune checkpoints and inflammatory mediators, offering avenues to improve melanoma treatment outcomes (Yan et al., 2023; Pezzani et al., 2023).

Materials and methods

Animals

For this study, twenty 6- to 8-week-old male C57BL/6 mice weighing 12–14 grams were obtained from the Pasteur Institute. They were housed in the animal facility at Baqiyatallah University of Medical Sciences Laboratory in Tehran. After a one-week acclimation period under controlled conditions (22±1.4°C, 55% humidity, 12-hour light/dark cycles), the mice were randomly assigned to one of four groups with five mice each: a melanoma tumor control group (MT), and groups receiving either aerobic training (AT), pineapple supplement (PS), or a combination of both (MT+AT+PS) alongside the tumor. The animals were housed in groups of four in polycarbonate cages and had continuous access to standard food and water. All procedures adhered to ethical standards for animal research, including having a single handler and following guidelines for painless euthanasia and surgery. The study was approved by Azad University of Sciences and Research under ethics code IR.SSRC.REC.1401.342.

Culture of melanoma cells and tumor implantation

The B16F10 melanoma cells were obtained from the Pasteur Institute of Iran's cell bank and grown in RPMI-1640 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin. While the injection of cell suspensions is a common method for establishing tumors, the present study utilized the implantation of tumor fragments to better preserve the original tumor microenvironment (TME), including stromal components and cell-cell interactions, which is more representative of clinical tumor growth.

To prepare tumors for implantation, tissue was harvested from donor mice. After the donor mice were euthanized, the tumor on the flank was sterilized with ethanol and surgically removed. The extracted tissue was placed in sterile phosphate-buffered saline (PBS) and cut into small fragments of 2-3 mm^3. Non-tumor elements like fat and blood vessels were carefully excised to purify the tumor pieces, which were then kept in PBS for implantation.

For the surgery, mice were anesthetized with an intraperitoneal injection of a ketamine-xylazine mixture. Once anesthetized, a small incision was made on the shaved flank, and a subcutaneous channel was formed. A single tumor fragment was placed into this channel, and the incision was sealed with surgical adhesive and a clip. Post-surgery, the mice recovered individually in a warm environment, and the wound was treated with povidone-iodine.

The mice, housed in groups of four after a one-week acclimatization period, were randomly divided into weight-matched groups. Tumor growth was monitored weekly. The experimental treatment began one week after implantation, once tumors were palpable. After six weeks, the study concluded with final blood collection, tissue sampling, and measurements of body weight and tumor volume (Dashti et al., 2014) (Table 1).

Pineapple extract

The study employed a dry extract made with ethanol from the pulp of pineapple (Ananas comosus). In summary, the fresh fruit was cleaned and peeled, after which the pulp was cut into thin rings. These rings were dehydrated for 72 hours in a shaded, well-ventilated space at room temperature to avoid contamination and the breakdown of photochemicals. The dried pineapple was subsequently pulverized into a consistent powder using a grinder. The extraction process used was the cold-soaking technique, which involved combining seven grams of the pineapple powder

Table 1. Tumor weight at different groups of study (means)

groups	MT	MT+RT	MT+PE	MT+RT+PE
Means (g)	0.37	0.24	0.23	0.29

MT: Melanoma Tumor, RT: Resistance Training, PE: Pineapple extract

with 50 mL of 85% ethanol in water and letting it sit at 4°C for 24 hours, with periodic mixing. This mixture was filtered, and the liquid portion was then placed in a water bath at 37°C to evaporate off all the solvent. The final dry extract was kept in storage at -20°C until it was needed. To ensure consistency in the active compounds, the total bromelain activity in the extract was measured via the casein digestion unit (CDU/mg) method, as referenced from Gholamian et al. (2020). For the experiment with animals, the dry extract was dissolved in normal saline right before it was given. The mice were administered the pineapple extract orally at a dosage of 300 mg of dry extract per kilogram of their body weight. The control animals were given the same quantity of normal saline (Gholamian et al., 2020).

Aerobic training

The aerobic training program in the present study consisted of six weeks of treadmill running, divided into three two-week periods. The first two weeks were the familiarization phase with the treadmill, in which the mice trained at a speed of 5 to 8 meters per minute, which was gradually increased, for two weeks, 5 days a week, one session per day for 20 minutes. In the following two weeks, as the mice's ability increased, a continuous training program was performed, initially at a speed of 10 and then at 12 meters per minute for 25 minutes per session, and finally, in the last two weeks, the speed reached 14 and then 16 meters per minute, and the mice trained for 30 minutes per session. In order to eliminate the effect of treadmill stress on the variables under study, the mice in the control group were also placed on the treadmill for the duration of the exercise training program (between 20 and 30 minutes) (Vieira et al., 2007).

