

Mechanism of Manganese Dioxide Nanomaterials Activating Stem-like CD8⁺ T Cells via Regulating Extracellular K⁺ Concentration

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Research Article

Keywords:

Posted Date: July 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10225255/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background

CD8⁺ T cells serve as the core effector cells of anti-tumor immune responses. Tumor cell necrosis leads to abnormally elevated extracellular potassium (K⁺) levels in the tumor microenvironment, which triggers autophagy and metabolic dysfunction in CD8⁺ T cells. Moreover, high-K⁺ stress reshapes histone epigenetic modifications, upregulates transcription factor 7 (TCF7) expression, sustains the stem-like properties of CD8⁺ T cells, and ultimately facilitates tumor immune escape.

Methods

MnO₂ nanomaterials were successfully fabricated, and their interaction with K⁺ was characterized via energy-dispersive X-ray spectroscopy (EDX). A series of experimental approaches, including UV–visible spectroscopy, LC-MS/MS, Western blotting, in vitro cell functional assays, and an in vivo melanoma mouse model, were applied to systematically evaluate the regulatory effects of MnO₂ nanomaterials on K⁺-mediated tumor immune dysfunction.

Results

High-K⁺ stress markedly impaired CD8⁺ T cell activity, suppressed the synthesis and accumulation of essential amino acids, and induced aberrant upregulation of TCF7, thereby disrupting the stemness homeostasis of CD8⁺ T cells. Intervention with MnO₂ nanomaterials effectively reversed these K⁺-induced detrimental phenotypes by downregulating excessive TCF7 expression, correcting abnormal stem-like properties, and restoring the immune activity of CD8⁺ T cells. In vivo experiments further verified that MnO₂ nanomaterials significantly prolonged the survival of melanoma-bearing mice.

Conclusion

MnO₂ nanomaterials can efficiently chelate extracellular K⁺ and alleviate local high-K⁺ stress in the tumor microenvironment. This intervention ameliorates metabolic disorders and nutritional deficiency in CD8⁺ T cells, restores the normal function of stem-like T cells, and ultimately strengthens anti-tumor immune efficacy. This study proposes a novel ion-regulation-based strategy for tumor immunotherapy and demonstrates the great potential of MnO₂ nanomaterials as a promising carrier for tumor immunotherapy.

Introduction

Cancer remains a major threat to human health worldwide. Cancer remains a major threat to human health worldwide. In 2022, approximately 20 million new cancer cases and 9.7 million cancer-related

deaths were reported globally, and the incidence is projected to rise substantially by 2050^[1, 2]. In China, the National Cancer Center estimates 4.82 million new cancer cases and 2.57 million deaths in 2022^[3]. Although surgery, radiotherapy, chemotherapy, targeted therapy and immunotherapy have improved cancer treatment^[4–7] therapeutic efficacy is still limited by the complexity of the tumor microenvironment, immune escape and treatment resistance^[8]. Therefore, new therapeutic strategies and targets are urgently needed.

In the anti-tumor immune response, CD8⁺ T cells, as the core effector cells, play a key role by directly recognizing and killing tumor cells, secreting effector factors such as *IFN-γ* and *TNF-α*, and their functional status directly determines the occurrence, development and prognosis of tumors^[9–13]. However, in the tumor microenvironment, continuous antigen stimulation, nutrient deprivation, imbalance of inflammatory factors and other factors will induce the depletion of CD8⁺ T cells, which is manifested as decreased proliferation ability, decreased cytotoxicity, decreased secretion of effector factors, and high expression of immune checkpoint molecules such as PD-1, Tim-3 and TIGIT, which ultimately leads to the decrease of anti-tumor immune response efficiency and immune escape of tumor cells. It has been found that the exhausted CD8⁺ T cell population is highly heterogeneous, and there is a class of stem cell-like CD8⁺ T cell subsets with high expression of transcription factor TCF7/TCF-1 and low expression of immunosuppressive receptors. This subgroup has self-renewal ability and differentiation potential, which is the key to maintaining persistent anti-tumor immune response^[9, 14–23]. The maintenance and activation of its stem state have become a research hotspot in the field of immunotherapy. Further studies have shown that the increase of extracellular K⁺ concentration in the tumor microenvironment is one of the important factors leading to the dysfunction of CD8⁺ T cells^[24–29]. Extracellular high K⁺ can lead to a significant decrease in glucose metabolism and essential amino acid content of CD8⁺ T cells, which makes the cells in a state of nutrient starvation and induces autophagy. At the same time, it inhibits the methylation of histone markers in stem cell-related programs, promotes the transformation of CD8⁺ T cells to stemness, and ultimately leads to a decrease in its anti-tumor ability. Therefore, regulating the concentration of K in the tumor microenvironment and reversing the dysfunction and abnormal stemness of CD8⁺ T cells have become a potential way to improve the effect of tumor immunotherapy.

