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Propranolol attenuates social isolation-associated anxiety-like behavior, immune dysfunction, and melanoma progression in mice, with involvement of β -adrenergic signaling

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Abstract: Chronic psychosocial stress, such as social isolation, is highly prevalent among cancer patients and has been associated with emotional disturbances including anxiety and depression as well as accelerated tumor progression and immune evasion through systemic remodeling of the neuro-endocrine-immune network. However, the key molecular bridge and actionable targets remain incompletely elucidated. In this study, using a murine model of social isolation stress established by 4-week single-cage housing in male C57BL/6 mice bearing B16F10 melanoma cells in the footpad, we investigated the role of β_2 -adrenergic receptor (β_2 -AR) signaling in stress-induced anxiety-like behaviors and tumor immunosuppression. Our results demonstrated that socially isolated mice exhibited anxiety-like phenotypes, characterized by decreased exploration in the central zone of the open field and decreased open arm entries and time in the elevated plus maze, accompanied by lower serum norepinephrine and corticosterone levels, suggesting neuroendocrine alterations under prolonged chronic stress. Concurrently, tumor growth was accelerated in isolated mice, with decreased intratumoral CD8⁺ leukocyte infiltration and secretion of IFN- γ and granzyme B, as well as decreased splenic CD8⁺ leukocyte counts, whereas protein expression of β_2 -AR, SOX10, and PD-L1 in tumor tissues was upregulated. Administration of the non-selective β -blocker propranolol (40 mg/kg, intragastric gavage for 8 days) was associated with improved anxiety behaviors, restored endocrine parameters, increased CD8⁺ leukocyte effector functions, decreased SOX10/PD-L1 expression, and reduced tumor growth while increasing thymic weight. However, co-administration of the β -adrenergic agonist isoproterenol (2 mg/kg/d, intraperitoneal injection) attenuated the effects of propranolol, with re-emergence of anxiety manifestations, immunosuppression, and rapid tumor progression, consistent with the involvement of β -adrenergic signaling. Collectively, social isolation stress is associated with melanoma progression and immune alterations via altered β_2 -AR-mediated SOX10–PD-L1 expression while also being associated with anxiety-related behaviors; propranolol, through β -adrenergic blockade, demonstrated effects on emotional regulation and antitumor immune function, providing a preclinical basis for further investigation in melanoma patients with significant social isolation and psychological distress.

Keywords: propranolol; β_2 -adrenergic receptor signaling pathway; social isolation stress; melanoma immune evasion; anxiety-like behaviors

1. Introduction

Chronic psychological stress is considered a risk factor promoting tumor initiation and progression. Social isolation, as a prototypical chronic stressor, is prevalent among cancer patients and has been associated with psychiatric

abnormalities such as anxiety and depression, through activation of the sympathetic nervous system, alterations in catecholamine secretion, remodeling of the tumor immune microenvironment, and accelerated immune evasion[1,2]. The conceptual relationship between objective social isolation, subjective loneliness, lack of social support, and psychological distress has been increasingly recognized, yet these constructs are distinct and should not be conflated: objective isolation refers to the physical absence of social contacts, whereas subjective loneliness reflects the perceived discrepancy between actual and desired social relationships. Melanoma is a highly aggressive skin malignancy with pronounced immune-evasive properties, commonly arising from cutaneous and mucosal sites[3]. Existing evidence[4,5] suggests that activation of the β_2 -adrenergic receptor (β_2 -AR) signaling pathway may constitute a molecular mechanism linking stress to cancer progression, which may suppress cytotoxic T cell function, recruit immunosuppressive cells, and impair antitumor immune surveillance. Propranolol (Pro), a non-selective β -blocker, antagonizes β -adrenergic signaling and has been reported to have potential effects on antitumor and neuroregulatory actions[6,7]. However, whether it can affect social isolation stress-associated melanoma progression and immune alterations while concurrently influencing stress-associated anxiety-like behaviors, and the precise underlying mechanisms, remain to be fully elucidated. Current clinical and basic research predominantly focuses separately on the impact of stress on tumors or the effects of β -blockers on tumors, with few studies simultaneously examining their effects on tumor immunity and stress-related behaviors. Accordingly, this study was designed to investigate the mechanistic basis by which propranolol, through β -adrenergic blockade, affects social isolation stress-associated melanoma progression and immune alterations and influences anxiety-like behaviors.