Laboratory measurements

Blood samples, tissue biopsies, and measurements of body weight and tumor volume were taken from the mice 48 hours after their final training session, which was followed by a 12-hour fasting period. Tumor tissue was also collected for gene expression analysis. This tissue was rapidly frozen in liquid nitrogen and stored at -80°C. IL-10 levels were measured using an ELISA kit from Mabtech (Cat N: BMS614, Switzerland).

To examine PD1 gene expression, total RNA was isolated from stabilized tumor tissue samples using a RiboEx kit (GeneAll). RNA quality and concentration were verified using a NanoDrop spectrophotometer and 1% agarose gel electrophoresis. Following confirmation of RNA integrity, cDNA was synthesized with a FIRE Script RT kit (Solis BioDyne) and stored at -20°C until use. Gene-specific primers for human PD1 (PDCD1) and the housekeeping gene GAPDH were designed using Primer3 software and supplied by Pioneer Biotechnology (primer sequences are provided in Table 2). The primers were explicitly designed against and confirmed to match the human gene sequences, as the tumor tissue under investigation was of human origin.

Quantitative real-time PCR (qPCR) reactions were performed in a 20 µl volume using AMPLIQON RealQ Plus 2x Master Mix Green, primers, cDNA template, and nuclease-free water. Amplification was carried out on a Rotor-Gene Q cycler (QIAGEN) with the following program: an initial enzyme activation at 95°C for 15 minutes; followed by 40 cycles of denaturation at 94°C for 10 seconds and a combined annealing-extension at 60°C for 30 seconds.

Gene expression was normalized to the GAPDH reference gene. The use of GAPDH as a single housekeeping gene was justified as it demonstrated stable and consistent expression across our sample set in preliminary validation experiments, and it is widely established as a reliable control in studies of human tumor tissue. Finally, the specificity of the PCR amplification was confirmed by analyzing the products on a 1% agarose gel.

The expression level of the desired gene was calculated with the formula $2^{-\Delta\Delta CT}$ in the following way.

(ΔCt= Ct Target-Ct Housekeeping)

In the next step, we subtract the ΔCt of each sample from the sample that needed to be compared, and multiply the negative number obtained to the power of two and obtain the relative expression of MFN1/DRP1 genes.

($\Delta\Delta$Ct=ΔCt Target-ΔCt Reference) $\Delta\Delta$Ct- E= 2

Statistical analysis

Data are expressed as mean ± standard deviation. After confirming data normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test), inter-group differences were analyzed by one-way ANOVA with a Tukey post hoc test. Associations between variables were assessed using Pearson correlation. For all analyses, statistical significance was set at p≤ 0.05, and Graph Pad Prism version 9 was used.

Results

Table 2. Primer sequences

Gene	Sequence
Mus musculus programmed cell death 1 (Pdcd1) PD1	Forward: 5′- AGCAAGGACGACACTCTGAA -3′
	Reverse: 5′- GCTCTGGTGTCTTCTCTCGT -3′
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Forward: 5′-ACCACAGTCCATGCCATCAC-3′
	Reverse: 5′-TCCACCACCCTGTTGCTGTA-3′

PD1 gene expression

Changes in PD1 gene expression in melanoma tumors are shown in Figure 1. Based on the results of ANOVA statistical test, there is a significant difference in PD1 gene expression in melanoma tumors between different research groups ($F=1272$, $p<0.0001$). The results of Tukey's post hoc test showed that all treatment groups including MT+RT, MT+PS and MT+RT+PS showed a significant decrease in PD1 gene expression in melanoma tumors compared to the MT group ($p<0.0001$ for all). However, the reduction of PD1 gene in the MT+RT group was less than that in the MT+PS ($p=0.0034$) and MT+RT+PS ($p=0.0132$) groups (Figure 1).

Serum IL-10

Changes in serum IL-10 levels are shown in Figure 2. Based on the results of the ANOVA test, there was a significant difference in serum IL-10 levels between the different research groups ($F=4.530$, $p=0.0177$). The results of the Tukey post hoc test showed that just MT+RT+PS group showed a significant decrease in serum IL-10 levels compared to the MT group ($p=0.0126$). Other groups also showed a decrease in serum IL-10 levels, but this decrease was not significant ($p>0.05$).

