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Grain-sized moxibustion activates dendritic cells to enhance the antitumor immunity of cancer vaccines

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Abstract

Background Moxibustion, a traditional Chinese medicine (TCM) therapy, employs thermal stimulation from the combustion of *Artemisia argyi* H.Lév. & Vaniot at acupoints to treat "deficiency-cold syndromes" (*xu han zheng*), historically linked to immune dysfunction and chronic inflammation. Modern pharmacological studies showed that grain-sized moxibustion (gM) enhances innate immune surveillance such as natural killer (NK) cell recruitment. However, its synergy with vaccine-induced adaptive immunity remains unexplored. Guided by the TCM principle of *fu zheng qu xie* ("fortify the host to dispel pathogens"), this study investigated whether gM augments cancer vaccine efficacy and validate the mechanistic basis of thermal acupoint stimulation in amplifying adaptive antitumor immunity.

Methods In tumor-bearing mice model, gM was applied to the ST36 (Zusanli) acupoint. Adjuvant effects on the cancer vaccine were evaluated through flow cytometry, β -adrenergic receptor blockade, and cell depletion.

Results gM synergized with the cancer vaccine, significantly suppressing tumor growth. Mechanistically, gM inhibited β -adrenergic signaling, driving DC maturation and subsequent coordination of CD4⁺ T cell, CD8⁺ T cell and NK cell responses. CD4⁺ T cells as primary effectors, with NK cells playing a secondary role. Propranolol mirrored gM's effects, further enhancing DC activation and tumor suppression when combined with vaccination.

Conclusion Both gM and β -blockers enhance cancer vaccine efficacy through β -adrenergic suppression and maturation of DC. These findings mechanistically bridge TCM's *fu zheng qu xie* strategy with modern immunotherapy, positioning β -adrenergic modulation as a convergent target for traditional and pharmacological interventions.

Keywords Cancer vaccine, Grain-sized moxibustion, β -adrenergic receptor, CD4⁺ T cells, Dendritic cells

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Introduction

Cancer vaccines aim to prime antigen-specific immunity against tumors by delivering tumor antigens (e.g., inactivated cells, lysates, or peptides) to antigen-presenting cells (APC) [1–6]. Despite preclinical success, clinical efficacy remains limited by inadequate T-cell activation and immunosuppressive tumor microenvironments [7, 8]. Current strategies—adjuvant optimization [9], neo-antigen discovery [10, 11], and checkpoint inhibitor combinations [12, 13]—have yet to fully overcome these barriers [14], necessitating innovative approaches that integrate traditional medical wisdom.

Traditional Chinese medicine (TCM) offers a millennia-old framework for immune modulation. Among its modalities, moxibustion—thermal stimulation of acupoints via combustion of *Artemisia argyi* H.Lév. & Vaniot (verified via <http://www.theplantlist.org>, accessed on 2025-03-04)—has been systematically documented since the *Huangdi Neijing* (475–221 BCE) for treating *xuhan zheng* (“deficiency-cold syndrome”) [15, 16], a condition marked by chronic inflammation and immune dysfunction [17, 18]. TCM theory posits that moxibustion restores balance through dual actions: tonifying host resistance (*zhengqi*) by warming meridians and dispelling pathogenic factors (*xieqi*) via purgation [19]. Modern studies attribute its effects to thermal, radiative, and pharmacological properties of moxa combustion [20], with demonstrated roles in analgesia, immunomodulation, and anti-aging [21–25]. Critically, recent trials highlight moxibustion’s potential in cancer care, particularly for managing chemotherapy-induced fatigue and immune suppression [26, 27].

Grain-sized moxibustion (gM), a refined technique using cone-shaped moxa, was historically prescribed in *Shenji Zonglu* (eleventh century CE) for consumptive diseases (*xulao*) characterized by progressive immune decline—a profile analogous to cancer-related immunosuppression. Building on this legacy, we hypothesize that gM enhances cancer vaccine efficacy through two convergent mechanisms: (1) APC Activation: gM-induced local inflammation may enhance APC maturation and antigen presentation, amplifying T-cell priming; (2) TME Remodeling: By recruiting NK cells and reducing immunosuppressive cytokines (e.g., IL-10, TGF- β), gM could facilitate T-cell infiltration into tumors.

This study explores the theoretical interface between TCM’s Fuzheng Quxie principle (reinforcing vital qi and eliminating pathogenic factors) and tumor immunology by drawing parallels between Yang-deficiency cold coagulation pathogenesis and the cold tumor phenotype [28, 29]—the former describing TCM’s pathological cold accumulation in cancer evolution [30, 31], the latter referring to modern immunology’s characterization

of immune-excluded malignancies, thereby highlighting the therapeutic relevance of Yang-strengthening therapy in modulating tumor microenvironmental frigidity. Notably, cancer vaccine represents a modern approach to converting immunologically inert (“cold”) tumors into immunoreactive (“hot”) lesions [29], while gM is as one Yang-strengthening therapy to combat pathological cold accumulation [26, 27, 32]. By integrating gM into cancer vaccine regimens, we establish a bidirectional translation model where ancient therapeutic wisdom informs contemporary immunotherapy.

Materials and methods

Cell lines and cell culture

The Lewis Lung Carcinoma (LLC) tumor cells [33] were bought from the Cell Bank of the Chinese Academy of Science and cultured in high-glucose DMEM (SH30243.01, HyClone) supplemented with 10% fetal bovine serum (S1580-500, Biowest) and 1% penicillin–streptomycin (60162ES76, YEASEN) in a humidified incubator at 37 °C with 5% CO₂.

Bone marrow dendritic cell generation

DC were generated from bone marrow progenitors according to a published method [34]. Briefly, bone marrow cells were isolated from the femurs and tibias of 5–6 week-old C57BL/6 mice by flushing with sterile saline solution. The suspension was then filtered through a 70 μ m mesh and centrifuged at 1000 rpm for 5 min to collect the cells. The red blood cells were removed with red blood lysis buffer (Biolegend, catalog #420301) for 5 min at room temperature. The remaining cells were suspended and cultured in 100 mm Petri dishes at 5×10^5 /mL with 10 mL specialized medium (RPMI1640 supplemented with 10% FBS, 20 ng/mL murine GM-CSF (576304, Biolegend), 10 ng/mL murine IL-4 (574304, Biolegend) and 1% penicillin–streptomycin). On day 3, the same volume of fresh medium was added to the Petri dish. On day 6, the medium was changed with fresh medium. On day 7, immature DC were harvested and used for the next assays.

Cancer vaccine preparation

Tumor cell lysate vaccines (TCL) and DC vaccines (DCV) were prepared according to previously established protocols [35]. For the TCL, LLC cells were harvested at a concentration of 2×10^7 cells/mL and transferred to a cryovial. The cells were lysed through three cycles of freeze-thawing, alternating between liquid nitrogen and a 37 °C water bath. Subsequently, the lysate supernatant was obtained by centrifugation at 1000 rpm for 5 min and stored at –80 °C until further use.