2. Modeling and intervention methods

2.1. Experimental animals and modeling

Forty-six SPF-grade male C57BL/6 mice, 3 weeks old (weaned), weighing 10–12 g, were purchased from the Kunming Institute of Zoology, Chinese Academy of Sciences [License No. SCXK (Dian) K2025-0001]. All mice were acclimatized in the animal facility for 1 day under controlled conditions: temperature (22±2)°C, relative humidity 50%–60%, 12 h/12 h light/dark cycle, with free access to food and water. This short acclimatization period and the use of post-weaning animals represent limitations on the generalizability of the findings.

Following acclimation, all 46 mice were subcutaneously inoculated in the footpad with B16F10 melanoma cells (Xiamen Yimo Biotechnology Co., Ltd.). B16F10 cells in the logarithmic growth phase were digested with 0.25% trypsin, resuspended in sterile PBS, and adjusted to a concentration of 2×10^7 cells/mL. Each mouse received a subcutaneous injection of 50 μ L of cell suspension (containing 1×10^6 cells) into the left hind footpad. The injection site was disinfected with 75% ethanol prior to injection, and a micro-insulin syringe was used with the bevel fully inserted subcutaneously, followed by slow injection and gentle compression for a few seconds to prevent extravasation.

On the day after inoculation, the 46 mice were allocated by housing conditions: 12 mice were assigned to the group-housed control group (Con group), housed 4 per cage in 3 cages (cage dimensions: 29 cm $\times$ 17.8 cm $\times$ 16 cm) for 4 weeks of continuous group housing; the remaining 34 mice were subjected to social isolation

stress, housed individually in single cages (same dimensions), for 4 weeks of continuous isolation. During isolation, all mice had no social contact (including visual, olfactory, and tactile isolation), with only minimal environmental perception through cage gaps. After 4 weeks of isolation, all 34 isolated mice underwent a sucrose preference test for model assessment: mice were water-deprived for 24 h in single cages, then given one bottle of 1% sucrose solution and one bottle of plain water for 1 h of free access, and sucrose preference rate was calculated (sucrose consumption / total fluid consumption $\times$ 100%). A sucrose preference rate $<60\%$ was used as the criterion for social isolation stress model establishment; this threshold was pre-specified. Two mice that did not meet the criteria were excluded, resulting in 32 socially isolated model mice ultimately enrolled. Baseline data for the excluded animals are not presented.

2.2. Intervention protocol

The 32 successfully modeled socially isolated mice were allocated into three groups: social isolation model group (SI group, $n=11$), social isolation + propranolol intervention group (SI+Pro group, $n=11$), and social isolation + propranolol + β -adrenergic agonist isoproterenol group (SI+Pro+ISO group, $n=10$). The group-housed control group (Con group, $n=12$) was also included. All drug interventions commenced on the day after sucrose preference assessment. The experimental timeline is as follows: day 0 – tumor inoculation and initiation of housing conditions; days 0–27 – 4-week isolation or group housing; day 28 – sucrose preference test (model assessment); days 29–36 – drug intervention (8 days); day 37 – behavioral tests (open field and elevated plus maze); day 37 (immediately after behavioral tests) – blood collection and euthanasia; day 37 – tumor and thymus dissection and measurement. Tumor volume was recorded every 3 days from day 0 to day 21 post-inoculation.

The specific dosing regimens were as follows: (1) SI+Pro group: propranolol (Sigma-Aldrich, USA) was administered by intragastric gavage at 40 mg/kg, prepared as a 4 mg/mL working solution in saline, with a gavage volume of 10 mL/kg, once daily at 8:00 AM for 8 consecutive days (total of 8 doses); (2) SI+Pro+ISO group: received the same dose of propranolol by gavage (40 mg/kg, once daily for 8 days), and 30 min prior to each propranolol gavage, received an intraperitoneal injection of the β -adrenergic agonist isoproterenol (Sigma-Aldrich, USA) at 2 mg/kg/d, prepared as a 0.2 mg/mL working solution in saline, with an injection volume of 10 mL/kg, once daily for 8 consecutive days; (3) SI group and Con group: as vehicle control groups, received an equal volume of saline by gavage and equal-volume intraperitoneal saline injections at the same frequency (10 mL/kg for each route). The SI and Con groups thus received both gavage and intraperitoneal injection sham procedures to control for handling and injection stress. All gavage procedures were performed using a gavage needle inserted orally into the esophagus, ensuring the needle entered the stomach before slow injection, avoiding tracheal misplacement. During the intervention period, all groups maintained their original housing conditions (group or single isolation). On the day after the last intervention, behavioral tests including open field test and elevated plus maze were conducted, with the open field test performed first, followed immediately by the elevated plus maze test, with no interval between the two tests. Blood samples were collected immediately after completion of both behavioral tests.