Pearson's correlation

In the present study, Pearson correlation statistical method was also used to investigate the relationship between the two study variables (Figure 3). Based on these results, it was determined that there was no correlation between PD1 gene expression in melanoma and serum IL-10 levels in different research groups ($p>0.05$). Correlation and significance values are also shown in Figure 3 (a-d).

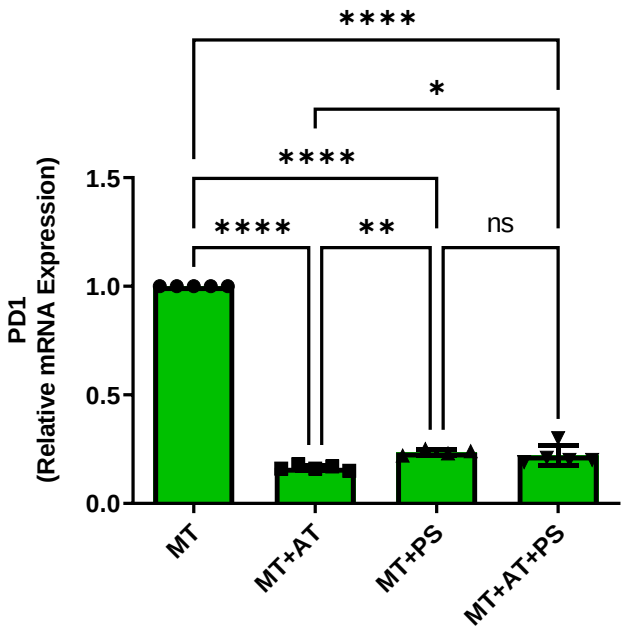

Figure 1. Expression of PD1 at melanoma tumor at different groups of study. Data were show as means ± SD (n=5). Significant difference: *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001. MT: Melanoma Tumor, RT: Aerobic Training, PS: Pineapple Supplement

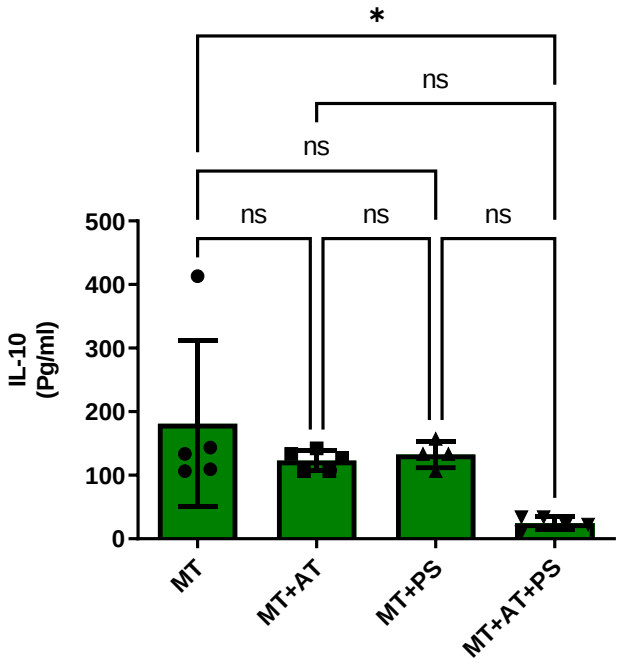

Figure 2. Serum IL-10 at different groups of study. Data were show as means ± SD (n=5). Significant difference: *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001. MT: Melanoma Tumor, RT: Aerobic Training, PS: Pineapple Supplement

Discussion

The significant reduction of PD-1 gene expression in melanoma tumors following aerobic training, pineapple supplement, and their combination confirms that exercise and nutritional interventions modulate tumor immune checkpoints, enhancing anti-tumor immunity. Exercise is hypothesized to modulate the aryl hydrocarbon receptor (AhR) pathway, which influences PD-1 expression on CD8+ T cells; physical activity decreases AhR activation by reducing kynurenine levels, resulting in lowered PD-1 expression and restoration of T cell effector function (Schenk et al., 2020). Moreover, exercise acts as a hypoxia modulator within the tumor microenvironment, improving oxygenation, which can alter immune cell infiltration and reduce PD-1 mediated immunosuppression (Yan et al., 2023; Jia et al., 2021).