Nanomaterials have broad prospects in the field of tumor therapy due to their unique physical and chemical properties^[30–36]. The strong reducing environment caused by abnormal metabolism of tumor tissues (intracellular glutathione concentration is significantly higher than extracellular and normal cells^[37]) provides new ideas for the design of reduction-responsive nano-drug carriers^[37–39]. Our preliminary experiments show that MnO₂ nanomaterials have good biocompatibility, which can effectively chelate and reduce the extracellular K⁺ concentration, promote the transformation of CD8⁺ T cells to stem cells, and have no toxic effect on cells. Based on this, the purpose of this study was to investigate the effect of nanomaterial MnO₂ on the concentration of K⁺ in extracellular or tumor tissue, enhance cell metabolism, reduce TCF7/TCF-1 expression, and activate stem-like CD8⁺ T cells. Through the establishment of CD8⁺ T cell culture system and melanoma mouse model, the effects of MnO₂ on K⁺

concentration were investigated from the aspects of molecules, cells, tissues and animals by means of nanomaterial MnO_2 intervention, CCK-8 cell proliferation experiment, immunofluorescence localization, flow cytometry analysis and amino acid mass spectrometry analysis. The mechanism of MnO_2 activating $\text{CD8}^+\text{T}$ cells was revealed, and the effect of MnO_2 on K^+ induced $\text{CD8}^+\text{T}$ cells was revealed, which provided theoretical basis and experimental support for the development of new tumor immunotherapy strategies, and was expected to open a new path for cancer treatment.

Experimental materials and methods

Synthesis and Characterization of MnO_2 Nanomaterials

$\alpha\text{-MnO}_2$ nanomaterials were synthesized *via* a hydrothermal method. Potassium permanganate and manganese sulfate monohydrate were dissolved in deionized water until complete dissolution, followed by the dropwise addition of concentrated sulfuric acid. The resulting mixture was transferred into a Teflon-lined stainless-steel autoclave, sealed, and heated. After the reaction, the obtained brownish-black precipitate was collected, yielding the $\alpha\text{-MnO}_2$ product.

The crystal structure of the sample was characterized by X-ray diffraction (XRD). In the recorded XRD pattern, all diffraction peaks matched well with those of tetragonal $\alpha\text{-MnO}_2$ (JCPDS standard card 44-0141). The characteristic peaks located at 12.7° , 18.1° , 28.8° , 37.5° , 42.0° , 49.9° , 56.7° , and 60.3° correspond to the (110), (200), (310), (211), (301), (411), (600), and (521) crystal planes, respectively, with a space group of $I4/m$. No impurity peaks were detected, confirming that the product is pure-phase $\alpha\text{-MnO}_2$.

To further elucidate the morphology, crystal structure, and growth orientation of the $\alpha\text{-MnO}_2$ nanorods, systematic analyses including low-magnification morphology imaging, high-resolution lattice imaging, and selected area electron diffraction (SAED) were carried out using transmission electron microscopy (TEM). Low-magnification TEM images reveal that the product exhibits a uniform one-dimensional rod-like morphology with good dispersion and no evident agglomeration. The nanorods possess smooth surfaces and well-defined contours, with lengths ranging from 50 to 150 nm and an average diameter of approximately 10 nm, showing typical characteristics of nanorods. High-resolution TEM (HRTEM) images further disclose the atomic-scale structural details of individual nanorods. In the direction perpendicular to the nanorod axis, a set of lattice fringes parallel to the axis displays a d-spacing of 0.28 nm, which agrees precisely with the interplanar spacing of the (001) planes of tetragonal $\alpha\text{-MnO}_2$. This lattice image directly indicates that the nanorods grow preferentially along the [001] crystallographic direction, with the side surfaces mainly enclosed by facets^[12]. Selected area electron diffraction performed on a single nanorod along its length yielded a pattern composed of regular and sharp diffraction spots, further confirming the single-crystalline nature of the nanorod. This SAED pattern can be completely indexed to the diffraction pattern of tetragonal $\alpha\text{-MnO}_2$ along the $[1-10]$ zone axis, consistent with the analysis of the high-resolution images.