For the DCV, bone marrow-derived dendritic cells were cultured at a density of 1×10^6 cells/mL and co-incubated with 100 μ L of TCL (100 μ g/mL) for 24 h. After co-culture, the DC were pelleted by centrifugation and then resuspended in 100 μ L of phosphate-buffered saline (PBS) within a centrifuge tube, thus forming the DCV preparation.

The cancer vaccine was subcutaneously injected into the upper back of mice (contralateral to the tumour) on days 1, 8 and 15 after tumor modelling [11, 36]. The usage of TCL is 10 μ g per mouse and DCV is 1×10^6 dose per mouse.

Grain-sized moxibustion

Grain-sized moxibustion was prepared and administered following a previously established protocol [26]. The moxa wool (47289, DAYUJP) was meticulously crafted into small cones (diameter 0.8 cm * height 0.9 cm), which were then positioned on the bilateral Zusanli (ST 36) acupoints located on the mice. The ST 36 acupoints are situated 2 mm below and lateral to the anterior tubercle of the tibia. Once in place, the moxa cones were ignited and permitted to burn slowly. The cones were promptly removed upon complete combustion. For the sham group, grain-sized moxibustion was performed at the mouse tail to avoid other potential effect.

The moxibustion intervention commenced on the day of tumor modeling and was subsequently repeated every other day until the conclusion of the experiment. Each acupoint was treated three times to ensure consistent and effective stimulation and the total treatment time of one acupoint is about 30 s.

Mice

Male C57BL/6 mice (6 week of age) were purchased from Vital River and bred under specific pathogen-free (SPF) environmental conditions. All procedures were approved by the Institutional Animal Care and Use Committee at Shanghai University of Traditional Chinese Medicine and the methods were performed in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Subcutaneous transplanted tumor model

A total of 5×10^5 LLC tumor cells were subcutaneously injected into the upper back of the mice. Tumour sizes were monitored every 2 days by using an electronic caliper, and tumour volumes were calculated by the following formula: $V = 0.5 \times a \times b^2$, where V = tumour volume, a = maximum tumour diameter, and b = minimum tumour diameter. After 3 weeks or when the tumour

volume reached 2000 mm³, the mice were sacrificed by inhalation of CO₂. The tumours were excised, weighed, and photographed.

Immune cells depletion

For NK cell depletion, an anti-mouse NK1.1 antibody (PK136, 108702) was intraperitoneally injected (100 μ g per mouse) every 7 days [26]. For CD4⁺ T cells and CD8⁺ T cells depletion, anti-CD4 (GK1.5, 100402) and anti-CD8 (2.43, 100,702) antibodies were intraperitoneally injected (100 μ g per mouse) every 3 days [37, 38]. Purified Mouse IgG2a, κ Isotype Ctrl Antibody (400202) was used as a control.

β -adrenoceptor blockade

For β -adrenoceptor blockade, propranolol hydrochloride (Prop) was dissolved in PBS and intraperitoneally injected at a concentration of 15 mg/kg/d beginning on the day of tumor modeling [39]. Propranolol hydrochloride (H32020133) was purchased from Jiangsu Yabang Aipusen Pharmaceutical Co., Ltd. β -AR agonist Epinephrine hydrochloride (E4642) was provided by Sigma. PKA Inhibitor Fragment (6-22) amide TFA (HY-P1290A) and BAY11 (HY-10257) were purchased from MCE.

Immunophenotype analysis

Tumors, spleens, and tumor-adjacent lymph nodes were isolated from the tumour-bearing mice to measure the levels of T cells, NK cells and DC by flow cytometry [26, 35]. Compared with spleens and lymph nodes, tumours were cut into small pieces with scissors and digested with 0.1% collagenase (40507ES60, Yeasen) for two hours. Then, all tissues were filtered through a 70 μ m mesh to make single-cell suspensions. Erythrocytes in tumors and spleens were removed with red blood lysis buffer (420,301, Biolegend). The cells were exposed to appropriate fluorescence-conjugated antibodies at 4 °C for 30 min in the dark and then washed and suspended in PBS containing 1% FBS. The data were obtained with a CytoFLEX LX instrument (Beckman) and analysed with FlowJo software.

Flow cytometry

For analysis of immune cells in spleen, lymph node and tumour, CD45⁺ cells were firstly sorted from all living cells, and then CD3⁺ T cells and CD3⁻NKp46⁺ NK cells were sorted from CD45⁺ cells. CD4⁺ T helper cells and CD8⁺ cytotoxicity T cells were sorted from CD3⁺ cells. For analysis of DC, CD11c⁺ Cells were sorted from CD45⁺ cells, then MHCII and CD86 expression were analysed in CD11c⁺ cells. To determine the proportion of IFN- γ ⁺ CD4⁺ T cells, splenocytes were isolated from mice, and red blood cells were lysed. The remaining cells

were counted and co-cultured with LLC cells at a ratio of 3:1 for 6 h. Intracellular cytokine production was blocked using Brefeldin A (BFA, HY-16592, MCE). Following the incubation period, the splenocytes were harvested and stained for CD3 and CD4, as well as intracellular IFN- γ . The frequency of IFN- γ ⁺CD4⁺ T cells was then analyzed. To validate that DC can be affected by propranolol, the expression of β -AR also was detected. The following fluorophore-conjugated antibodies were used: PE/Cyanine7-conjugated anti-mouse CD45 (147704), FITC-conjugated anti-mouse CD4 (100406), PE-conjugated anti-mouse CD3 (100206), PerCP/Cy5.5-conjugated anti-mouse CD8 α (100734), APC-conjugated anti-mouse CD335/NKp46 (137608), APC-conjugated anti-mouse CD11c (117309), FITC-conjugated anti-mouse I-A/I-E (MHCII, 107606), PE-conjugated anti-mouse CD86 (105106), APC-conjugated anti-mouse IFN- γ (505810), PE Donkey anti-rabbit IgG Antibody (406421) were purchased from Biolegend Ln. β 1 Adrenergic Receptor Polyclonal Antibody (PA5-95742) was purchased from Invitrogen.

Statistical analysis

All the data are presented as the means \pm standard errors of the means (SEM) and were analysed with SPSS statistical software. One-way analysis of variance, two-way analysis of variance and independent-samples t tests were used to assess statistical significance. P value < 0.05 was considered to indicate statistical significance.