Bromelain, the active enzyme complex in pineapple extract, exhibits multifaceted anti-cancer mechanisms including immunomodulation, induction of tumor cell apoptosis, and suppression of pro-inflammatory cytokines (Pezzani et al., 2023). Bromelain enhances the cytotoxicity of lymphocytes and monocytes, increases IL-2 and TNF-α production, and improves

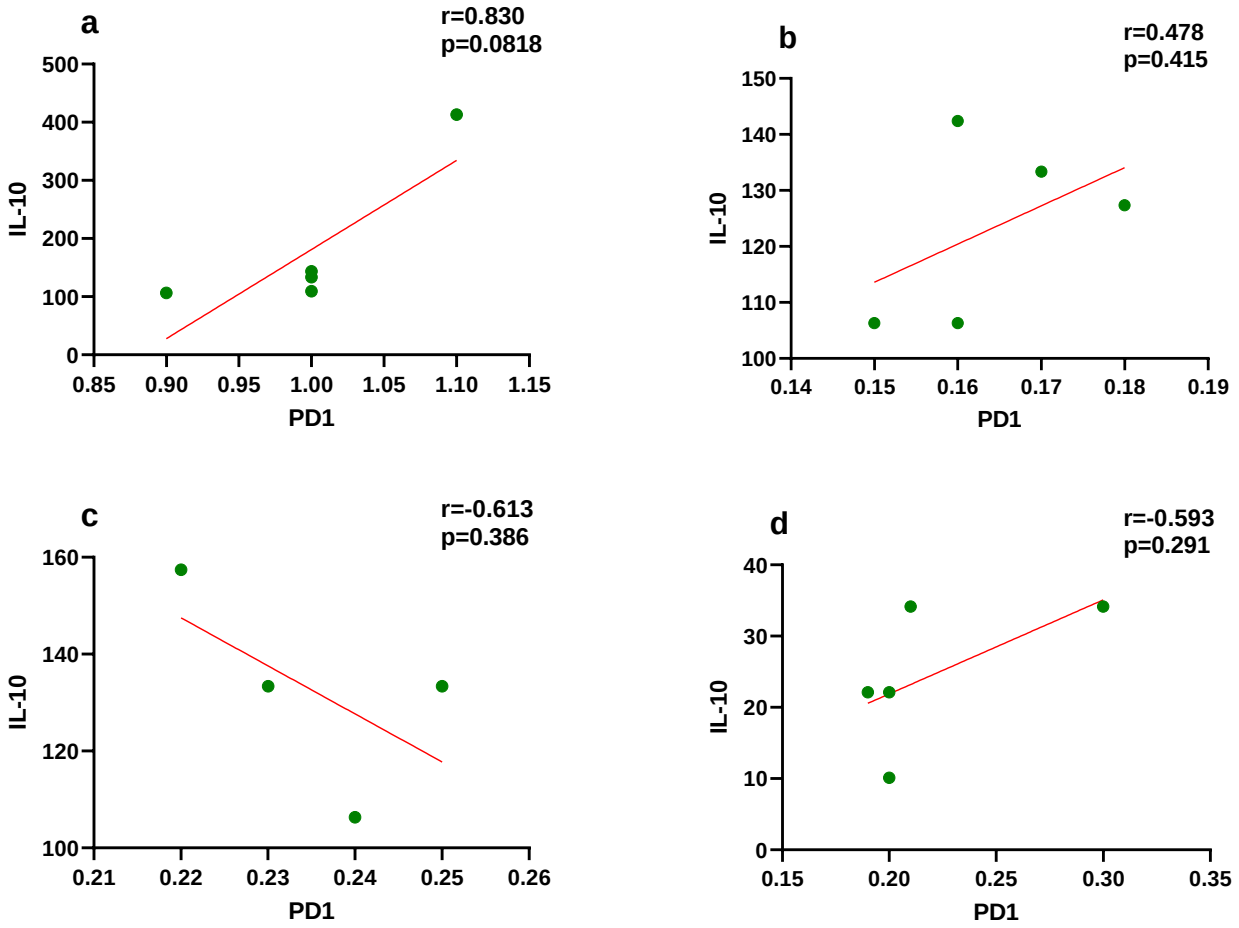

Figure 3. Pearson's correlation analysis was performed to analyze the correlation between the level of melanoma PD1 gene and serum IL-10 ad MT (a), MT+AT (b), MT+PS (c), and MT+AT+PS (d) groups. R2, Pearson's correlation coefficient. Data were show as means ± SD. Significant difference: *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001. MT: Melanoma Tumor, RT: Aerobic Training, PS: Pineapple Supplement

immune cell adhesion through modification of surface molecules such as CD44 and CD2, promoting anti-tumor immune responses (Khosravi et al., 2024; Rathnavelu et al., 2016). Additionally, bromelain inhibits NF-KB signaling and reduces immunosuppressive cytokines, which may indirectly reduce PD-1 expression within tumors (Kumar et al., 2023).