3. Observational indicators

3.1. Open field test

A homemade 60-cm cube open field without a lid was used, with interior walls and floor covered with matte black wallpaper. Using SMART 3.0 behavioral analysis software, the floor was divided into 16 grids, with the central 4 grids designated as the central zone and the peripheral 12 grids as the peripheral zone. Mice were placed in the center of the arena, and the video system recorded total distance traveled, central zone entries, and central zone duration (reported as percentage of total time) over 5 min. After each test, feces were removed and the apparatus was wiped with 75% ethanol to eliminate odor cues. All behavioral tests were conducted between 9:00 AM and 12:00 PM under consistent lighting conditions (dim light, ~10 lux), with ambient noise minimized. Investigators performing behavioral analysis were blinded to group assignments.

3.2. Elevated plus maze test

The elevated plus maze comprised two open arms (50 cm × 10 cm), two closed arms (50 cm × 10 cm × 30 cm), and a central platform (10 cm × 10 cm), elevated 30 cm above the floor. Mice were placed on the central platform facing an open arm, and the video system recorded open arm entries, open arm duration, closed arm entries, and total arm entries over 5 min. After each trial, feces were removed and the apparatus was wiped with 75% ethanol. Data are reported as percentage of open arm entries (open entries / total entries × 100%) and percentage of time in open arms (open time / total time × 100%) to control for differences in overall locomotor activity.

3.3. Tumor volume, tumor weight, and thymus weight

Dynamic tumor volume measurement: from the day of B16F10 inoculation (day 0), tumor length and width were measured using a vernier caliper every 3 days, and tumor volume was calculated according to the formula $V = 1/2 \times \text{length} \times \text{width}^2$, with continuous recording until day 21 post-inoculation. All measurements were performed under fixed lighting and standardized handling to minimize human error. Tumor weight and thymus weight measurement: at the experimental endpoint (day 37, i.e., day 21 post-inoculation and day 8 post-intervention), mice were anesthetized and euthanized by cervical dislocation, and primary footpad melanoma tumors and bilateral thymus glands were completely dissected, with adherent adipose tissue removed. Tumor weight and thymus weight were measured using a precision electronic balance (Shanghai Yinzhi Scientific Instrument Co., Ltd., model PB3001-S/FACT). Thymus index was calculated as thymus weight (mg)/body weight (g).

3.4. Stress hormone levels

Blood samples were collected immediately after completion of behavioral tests by retro-orbital bleeding within 2 min of removal from the maze to minimize handling-induced hormone fluctuations, allowed to clot for 30 min, then centrifuged at 4°C, 12,000 rpm for 10 min (radius 10 cm). Serum was stored at -80°C. Serum norepinephrine (NE) and corticosterone (CORT) levels were measured using enzyme-linked immunosorbent assay kits (Tianjin Boji Biotechnology Co., Ltd.; catalog numbers: NE – E-EL-0045c, CORT – E-EL-0014c; detection range: NE 10–2000 pg/mL, CORT 10–1000 ng/mL; intra-assay CV < 8%, inter-assay CV < 10%) according to the manufacturer's protocols.

3.5. CD8⁺ T cell proportions and functional molecules

Single-cell suspensions were prepared from mouse melanoma tissues and

spleens. Spleens were ground, filtered, and subjected to erythrocyte lysis; tumor tissues were digested with collagenase and DNase, then filtered. After washing with PBS, cells were stained with viability dye to exclude dead cells, then surface-stained with anti-CD45 (Leukocyte Common Antigen) and anti-CD8 fluorescent antibodies and anti-CD3/TCR β antibodies (BD Biosciences; clones: CD45 – 30-F11, CD8 – 53-6.7, CD3 – 145-2C11) at 4°C in the dark, stimulated, then fixed, permeabilized, and intracellularly stained with anti-IFN- γ and anti-granzyme B antibodies (BD Biosciences; clones: IFN- γ – XMG1.2, granzyme B – GB11). Fluorescence-minus-one (FMO) controls were used for gating. Compensation was performed using single-stained beads. Samples were acquired on a flow cytometer (Beijing Beijia Meiyi Biotechnology Co., Ltd.; model SONY SH800SEP), and data were analyzed with FlowJo software to calculate tumor-infiltrating CD8 $^{+}$ T cells (CD45 $^{+}$ CD3 $^{+}$ CD8 $^{+}$)/CD45 $^{+}$, IFN- γ $^{+}$ /CD8 $^{+}$, granzyme B $^{+}$ /CD8 $^{+}$, and splenic CD8 $^{+}$ T cells/CD45 $^{+}$.