Cell culture

Human leukemia T lymphocytes CD8⁺ T cells (CloneE6-1) and mouse CD8⁺ T cells were purchased from Beijing Huizhiheyuan Co., Ltd.; blood disease T lymphocytes CD8⁺ T cells (CloneE6-1) and mouse CD8⁺ T cells. RPMI-1640 medium supplemented with 10% inactivated fetal bovine serum (HIFCS, Gibco) and 1% double antibody was cultured in 5% CO₂ and 37°C incubator. Four groups were set up as control group(using conventional medium), K- α -MnO₂ group (adding MnO₂ and K for intervention), α -MnO₂ group (adding MnO₂ for intervention), and α -K group(adding K for intervention). The concentration of MnO₂ nanomaterials was configured according to the optimal concentration during the synthesis of nanomaterials in the early stage. The sterile distilled water was used for dissolution configuration. The concentration of MnO₂ was 12.5 mg/ml, and 4 μ l of MnO₂ was added to each ml of medium. According to the literature research, the extracellular concentration was maintained at 40 mM and the intervention time was 10 days^[25].

Metabolomics LC-MS analysis

The instruments used in this experiment were Waters Acquity UPLC liquid chromatograph and ABSCIEX5500QQQ-MS mass spectrometer. The chromatographic column was Acquity UPLC Amide BEH (1.7 μ m, 2.1mm $\times$ 100mm).

After 10 days of cell culture, the four groups of cells were blowed separately, and the precipitate was collected by centrifugation at 1000 rpm. The precipitate was frozen in a refrigerator at -80°C for later use, and the sample collection and preservation were completed. During the determination of amino acid content in cells, the frozen samples were taken out and thawed at 4°C. After weighing all the samples, 600 μ L of 80% methanol water(containing 50ng/mL tryptophan-d5 internal standard)was added, followed by 180 s vortex oscillation, 60HZ frequency 200s grinding, followed by ultrasonication at 4°C for 30 min, standing for 30 min, and then centrifuged at 4 °C, 12000 rpm for 10 min. The supernatant was taken directly on the machine, and diluted with 80% methanol water containing the same internal standard 100 times and detected on the machine to complete the extraction and processing of metabolites. At the same time, 10 μ L supernatant was taken from each sample and mixed to prepare a QC sample.

CCK-8 cell activity assay

Cells were collected after 10 days of intervention, and the concentration of cell suspension was adjusted. 100 μ L of cell suspension was added to each hole of 96-well plate, so that the density of cells to be tested in each hole was between 1,000 and 10,000 cells/hole. Subsequently, 96-well plates were placed in a culture environment containing 5% CO₂ at 37°C for 24 hours. After the culture, 10 μ L CCK-8 solution was added to each well and continued to be placed in the cell culture box for 2 hours. After incubation, the absorbance of each well was measured by a microplate reader at a wavelength of 450 nm to complete the cell activity detection.

Western Blot experiment.

CD8⁺ T cells from each group were collected after intervention. Cells were lysed on ice for 30 min with RIPA buffer containing protease and phosphatase inhibitors, and protein supernatants were obtained by centrifugation at 12,000 rpm for 15 min at 4°C. Protein concentrations were determined using the BCA assay. Equal amounts of protein were denatured, separated by SDS-PAGE, transferred to membranes, and blocked with 5% skim milk for 1 h at room temperature. Membranes were incubated overnight at 4°C with rabbit anti-TCF-1 antibody (T2020-03, USBiological, 1:1000) and mouse anti-GAPDH antibody (Servicebio, 1:2000), followed by HRP-conjugated secondary antibodies. Signals were developed using ECL reagent, and band intensities were quantified with ImageJ using GAPDH as the internal control.