Results

Grain-sized moxibustion promotes the immune response of cancer vaccines to inhibit tumor growth

In our previous study, we demonstrated that grain-sized moxibustion (gM) stimulation at the ST36 acupoint in tumor-bearing mice could elevate the level of natural killer (NK) cells, thereby inhibiting tumor growth [26]. To sustain the therapeutic effects of gM, we continued to administer stimulation at the ST36 site (Fig. 1A). For the preparation of cancer vaccines, we developed both vaccines: tumor cell lysate vaccines (TCL) and dendritic cell vaccines (DCV) (Fig. 1B). To evaluate the impact of gM on the efficacy of cancer vaccines, we administered the TCL/DCV to mice with lung carcinoma (LLC) at Days 0, 7, and 14, and performed gM every two days starting from Day 0 (Fig. 1C). Our findings indicated that the TCL alone did not significantly inhibit tumor growth compared to the control group, whereas the DCV demonstrated improved efficacy (Fig. 1D and H). When combined with gM, the TCL achieved efficacy comparable to that of the DCV (Fig. 1D–F, H–J), suggesting that gM can enhance the therapeutic efficacy of cancer vaccines, particularly the TCL. With respect to safety, there were no

substantial changes in the body weights of mice across all groups (Fig. 1G, K).

To investigate whether gM potentiates the anti-tumor effects of cancer vaccines through enhanced immune responses, we assessed the lymphocyte populations in tumor-bearing mice. Our results revealed that the combination of gM with the TCL/DCV significantly increased the levels of NK cells, as well as CD4⁺ and CD8⁺ T cells, both in the spleen (Fig. 2A–D) and the tumor tissue (Fig. 2E–G). To further prove that gM enhances the anti-tumor immune response of cancer vaccines, we also detected IFN- γ secreted by T cells, which is increased in the treatment groups (Figure S1). These findings suggest that gM enhances the anti-tumor immune response of cancer vaccines, with NK cells, CD4⁺, and CD8⁺ T cells potentially contributing to the synergistic tumor regression.

Grain-sized moxibustion enhances cancer vaccine partly depends on NK cells

Our previous research established that NK cells are the primary effector cells mediating the inhibition of tumor growth by gM treatment [26]. To explore whether gM potentiates the efficacy of cancer vaccines through the action of NK cells, we depleted NK cells in tumor-bearing mice using an anti-PK136 antibody via intraperitoneal injection (Fig. 3A, B). Notably, the depletion of NK cells partially reversed the anti-tumor effects of the combination therapy; however, there remained a statistically significant difference in tumor growth suppression when compared to the group treated with PK136 alone (Fig. 3C–F). These findings suggest that while NK cells contribute to the therapeutic effects of the combination therapy, they do not play an exclusive role. The persistence of anti-tumor activity despite NK cell depletion indicates that other immune cells are likely involved in the mechanism of action of the gM-enhanced cancer vaccine.

CD4⁺ T cells are the main effector cells in combination therapy

Numerous studies have highlighted the pivotal role of T cells, particularly CD8⁺ T cells, in the efficacy of cancer vaccines [40]. To delineate the primary effector cells involved, we depleted CD4⁺ and CD8⁺ T cells using the specific antibodies GK1.5 and 2.43, respectively (Fig. 4A, B). In comparison to the IgG control group, the depletion of CD8⁺ T cells led to an increase in tumor growth, highlighting the beneficial role of CD8⁺ T cells in tumor suppression (Fig. 4C–E). However, in the group receiving the combination therapy, the absence of CD8⁺ T cells only partially reversed the anti-tumor effects (Fig. 4C–E). In contrast, the