3.6. Expression of β_2 -AR, SOX10, and PD-L1

Melanoma tissues were isolated, and protein extraction reagent was added for thorough grinding and lysis. Protein concentration was determined, and samples were mixed with loading buffer and boiled for denaturation. Proteins were separated by 10% SDS-PAGE at 100 V constant voltage for 90 min, then transferred onto PVDF membranes at 300 mA constant current for 100 min. After blocking at room temperature for 2 h, membranes were incubated with primary antibodies (anti- β_2 -AR, anti-SOX10, anti-PD-L1; all from Cell Signaling Technology; dilution 1:1000) overnight at 4°C, washed three times with TBST, then incubated with HRP-conjugated secondary antibodies (dilution 1:3000) for 2 h at room temperature, followed by three TBST washes. Chemiluminescence was developed using ECL substrate, and images were captured with a fully automated chemiluminescence imaging system. Densitometric analysis was performed using ImageJ software, with GAPDH as the internal reference, to calculate relative protein expression levels. All reagents were purchased from Shanghai Kepeirui Biotechnology Co., Ltd.

3.7. Statistical analysis

Data were analyzed using SPSS 25.0 software. Normality was assessed by Shapiro-Wilk test and homogeneity of variance by Levene's test. For data meeting normality and homogeneity, comparisons between two groups were performed using independent-sample t-tests, and comparisons among multiple groups were performed using one-way ANOVA followed by LSD-t test for post-hoc pairwise comparisons. For tumor volume longitudinal data, a linear mixed-effects model was used with group, time, and group $\times$ time interaction as fixed effects, and animal as random effect. Data were expressed as mean $\pm$ standard deviation. Exact P-values, effect sizes (Cohen's d or partial η^2), and 95% confidence intervals are reported where applicable. $P < 0.05$ was considered statistically significant; all tests were two-sided. No correction for multiple comparisons was applied; results should therefore be interpreted as exploratory.

4. Results

4.1. Comparison of open field test results among groups

As shown in **Figure 1**, compared with the Con group, the SI and SI+Pro+ISO groups exhibited increased total distance traveled, decreased central zone duration (percentage of total time), and decreased central zone entries. Compared with the SI group, the SI+Pro group showed decreased total distance traveled, increased central

zone duration (percentage), and increased central zone entries. Compared with the SI+Pro group, the SI+Pro+ISO group demonstrated increased total distance traveled, decreased central zone duration (percentage), and decreased central zone entries, with all differences statistically significant ($P < 0.05$). Data points from individual animals are overlaid on all figures, and n values are indicated.

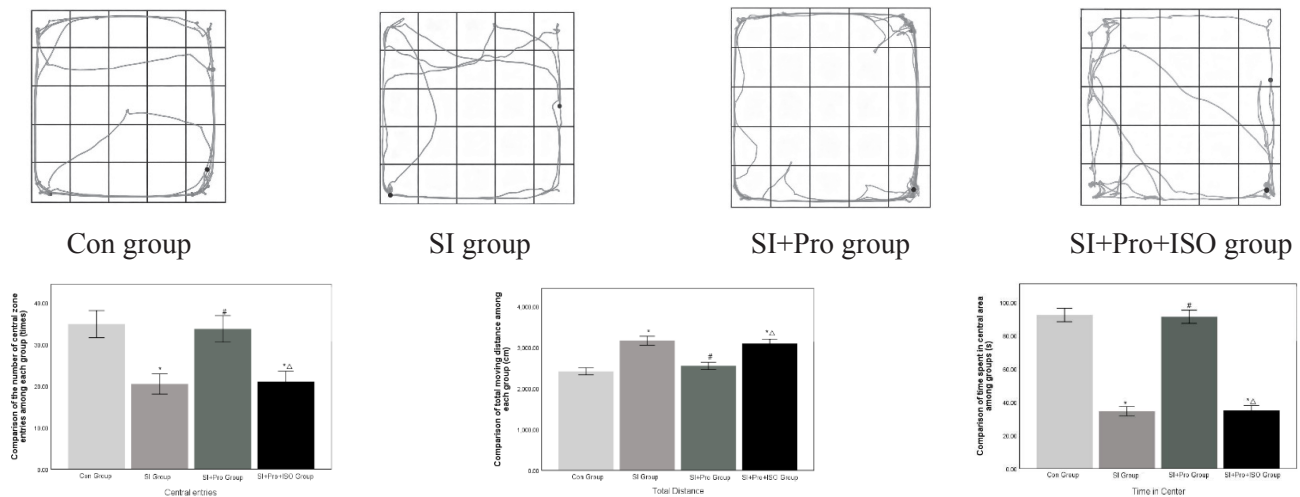


Figure 1. Comparison of open field test results among groups

Note: vs. Con group, * $P < 0.05$; vs. SI group, # $P < 0.05$; vs. SI+Pro group, $\Delta P < 0.05$.