Construction of tumor mouse model

Sixteen male C57BL/6J mice were used to establish the melanoma model. B16F10 melanoma cells were cultured *in vitro*, resuspended at 3×10^6 cells/100 μ L, and subcutaneously injected into the right flank of each mouse. Tumor growth was monitored daily, and treatment was initiated when tumor volume reached approximately 50–100 mm³. All animal experimental protocols were reviewed and approved by the Animal Ethics Committee of Shandong Sport University (approval number: 2024043) and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Nanomaterials for animal *in vivo* intervention

After tumor formation, C57BL/6J male mice were randomly divided into four groups: PBS control, α -K⁺, K⁺- α -MnO₂ and α -MnO₂. The corresponding interventions were administered for 14 days. MnO₂ was dissolved in sterile water and sterilized before use. The MnO₂ concentration was 12.5 mg/mL, the K⁺ concentration was 40 mM, and 25 μ L was injected each time for a total of six injections.

Extraction of tumor tissue

A total of 16 male C57BL/6J mice were used for tissue collection. Animals were fasted with free access to water for 12 h before sampling and anesthetized by intraperitoneal injection of 3% pentobarbital sodium at 1 mL/kg. After orbital blood collection, mice were euthanized by cervical dislocation. The abdominal cavity was opened, liver and lung tissues were excised, and subcutaneous tumor tissues were removed. Part of each tumor sample was fixed in 4% glutaraldehyde or 4% paraformaldehyde for histological analysis, and the remaining tumor tissue was embedded in OCT and stored at -80°C.

Immunofluorescence localization

After 15 days of intervention, surviving mice were euthanized and liver, lung and tumor tissues were collected. Tumor samples were embedded in OCT and frozen. Frozen sections with a thickness of 10–20 μ m were prepared, dried, dewaxed, hydrated and subjected to antigen retrieval in citric acid buffer. After blocking with goat serum, sections were incubated overnight at 4°C with rabbit anti-rat C63D9 primary antibody. Negative controls were incubated with 0.01 M PBS or rabbit serum instead of primary antibody. Sections were then incubated with the secondary antibody working solution, washed with PBS, stained with DAPI in the dark and mounted for fluorescence observation.

Statistical analysis

Statistical analyses were performed using SPSS 26.0. Data are presented as the mean $\pm$ standard error of the mean (SEM). Tumor growth curves and histological indices between two groups were compared using independent-sample t tests. Tumor volume and immunofluorescence-positive rates among the four intervention groups (PBS, α -K⁺, K⁺- α -MnO₂ and α -MnO₂) were analyzed by one-way analysis of variance (ANOVA), followed by least significant difference (LSD) multiple-comparison tests. Non-parametric tests were used for semi-quantitative immunofluorescence data when appropriate. $P < 0.05$ was considered statistically significant, and $P < 0.01$ was considered highly significant.

Results

Characterization of MnO₂ and K⁺-adsorbed MnO₂ nanomaterials

Figures 1a and 1b show the TEM images of MnO₂ nanomaterials before and after K⁺ adsorption, respectively. Figures 1c and 1d show the corresponding HRTEM images. The prepared MnO₂ exhibited a regular nanorod morphology with good dispersion, and K⁺ adsorption did not destroy the overall nanostructure. The HRTEM images further confirmed the crystalline structure of the material and the lattice changes associated with K⁺ adsorption.

The microstructure and crystal structure of MnO₂ nanomaterials before and after K⁺ adsorption was characterized by TEM and HRTEM (Fig. 1). The TEM image in Fig. 1a shows that MnO₂ nanorods had a regular morphology, complete structure and good dispersion. After K⁺ adsorption, the K⁺-MnO₂ nanomaterials maintained a similar nanorod morphology (Fig. 1b). HRTEM images (Fig. 1c, d) showed clear lattice fringes, confirming that K⁺ adsorption induced regular changes in the crystal structure while preserving favorable crystallinity.