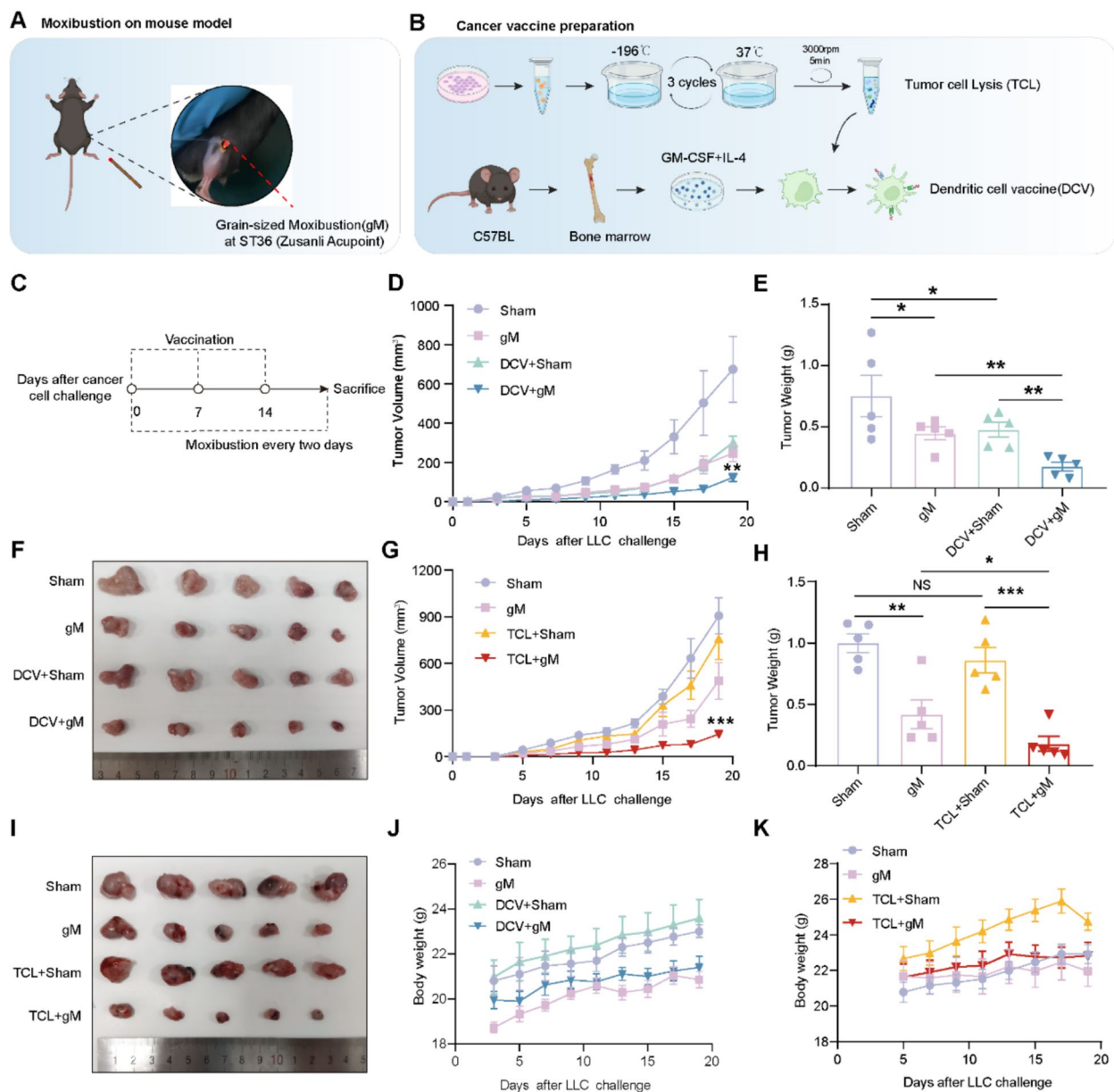


Fig. 1 The combination of gM with cancer vaccines improves tumor regression. **A** Grain-sized moxibustion (gM) at ST36 (acupoint zusanli) in a mouse model; **B** Cancer vaccine preparation involving tumor cell lysate (TCL) and dendritic cell vaccine (DCV); **C** Schematic outline of the intervention protocol used in this study. Briefly, 5×10^5 tumor cells were subcutaneously injected on day 0, and mice were subcutaneously immunized on days 0, 7, 14 and treated with gM at ST36 every two days; **D–F** Tumor growth curves/tumor weight/tumor size of mice from the Sham, gM, DCV+Sham and DCV+gM groups ($n=5$ mice per group); **G–I** Tumor growth curves/tumor weight/tumor size of mice from the Sham, gM, TCL+Sham and TCL+gM groups ($n=5$ mice per group). **J–K** The body weight of mice in different groups. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

complete reversal of the combination therapy's anti-tumor effect was observed only when $CD4^+$ T cells were depleted (Fig. 4C–E). These findings suggest that $CD4^+$ T cells may play a more critical role in the therapeutic efficacy of the combination therapy than either NK cells or $CD8^+$ T cells.

Furthermore, we analyzed the infiltration of $CD4^+$ and $CD8^+$ T cells within the tumor microenvironment and found that their levels were consistent with those detected in the spleen (Fig. 4F–G). This suggests a

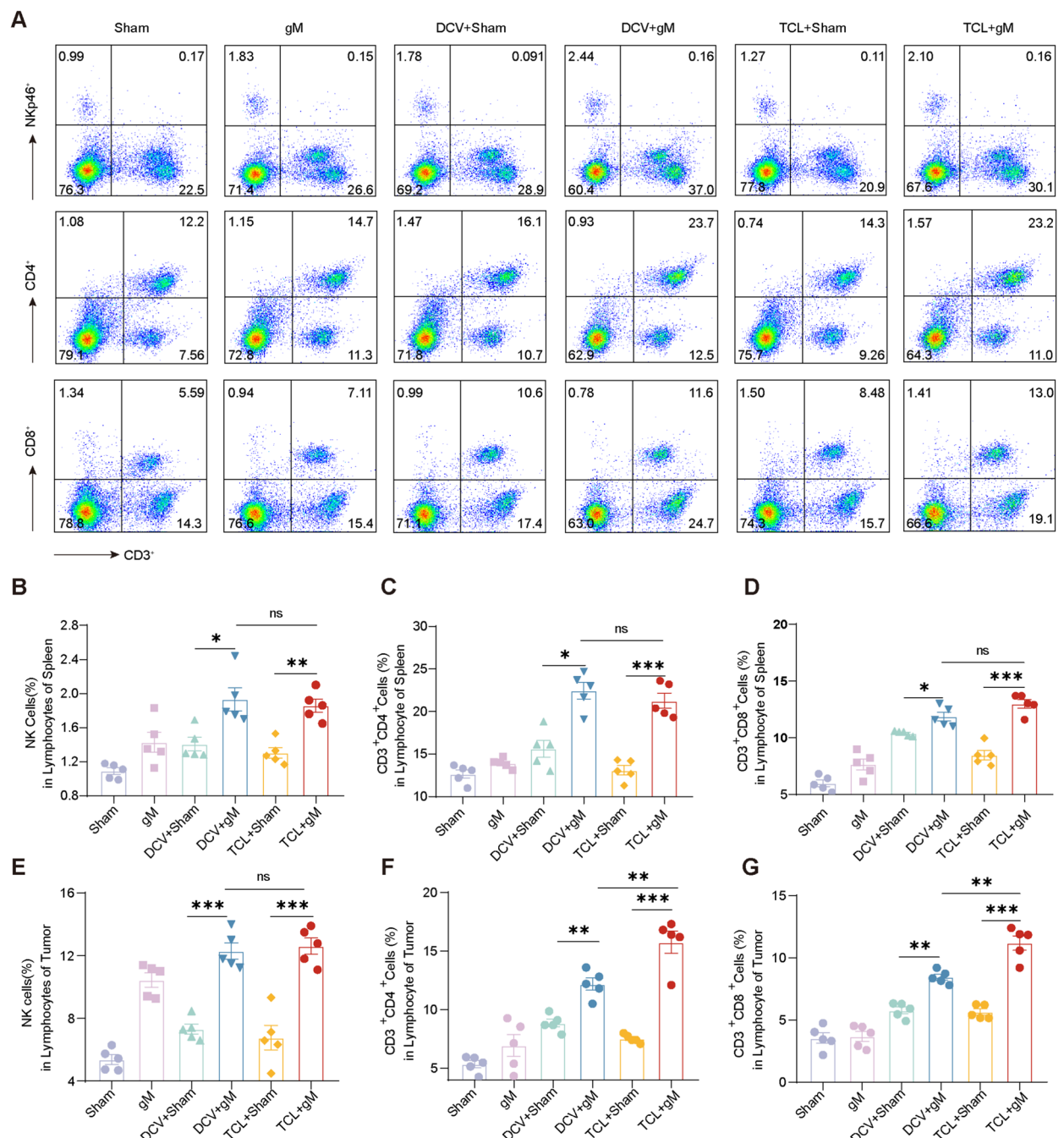


Fig. 2 The combination of gM with cancer vaccines enhances the immune response. **A–D** Flow cytometry analysis of NK, CD4⁺T and CD8⁺T cells in splenic lymphocytes from the Sham, gM, DCV + Sham, DCV + gM, TCL + Sham and TCL + gM groups (n = 5 mice per group); **E–G** Flow cytometry analysis of NK, CD4⁺T and CD8⁺T cells in tumor lymphocytes from the Sham, gM, DCV + Sham, DCV + gM, TCL + Sham and TCL + gM groups (n = 5 mice per group). "ns": no statistical significance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

correlation between the peripheral immune response and the cellular dynamics within the tumor, supporting the notion that CD4⁺ T cells are integral to the mechanism of action of the gM-enhanced cancer vaccine.