4.2. Comparison of elevated plus maze results among groups

As shown in **Figure 2**, compared with the Con group, the SI and SI+Pro+ISO groups showed increased closed arm entries, decreased open arm entries (percentage of total entries), and decreased open arm duration (percentage of total time). Compared with the SI group, the SI+Pro group exhibited decreased closed arm entries, increased open arm entries (percentage), and increased open arm duration (percentage). Compared with the SI+Pro group, the SI+Pro+ISO group displayed increased closed arm entries, decreased open arm duration (percentage), and decreased open arm entries (percentage), with all differences statistically significant ($P < 0.05$). Total arm entries are also reported to control for overall locomotor activity.

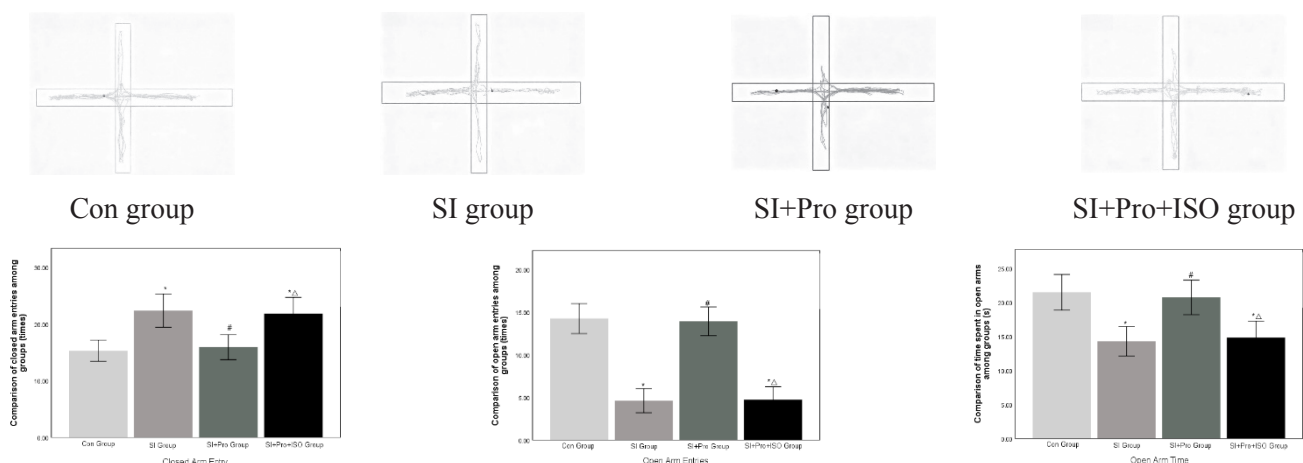


Figure 2. Comparison of elevated plus maze results among groups

Note: vs. Con group, * $P < 0.05$; vs. SI group, # $P < 0.05$; vs. SI+Pro group, $\Delta P < 0.05$

4.3. Comparison of tumor volume, tumor weight, and thymus weight among groups

As shown in **Figure 3**, at early time points (day 0 and day 3 post-inoculation), tumor volumes were not significantly different among the four groups. From day 7 onward, tumor growth was significantly accelerated in the SI and SI+Pro+ISO groups, with volumes higher than those in the Con and SI+Pro groups. Over time, inter-group differences progressively increased; by the experimental endpoint at day 21, tumor volumes in the SI and SI+Pro+ISO groups were significantly enlarged, whereas tumor growth in the SI+Pro group was reduced, with tumor volumes not significantly different from those of the Con group in post-hoc comparisons ($P < 0.05$ for group $\times$ time interaction in the linear mixed-effects model). Compared with the Con group, the SI and SI+Pro+ISO groups had increased tumor weight and decreased thymus weight and thymus index. Compared with the SI group, the SI+Pro group had decreased tumor weight and increased thymus weight and thymus index. Compared with the SI+Pro group, the SI+Pro+ISO group had increased tumor weight and decreased thymus weight and thymus index, with all differences statistically significant ($P < 0.05$).