The combined intervention exhibits greater PD-1 suppression likely due to complementary mechanisms of immune activation. Aerobic training mobilizes and activates immune effector cells, while bromelain enhances immune cell function and modifies the tumor microenvironment to reduce immunosuppression (Yan et al., 2023; Khosravi et al., 2024). Together, these interventions may synergistically restore effective T cell immunity by targeting distinct pathways converging on PD-1 regulation and tumor immune escape. Although our data focused on the tumor microenvironment and systemic circulation, the observed effects are likely underpinned by complex organ crosstalk. Skeletal muscle, as an endocrine organ, releases myokines (e.g., IL-6, IL-15) during aerobic training that can systemically modulate immune cell function and trafficking, while bioactive compounds from pineapple extract, absorbed via the gastrointestinal tract, can exert direct and indirect immunomodulatory effects on the liver and the systemic inflammatory milieu. This multi-organ signaling converges on the tumor to alter PD-1 expression and enhance immunogenicity, independent of systemic IL-10.

The isolated significant reduction in systemic IL-10 in the combined group, but not with each intervention alone, suggests complex immune modulation. This specific effect highlights a potential synergistic immunoregulatory interaction between aerobic training and bromelain that is not achieved by either intervention in isolation. Exercise induces transient increases in pro- and anti-inflammatory cytokines; chronic training often rebalances this toward reduced systemic inflammation and improved immune regulation (Shi et al., 2025). IL-10 produced in the tumor microenvironment by melanoma cells contributes to local immunosuppression; its systemic levels may not directly parallel tumor PD-1 expression, explaining the lack of correlation observed (Itakura et al., 2011; Salkeni et al., 2023).

Mechanistically, IL-10 can suppress dendritic cells and T cell activation, fostering tumor persistence (Wiguna et al., 2015), while its reduction systemically may relieve some immune suppression. Therefore, the combination therapy may act through a broader immunomodulatory network, where the reduction in systemic IL-10 complements the localized downregulation of PD-1 within the tumor. However, the independence of PD-1 expression from systemic IL-10 levels aligns with emerging paradigms that local tumor immune checkpoints are regulated by intrinsic signaling and microenvironmental factors distinct from circulating cytokines (Yan et al., 2023).

In summary, aerobic training and bromelain supplementation represent feasible lifestyle interventions modulating crucial immune checkpoints and the inflammatory milieu in melanoma. These findings encourage further mechanistic studies on the crosstalk between exercise, nutraceuticals, and immune pathways, potentially guiding integrative cancer immunotherapy approaches that enhance patient outcomes with minimal toxicity.

Conclusion

This study demonstrates that aerobic training combined with pineapple extract supplementation effectively reduces PD-1 gene expression in melanoma tumors, highlighting a synergistic enhancement of anti-tumor immune response. The combined intervention also significantly lowers systemic IL-10 levels, suggesting a complex immunomodulatory effect. These findings support the potential of integrating exercise and nutritional bioactive compounds as non-pharmacological strategies to improve melanoma immunotherapy outcomes by modulating immune checkpoints and the tumor microenvironment. Further research is warranted to optimize such integrative approaches in cancer treatment.

What is already known on this subject?

Therapeutic inhibition of PD-1 has transformed melanoma treatment by rejuvenating exhausted T cells and inducing durable anti-tumor immunity.

What this study adds?

The combined intervention also significantly lowers systemic IL-10 levels, suggesting a complex immunomodulatory effect.

Organ Cross-Talk Tips:

- Pineapple supplementation, acting through the gastrointestinal system, influences gene expression within the distant tumor microenvironment.
- Exercise and • pineapple intervention suggests that signals originating from multiple organ systems can converge to produce a more robust suppression of tumor immune evasion than any single intervention alone.

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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 This study received approval under the research ethics code IR.SSRC.REC.1401.342 from the Azad University of Sciences and Research.

Informed consent Animal study.