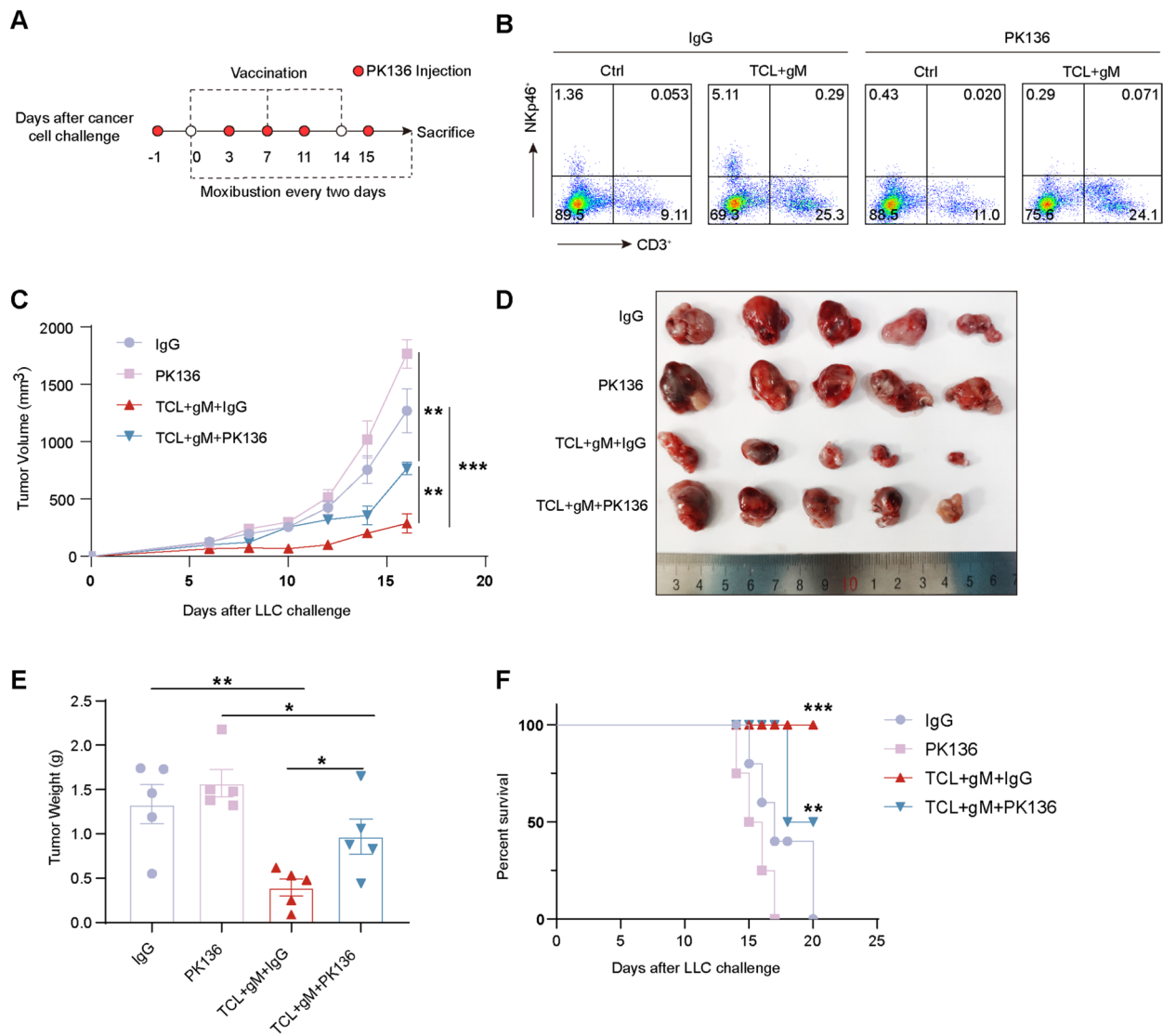


Fig. 3 The depletion of NK cells partly reversed the inhibitory effect of gM combined with the TCL vaccine on tumors. **A** Schematic outline of the NK depletion protocol (PK136; anti-NK antibody) used in this study; **B** Flow cytometry analysis of NK cells in splenic lymphocytes from the Ctrl and TCL + gM groups with/without PK136 treatment (n = 5 mice per group); **C–E** Tumor growth curves/tumor size/tumor weight of the IgG, PK136, TCL + gM + IgG and TCL + gM + PK136 groups (n = 5 mice per group). **F** Survival curves of the IgG, PK136, TCL + gM + IgG and TCL + gM + PK136 groups (n = 5 mice per group). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Grain-sized moxibustion promotes DC activation in tumor-draining lymph nodes by inhibiting β -adrenergic receptors

Tumor antigens are typically not recognized by T cells directly; they require uptake and presentation by DC for effective T cell activation. Upon engagement with peptide-Major Histocompatibility Complex class II complexes (MHC-II), along with the interaction of costimulatory molecules CD80/CD86 and cytokines produced by DC, naive CD4⁺ T cells are activated and differentiate into effector T cells (Fig. 5A). Essentially, the

functionality of CD4⁺ T cells is often contingent upon the activity of DC [41, 42]. To investigate whether gM facilitates the activation of CD4⁺ T cells through modulation of DC, we examined the expression of activation markers on DC within tumor-adjacent lymph nodes. Our findings revealed that gM administration alone significantly increased the expression of MHC-II and CD86 on DC. Additionally, the concurrent application of gM and the TCL vaccine resulted in even higher levels of MHC-II on DC (Fig. 5B, C), indicating that gM plays a crucial role in the activation of DC.