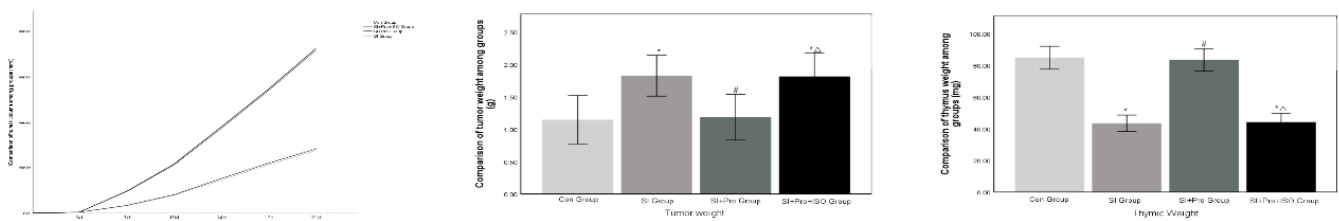


Figure 3. Comparison of tumor volume, tumor weight, and thymus weight among groups

Note: vs. Con group, * $P < 0.05$; vs. SI group, # $P < 0.05$; vs. SI+Pro group, $\Delta P < 0.05$.

4.4. Comparison of serum stress hormone levels among groups

As shown in **Figure 4**, compared with the Con group, the SI and SI+Pro+ISO groups had decreased NE and CORT levels. Compared with the SI group, the SI+Pro group showed increased NE and CORT. Compared with the SI+Pro group, the SI+Pro+ISO group exhibited decreased NE and CORT, with all differences statistically significant ($P < 0.05$).



Figure 4. Comparison of serum stress hormone levels among groups

Note: vs. Con group, * $P < 0.05$; vs. SI group, # $P < 0.05$; vs. SI+Pro group, $\Delta P < 0.05$.

4.5. Comparison of CD8⁺ T cell proportions and functional molecules among groups

As shown in **Figure 5**, compared with the Con group, the SI and SI+Pro+ISO

groups had decreased CD8⁺ T cells (CD45⁺CD3⁺CD8⁺)/CD45⁺, IFN- γ ⁺/CD8⁺, granzyme B⁺/CD8⁺, and splenic CD8⁺ T cells/CD45⁺. Compared with the SI group, the SI+Pro group showed increased CD8⁺ T cells/CD45⁺, IFN- γ ⁺/CD8⁺, granzyme B⁺/CD8⁺, and splenic CD8⁺ T cells/CD45⁺. Compared with the SI+Pro group, the SI+Pro+ISO group exhibited decreased CD8⁺ T cells/CD45⁺, IFN- γ ⁺/CD8⁺, granzyme B⁺/CD8⁺, and splenic CD8⁺ T cells/CD45⁺, with all differences statistically significant ($P < 0.05$).

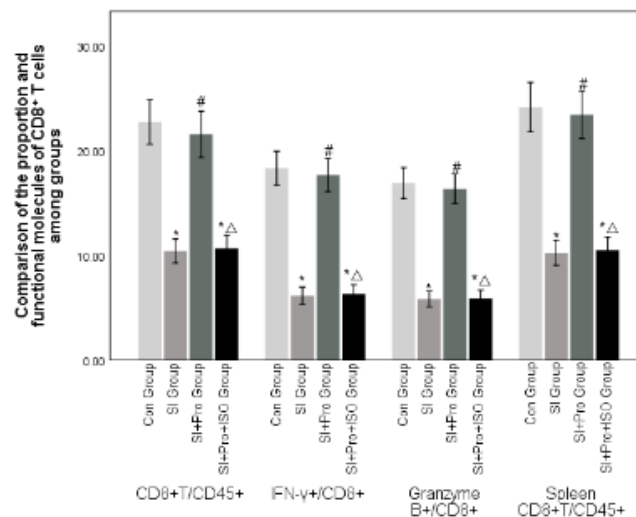


Figure 5. Comparison of CD8⁺ T cell proportions and functional molecules among groups

Note: vs. Con group, * $P < 0.05$; vs. SI group, # $P < 0.05$; vs. SI+Pro group, Δ $P < 0.05$.