Author contributions

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

References

Dashti, A., Ebrahimi, M., Hadjati, J., & Moazzeni, S. M. (2014). Identification and Characterization of Cancer Stem Cells in Mouse Malignant Melanoma. Pathobiology Research, 17(2), 27-37. URL: https://journals.modares.ac.ir/article_19016_16e12822445d7ba7d4433330dd22b91b.pdf

Gholamian, R., Lotfabadi, N. N., & Haghiralssadat, B. F. (2020). Evaluation of antioxidant and cytotoxic effects of liposomes containing pineapple fruit extract on Melanoma skin cancer (A375 Cell Line). Journal of Shahid Sadoughi University of Medical Sciences. doi: https://doi.org/10.18502/ssu.v28i2.3480

Hojman, P., et al. (2018). Molecular mechanisms linking exercise to cancer control. Trends in Molecular Medicine, 24(6), 524-541. URL: https://www.cell.com/cell-metabolism/fulltext/S1550-4131(17)30567-3

Itakura, E., et al. (2011). IL-10 expression by primary tumor cells correlates with tumor progression in melanoma. Cancer Immunology, Immunotherapy, 60(7), 983–993. doi: https://doi.org/10.1038/modpathol.2011.5

Jia, N., et al. (2021). The antitumor mechanisms of aerobic exercise: A review. Cancer Medicine, 10(10), 3405-3418. doi: https://doi.org/10.1002/cam4.4169

Khosravi, M. R., et al. (2024). The survey of antitumor effects of bromelain on neoplastic disease: Immunomodulatory and apoptotic mechanisms. Journal of Herbmed Pharmacology, 13(10), 850-860. doi: https://doi.org/10.34172/jhp.2024.48078

Kumar, V., Mangla, B., Javed, S., Ahsan, W., Kumar, P., Garg, V., & Dureja, H. (2023). Bromelain: A review of its mechanisms, pharmacological effects and potential applications. Food & function, 14(18), 8101-8128. doi: DOI https://doi.org/10.1039/D3FO01060K

Lei, Q., et al. (2020). Resistance mechanisms of anti-PD1/PDL1 therapy in cancer. Frontiers in Cell and Developmental Biology, 8, 672. doi: https://doi.org/10.3389/fcell.2020.00672

Mandalà, M. (2016). PD-L1 in melanoma: Facts and myths. Journal of Translational Medicine, 14, 381. doi: https://doi.org/10.2217/mmt-2016-0013

Pezzani, R., et al. (2023). Anticancer properties of bromelain: State-of-the-art and recent advances. Frontiers in Pharmacology, 14, 1108653. doi: https://doi.org/10.3389/fonc.2022.1068778

Rathnavelu, V., et al. (2016). Potential role of bromelain in clinical and therapeutic applications. Evidence-Based Complementary and Alternative Medicine, 2016, 1235497. doi: https://doi.org/10.3892/br.2016.720

Schenk, A., et al. (2020). Acute exercise impacts AhR and PD-1 levels of CD8+ T cells: Novel insights into immune checkpoint regulation. European Journal of Applied Physiology, 120(11), 2479–2487. doi: https://doi.org/10.1007/s00421-020-04552-w

Shi, X., et al. (2025). Exercise workload: A key determinant of immune health. Exercise Immunology Review, 31, 54-78. doi: https://doi.org/10.3389/fimmu.2025.1617261

Wiguna, A. P., et al. (2015). Role of IL-10 and TGF-β in melanoma progression. Asian Pacific Journal of Cancer Prevention, 16(7), 2859-2864. doi: https://doi.org/10.1111/exd.12629

Yan, H., et al. (2023). Exercise sensitizes PD-1/PD-L1 immunotherapy as a hypoxia modulator. Journal for ImmunoTherapy of Cancer, 11(10), e005088. doi: https://doi.org/10.3389/fimmu.2023.1265914

Zuazo, M., et al. (2017). Molecular mechanisms of programmed cell death-1 (PD-1) and its ligands: Therapeutic implications. Annals of Translational Medicine, 5(12), 238. doi: https://doi.org/10.21037/atm.2017.06.11

Vieira, R. P., Claudino, R. C., Duarte, A. C. S., Santos, Â. B., Perini, A., Faria Neto, H. C., . . . Carvalho, C. R. (2007). Aerobic exercise decreases chronic allergic lung inflammation and airway remodeling in mice. American journal of respiratory and critical care medicine, 176(9), 871-877. doi: https://doi.org/10.1164/rccm.200610-1567OC

Zhang, C., & Chen, L. (2025). Bibliometric and visualized analysis of global distribution and research frontiers in tumor immune escape. Frontiers in Immunology, 16, 1586120. doi: https://doi.org/10.3389/fimmu.2025.1586120