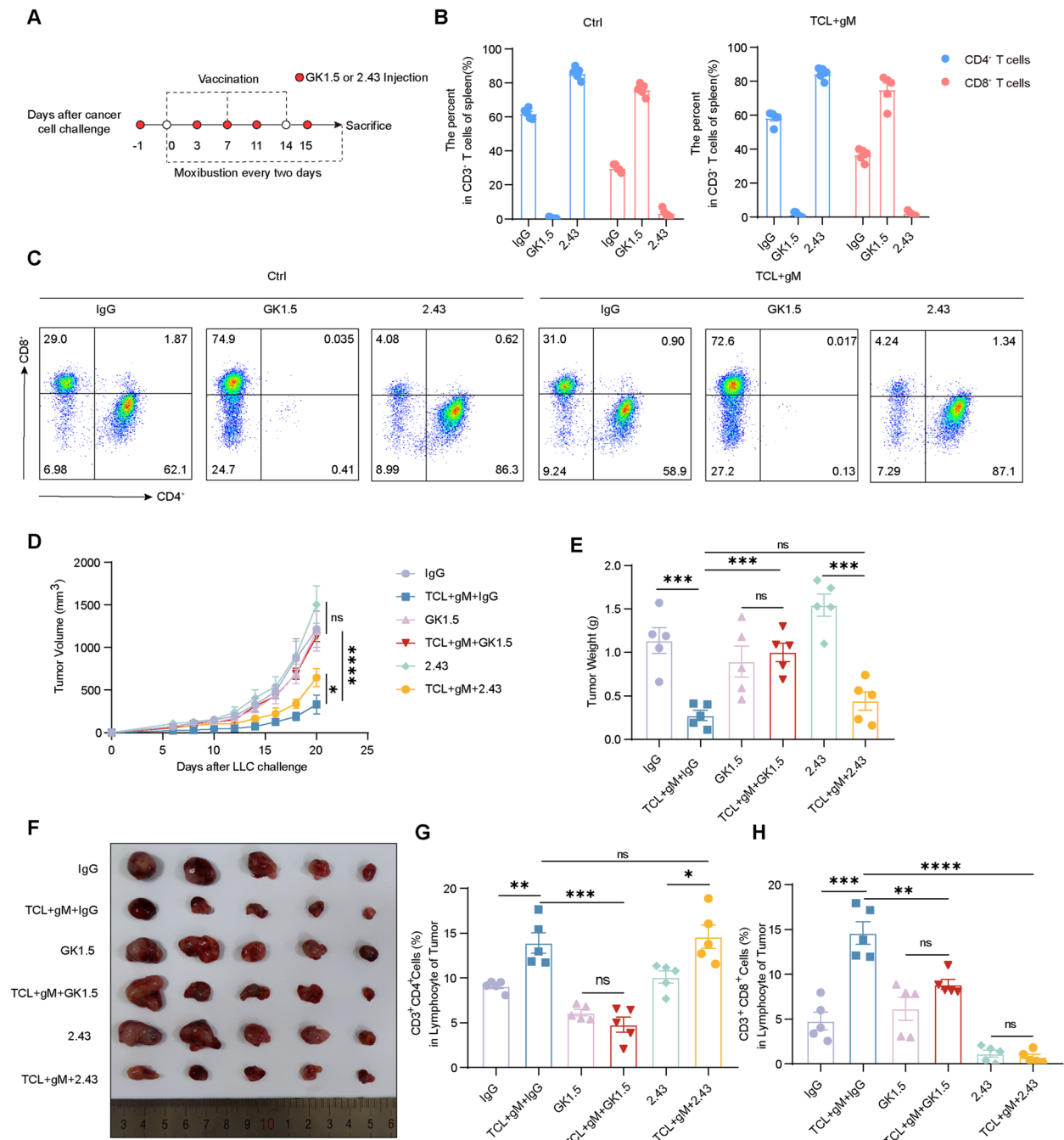


Fig. 4 The combination of gM with the TCL vaccine inhibits tumor growth via CD4⁺ T cells but not CD8⁺ T cells. **A** Schematic outline of the CD4⁺ T-cell or CD8⁺ T-cell depletion protocol (GK1.5: anti-CD4 antibody; 2.43: anti-CD8 antibody) used in this study; **B, C** Flow cytometry analysis of CD4⁺/CD8⁺ T cells in splenic lymphocytes from the Ctrl and TCL + gM groups with/without GK1.5/2.43 (n = 5 mice per group); **D–F** Tumor growth curves/tumor Weights/tumor sizes from the Ctrl and TCL + gM groups with/without GK1.5/2.43 (n = 5 mice per group). **G, H** Flow cytometry analysis of CD4⁺/CD8⁺ T cells in lymphocytes from tumors from the Ctrl and TCL + gM groups with/without GK1.5/2.43 treatment. "ns": no statistical significance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

To elucidate the mechanism by which gM regulates DC function, we conducted RNA-sequencing on tumor samples from control and gM-treated groups, followed

by KEGG signaling pathway [43–45] analysis of the differentially expressed genes. Notably, among the top 10 enriched signaling pathways, four were found to be