4.6. Comparison of β_2 -AR, SOX10, and PD-L1 protein expression levels among groups

As shown in **Figure 6**, compared with the Con group, the SI and SI+Pro+ISO groups had elevated β_2 -AR, SOX10, and PD-L1 expression. Compared with the SI group, the SI+Pro group showed decreased β_2 -AR, SOX10, and PD-L1 expression. Compared with the SI+Pro group, the SI+Pro+ISO group demonstrated increased β_2 -AR, SOX10, and PD-L1 expression, with all differences statistically significant ($P < 0.05$).

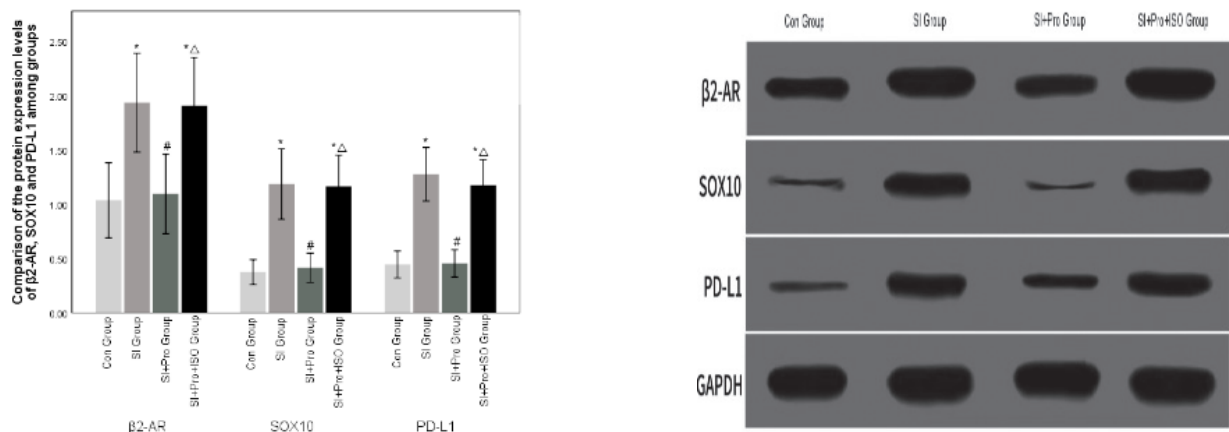


Figure 6. Comparison of β_2 -AR, SOX10, and PD-L1 protein expression levels among groups

Note: vs. Con group, * $P < 0.05$; vs. SI group, # $P < 0.05$; vs. SI+Pro group, Δ $P < 0.05$

5. Discussion

Social isolation, as a prototypical chronic psychological stressor, is widely prevalent among cancer patients and has been associated with anxiety-related disorders such as anxiety and depression as well as accelerated tumor progression through systemic remodeling of the neuro-immune-endocrine network[8-9]. In this study, utilizing a male C57BL/6 mouse model of social isolation stress with melanoma, we observed coordinated alterations in β_2 -AR, SOX10, and PD-L1 expression in stress-induced anxiety-like behaviors and immune alterations, and found that propranolol was associated with effects on emotional behavior and antitumor immune function through β -adrenergic blockade. The discussion is elaborated from three perspectives: neuroendocrine alterations of psychological stress, effects of social isolation on emotion and immunity, and the β -adrenergic-mediated molecular mechanisms.

5.1. Neuroendocrine alterations underlying social isolation stress-induced anxiety-like behaviors

Social isolation deprives social animals of normal social interaction, which the central nervous system perceives as a sustained survival threat, potentially driving activation of the stress circuitry involving the amygdala, hippocampus, and prefrontal cortex[10]. In the present study, SI mice exhibited decreased exploration in the central zone of the open field and in the open arms of the elevated plus maze, along with increased avoidance in closed arms, behavioral phenotypes consistent with anxiety-like states, confirming that social isolation successfully induced an anxiety-like state. Notably, serum NE and CORT levels in the SI group were lower than those in the Con group. This finding may suggest neuroendocrine alterations under prolonged chronic stress (4 weeks): in the initial stress phase, NE and glucocorticoids are released, but after sustained weeks, GR-mediated negative feedback in the hippocampus may elevate the response threshold of CRH neurons, adrenal cortex sensitivity to ACTH may decline, and NE stores in sympathetic terminals may become depleted[11,12]. Thus, decreased hormone levels do not necessarily indicate absence of stress but may reflect neuroendocrine changes induced by long-term stress, a phenomenon also reported in chronically lonely human populations[13]. Following propranolol intervention, the SI+Pro group showed improvement in anxiety behaviors, with NE and CORT increased to levels not significantly different from the Con group, while ISO was associated with reversal of this effect, consistent with the involvement of β -adrenergic signaling in stress-induced emotional and endocrine changes[9].