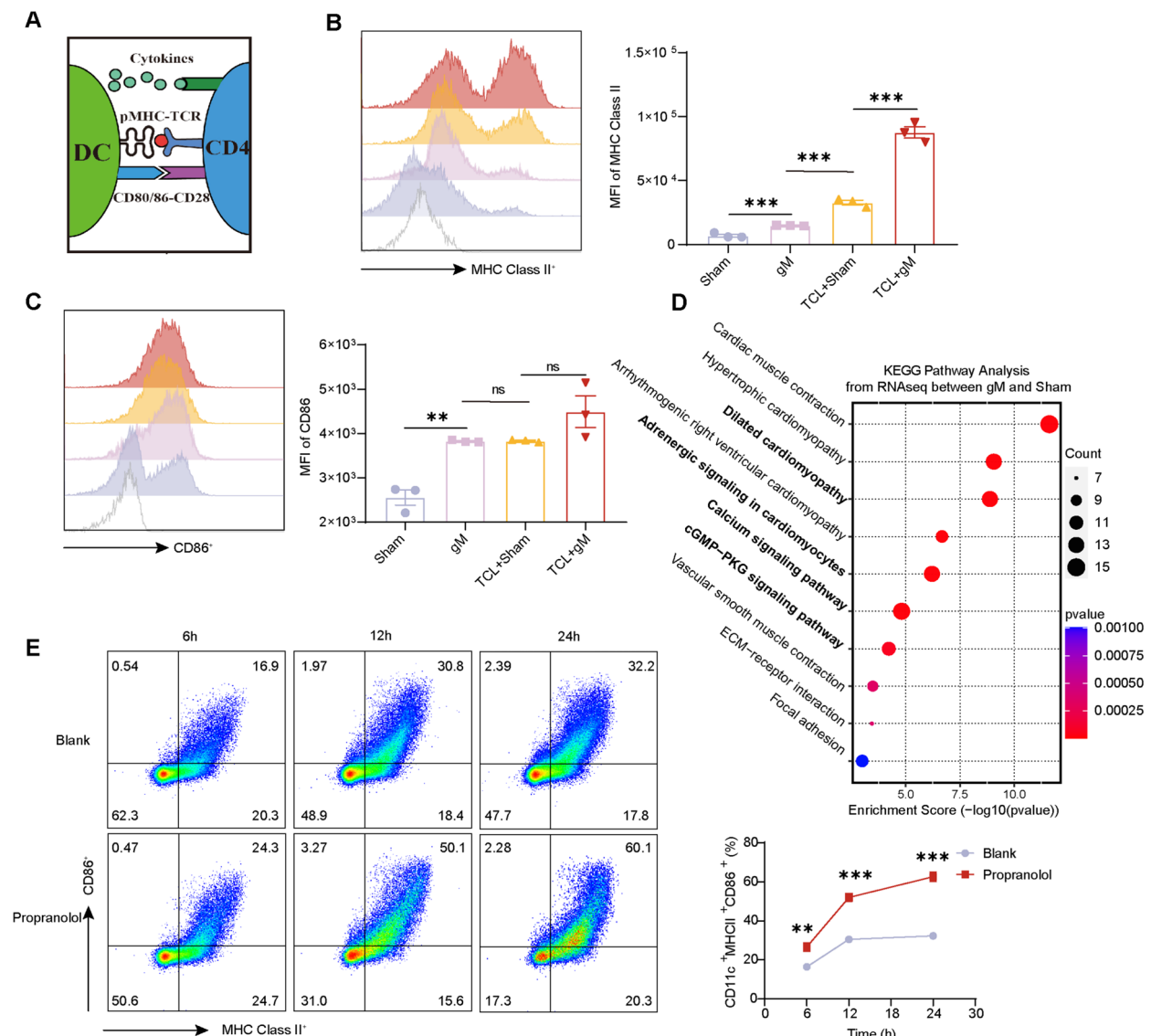


Fig. 5 The combination of gM with the TCL vaccine promotes DC activation in paracancerous lymph nodes by inhibiting the adrenergic signalling pathway. **A** Schematic outline of the interaction between dendritic cells and CD4⁺ T cells. **B** Flow cytometry analysis of MHC class II⁺ activation markers in CD11c⁺ dendritic cells from paracancerous lymph nodes from the Sham, gM, TCL + Sham and TCL + gM groups (n = 3 mice per group); **C** Flow cytometry analysis of the CD86 activation marker in CD11c⁺ dendritic cells of paracancerous lymph nodes from the Sham, gM, TCL + Sham and TCL + gM groups (n = 3 mice per group). **D** KEGG pathway analysis of RNA-seq data [26, 43–45] from tumors in the Sham and gM groups (n = 4 mice per group). Downstream pathways of the adrenergic receptor family are marked in bold. **E** Flow cytometry analysis of the percentage of MHC class II⁺CD86⁺ CD11c⁺ bone marrow-derived dendritic cells (BMDCs) treated with/without propranolol for 6–24 h. “ns”: no statistical significance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

down-regulated and associated with the β -adrenergic receptor pathway, implying that gM may suppress β -adrenergic receptor downstream signaling (Fig. 5D).

Propranolol, a well-known β -adrenergic receptor inhibitor, was utilized to test the hypothesis that gM activates DC by inhibiting β -adrenergic receptor signaling. Indeed, treatment of DC with propranolol for

6 h, 12 h, and 24 h in vitro led to an up-regulation of DC activation, as evidenced by increased expression of activation markers (Fig. 5E). And We also compared the effects of the β -agonist epinephrine on DC and found that it impairs DC maturation (Figure S3B-C). These results suggest that gM may activate DC through the modulation of β -adrenergic receptor signaling.

Propranolol enhances the anti-tumor effect of the TCL vaccine

Given that the effects of propranolol resemble those of gM, we postulated that the combination of propranolol with a TCL vaccine might elicit a more robust anti-tumor immune response. To test this hypothesis, tumor-bearing mice were vaccinated and concurrently administered daily intraperitoneal injections of propranolol (Fig. 6A). We then monitored tumor growth in the mice and compared the outcomes between the TCL+gM group and the TCL+gM+prop group. The findings indicated that the TCL+prop group exhibited a comparable antitumor

effect to that of the TCL+gM group, suggesting that propranolol can also potentiate the efficacy of the TCL vaccine (Fig. 6B, C).

Consistent with these observations, we employed flow cytometry to assess the proportions of mature DC in paracancerous lymph nodes and the infiltration of NK, CD4⁺, and CD8⁺ T cells within the tumor microenvironment across all groups. The data revealed that both propranolol and gM similarly enhanced the levels of these immune cells (Fig. 6D–G). Moreover, propranolol did not induce significant toxicity when administered alone or in combination with gM (Fig. 6H), indicating that

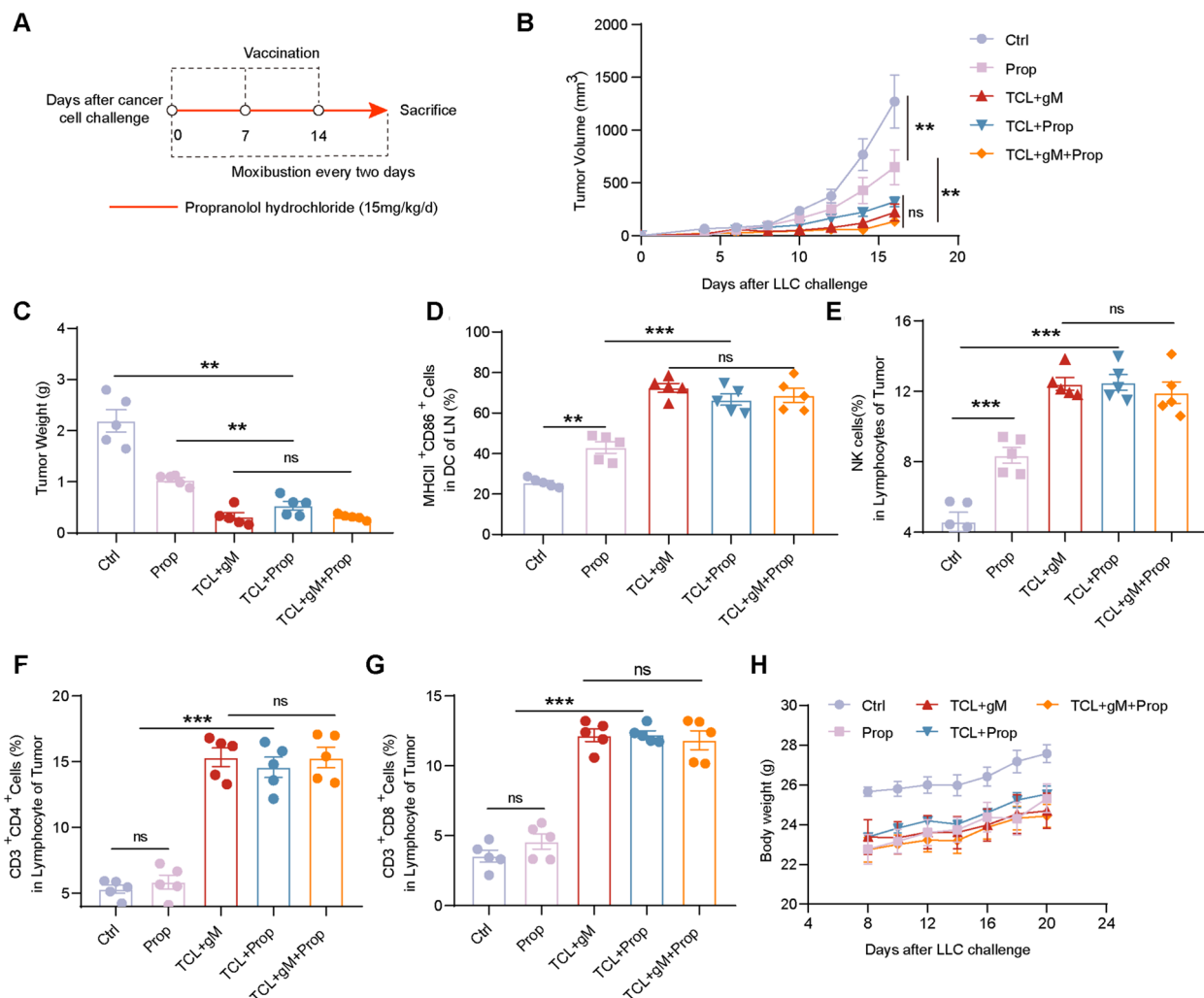


Fig. 6 The combination of propranolol with the TCL vaccine enhances antitumor immunity. **A** Schematic outline of propranolol (Prop) used in this study; **B, C** Tumor growth curves/tumor weights of the Ctrl, Prop, TCL+gM, TCL+Prop and TCL+gM+Prop groups ($n=5$ mice per group); **D** Flow cytometry analysis of the percentage of MHC class II⁺CD86⁺CD11c⁺ dendritic cells in the paracancerous lymph nodes of the Ctrl, Prop, TCL+gM, TCL+Prop and TCL+gM+Prop groups ($n=5$ mice per group); **E–G** Flow cytometry analysis of the percentages of NK, CD4⁺T and CD8⁺ T cells in the lymphocytes of tumors from the Ctrl, Prop, TCL+gM, TCL+Prop and TCL+gM+Prop groups ($n=5$ mice per group); **H** Body weights of the mice from the Ctrl, Prop, TCL+gM, TCL+Prop and TCL+gM+Prop groups ($n=5$ mice per group). "ns": no statistical significance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

propranolol may serve as a potential adjuvant for cancer vaccines.

Discussion

Poor clinical outcomes represent a significant challenge for cancer vaccines, and there is an urgent need to develop novel strategies to enhance their efficacy. In this study, we discovered that gM can inhibit the β -adrenergic receptor signaling pathway and augment DC function, thereby potentiating the antitumor immune response elicited by cancer vaccines and ultimately suppressing tumor progression.

Previous research has demonstrated that stimulation of β -adrenoceptors compromises the functionality of DC and cytotoxic CD8⁺ T lymphocytes [46–48]. The dysfunction of DC and CD8⁺ T cells, which serve as primary effector cells, likely contributes to the ineffectiveness of cancer vaccines. Therefore, inhibiting β -adrenoceptors may enhance vaccine efficacy. Several studies have demonstrated that the β -adrenoceptor antagonist propranolol can potentiate the effects of tumor vaccines when used as an adjuvant. For instance, Somayeh Ashrafi et al. reported that the combination of propranolol with a tumor vaccine suppresses tumor growth by modulating cytokine patterns within the tumor microenvironment [49]; Clara Daher et al. also demonstrated that propranolol can strongly improve the efficacy of an antitumor STxBE7 vaccine by enhancing the frequency of CD8⁺ T lymphocytes infiltrating the tumor (TIL) [46]. In this study, we also demonstrated that propranolol can be a good adjuvant for the TCL vaccine.

As a nonselective β -adrenergic receptor inhibitor, propranolol exhibits significant antitumor efficacy both as a monotherapy and in combination with vaccines. However, its clinical application is constrained by adverse effects (e.g., bradycardia) and contraindications (e.g., asthma) [50, 51]. As a kind of external treatment, moxibustion does not show these adverse effects, suggesting it may be a safer, more cost-effective adjuvant for tumor vaccines with potential for rapid clinical application.

Despite the established recognition of CD8⁺ T cells as predominant effector cells in numerous vaccine studies [52–54], their contribution was comparatively limited in this study (Fig. 4D–E). This phenomenon may be attributable to variations in tumor types, vaccine designs, or individual immune responses. Although we detected the upregulation of CD8⁺ T cells in the tumor and spleen under the combination therapy, its depletion did not affect tumor growth. Moreover, NK cell depletion partly impaired the antitumor effect of the combined therapy, which implies that NK cells play an important role in vaccine therapy [55, 56]. For CD4⁺ T cells, some studies have reported a role for neoantigen-specific CD4⁺ T-cell

responses in direct tumor clearance [57–59], and CD4⁺ T cells can exert direct antitumor effects independent of CD8⁺ T cells [60, 61]. These studies revealed that cytotoxic CD4⁺ T cells, which are involved in infection and autoimmunity, play a key role in tumors [62]. Our findings underscore the critical role of CD4⁺ T cells as pivotal antitumor effector cells in specific contexts, offering novel insights for further investigation into the underlying mechanisms.

In general, exogenous antigens such as TCL necessitate the presentation by mature DC to activate CD4⁺ T cells [63, 64]. In this study, we demonstrated that gM promotes DC maturation by inhibiting β -adrenoceptor signaling. However, two key questions remain unresolved. First, the precise mechanism by which gM inhibits the β -signaling pathway is not fully elucidated. While we conducted RNA sequencing of tumor tissues and used propranolol as a substitute for gM to indirectly support our hypothesis, the underlying molecular mechanisms require further investigation. Considering that acupuncture typically involves regulation of the nervous system, our subsequent investigation aims to explore whether moxibustion mediates the downregulation of adrenergic receptor signaling pathways by modulating neurotransmitter secretion through the sympathetic nervous system. Second, the impact of the β -signaling pathway on DC maturation remains unclear. Classical downstream components protein kinase A had little effects on DC differentiation. While inhibition of NF- κ B had similar effect as propranolol (Figure S3B–C). The specific mechanisms warrant additional exploration.

Abbreviations

APC	Antigen-presenting cells
DC	Dendritic cell
DCV	DC vaccines
gM	Grain-sized moxibustion
LLC	Lewis Lung Carcinoma
MHC-II	Major histocompatibility complex class II complexes
NK	Natural killer cells
PBS	Phosphate-buffered saline
Prop	Propranolol hydrochloride
SEM	Standard errors of the means
SPF	Specific pathogen-free
ST 36	Zusanli acupoints
TCL	Tumor cell lysate vaccines
TCM	Traditional Chinese medicine

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13020-025-01134-w>.

Additional file1 (DOCX 720 KB)

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Author contributions

W.S. and D.H. performed most of the experiments. C.G., C.F., J.L., L.W., and C.Y. participated in the experiments. H.W., C.Z. and S.Z. conceived the study and participated in its design and coordination. All the data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of the work, ensuring its integrity and accuracy.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate

All procedures were approved by the Institutional Animal Care and Use Committee at Shanghai University of Traditional Chinese Medicine (PZSHUTCM2306290003) and the methods were performed in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Consent for publication

We declare that the Publisher has the Authors' permission to publish the relevant contribution.

Conflict of interest

SZ has equity and consulting relationships with Base Therapeutics. No potential conflict of interest was reported by other authors.

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