5.2. Social isolation is associated with CD8⁺ T cell functional changes and altered immune responses

The core of tumor immune alterations lies in changes in CD8⁺ T cell effector function. In this study, the SI group exhibited decreased proportions of tumor-infiltrating CD8⁺ T cells/CD45⁺, IFN- γ ⁺, and granzyme B⁺ positive rates, as well as decreased splenic CD8⁺ T cells, indicating that social isolation was associated with reduced local antitumor immunity and reduced peripheral T cell measures. The potential mechanism is as follows: stress-released NE may continuously activate β_2 -AR expressed on tumor-infiltrating CD8⁺ T cells, potentially inhibiting TCR signal transduction and metabolic reprogramming through the cAMP-PKA signaling pathway, thereby affecting T cell proliferation, effector molecule secretion, and cytotoxicity[9,10]. Concurrently, decreased thymus weight reflects stress-associated thymic changes, possibly through effects on thymocyte apoptosis and on IL-7

secretion, affecting nascent T cell output[14]. After propranolol administration, CD8⁺ T functional indicators increased and thymus weight increased, whereas ISO was associated with attenuation of these effects.

5.3. Coordinated alterations in β_2 -AR, SOX10, and PD-L1 expression are associated with immune alterations in melanoma

In this social isolation model, tumor tissues in the SI group concurrently exhibited elevated β_2 -AR, SOX10, and PD-L1 protein expression, which were decreased by propranolol and increased by ISO. SOX10 is a neural crest lineage transcription factor critical for maintaining the malignant phenotype of melanoma[11,15]. Recent studies indicate that β -adrenergic signaling, via PKA phosphorylation, may activate downstream transcription factors to increase SOX10 transcriptional activity, and SOX10 has been reported to bind to the PD-L1 promoter region to increase its expression, suggesting a possible association among ADRB2, SOX10, and PD-L1[9,16]. This pattern of altered expression may contribute to effects on CD8⁺ T effector function (via β_2 -AR effects on T cells) and PD-L1-mediated effects on T cell activation[14]. In this study, the SI group showed accelerated tumor growth and increased tumor weight, the SI+Pro group exhibited reduced tumor growth with volumes not significantly different from the Con group, while the ISO group displayed rapid tumor progression, consistent with this signaling pattern as a potential molecular pathway through which stress is associated with melanoma progression.

5.4. Effects of propranolol and potential clinical implications

Through β -adrenergic blockade, propranolol was associated with emotional behavior improvement and immune function changes (increased CD8⁺ T function, decreased SOX10/PD-L1, and increased thymus weight), whereas ISO attenuated these effects, consistent with involvement of β -adrenergic signaling[17]. This study provides a preclinical basis for further investigation in melanoma patients with significant social isolation and anxiety, suggesting that psychological assessment may be incorporated into individualized cancer treatment regimens[18]. Limitations include the use of only male mice, young animals, adaptation period of only 1 day, footpad tumor effects on behavior, lack of female animals, lack of tumor-free isolation group, lack of ISO alone group, lack of drug receptor selectivity, single-time point hormone measurement, lack of behavioral pain control, no determination of depletion markers, whole tumor protein not providing information on cell origin, and lack of clinical sample validation. Additionally, future studies should extend to sex-difference investigations and clinical cohort validation.

In conclusion, social isolation stress was associated with anxiety-like behaviors and tumor progression via β -adrenergic pathway alterations, with mechanisms involving coordinated changes in β_2 -AR, SOX10, and PD-L1 expression and CD8⁺ T cell functional changes, and propranolol administration was associated with effects on this pathway.

Author contributions: Conceptualization, M.W. and S.W.; methodology, M.W.; software, M.W.; validation, M.W. and S.W.; formal analysis, M.W.; investigation, M.W.; resources, S.W.; data curation, M.W.; writing—original draft preparation, M.W.; writing—review and editing, S.W.; visualization, M.W.; supervision, S.W.; project administration, S.W. All authors have read and agreed to the published version of the manuscript.

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Ethical approval: This study was reviewed and approved by the Institutional Animal Care and Use Committee of Kunming Institute of Zoology (approval number: License No. SCXK (Dian) K2025-0001). All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and followed the ARRIVE guidelines.

Informed consent statement: Not applicable.

Data availability statement: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest: The authors declare no conflicts of interest.

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