Analysis of K⁺-MnO₂ and UV-visible spectra of K⁺-MnO₂

EDX elemental mapping (Fig. 2a) was performed to characterize the elemental composition of K-MnO₂ nanomaterials. The corresponding elemental analysis results revealed three dominant constituents, namely K, Mn and O. Specifically, potassium occupied a weight percentage of 3.29% and an atomic percentage of 2.55%; manganese accounted for 35.27 wt% and 63.9 at%; oxygen exhibited a weight fraction of 62.18% and an atomic fraction of 32.81%. All three elements displayed homogeneous spatial distribution, with no detectable signals originating from impurity elements. These observations verified the successful immobilization of K⁺ onto the MnO₂ nanoparticle surface and demonstrated the high purity of the as-prepared K-MnO₂ material.

UV-vis absorption spectroscopy (Fig. 2b) was further adopted to investigate the catalytic performance of K-MnO₂ for hydroxyl radical ($\cdot$ OH) generation under glutathione (GSH) coexistence. Methylene blue (MB), a chromogenic substrate degradable by $\cdot$ OH radicals, was employed as a colorimetric indicator to quantify $\cdot$ OH production. Control experiments confirmed that separate treatment with only MnO₂ or H₂O₂

barely altered the characteristic absorbance of MB. As illustrated in Fig. 2b, upon co-incubation of MB with H₂O₂, K-MnO₂ and a moderate dosage of GSH at mild acidic pH (pH = 6.5) for 40 min, a remarkable decline in MB absorbance was observed. In contrast, negligible absorbance attenuation of MB was detected when GSH was supplied at either excessively low or high concentrations, even with identical dosages of H₂O₂ and K-MnO₂.

Amino acid metabolomics analysis of CD8⁺ T cells in different treatment groups

Four experimental groups were established in this study, including the control group, K- α -MnO₂ group, α -MnO₂ group, and α -K group. Cells in each group were cultured with the corresponding treatments for 10 days, while cells in the control group were maintained in routine culture medium. Microscopic observations (Fig. 4a) revealed that the α -K group exhibited the lowest number of viable cells. Combined with the CCK-8 assay results (Fig. 4b), these findings confirmed that K⁺ induces autophagy in CD8⁺ T cells, thereby reducing cell viability. Notably, the K- α -MnO₂ group showed a decreased proportion of autophagic cells and a significant recovery in viable cell numbers. In addition, the α -MnO₂ group presented higher cell activity than the α -K and K- α -MnO₂ groups, yet its cell viability remained lower than that of the control group. It is speculated that MnO₂ alleviates high K⁺-induced stress by reducing intracellular K⁺ accumulation, thereby facilitating the restoration of stemness in CD8⁺ T cells.

Following different treatments, cells from all groups were harvested via centrifugation for subsequent amino acid metabolomic analysis, and the corresponding results are presented in Figs. 4c–4e. Compared with the control group, high-potassium intervention significantly remodeled the intracellular amino acid profiling of CD8⁺ T cells. Moreover, the combined treatment of K⁺ and α -MnO₂ further modulated the abundance of multiple metabolism-associated amino acids.

The levels of L-arginine and L-citrulline were upregulated in the α -K group, and were further elevated in the K⁺- α -MnO₂ combination group, indicating the activation of nitrogen metabolism and the arginine-citrulline cycle. Similarly, the combined treatment also increased the contents of L-histidine and L-valine, which are closely associated with immune activation, energy metabolism, and functional maintenance of T cells. These metabolic alterations were highly consistent with the improved cell viability validated by CCK-8 assays.

In contrast, the abundances of L-lysine and L-hydroxyproline were decreased in the K⁺- α -MnO₂ group. Given that these two amino acids are critical for protein and collagen synthesis, their downregulation suggests that the combined treatment reprograms the cellular metabolic phenotype, shifting the metabolic priority from structural substance synthesis to immune functional maintenance. Furthermore, treatment-specific variations in the levels of L-O-phosphoserine, D-tyrosine, L-threonine, and L-methionine were also observed, demonstrating that high-potassium stress and MnO₂ intervention jointly regulate phospholipid signaling, methylation metabolism, and other amino acid metabolic pathways in CD8⁺ T cells.

Collectively, amino acid metabolomic analysis verified that MnO₂ intervention effectively remodels the metabolic state of CD8⁺ T cells under high-potassium stress. By regulating amino acid homeostasis, especially the pathways involved in nitrogen metabolism, branched-chain amino acid metabolism, and immune activation, MnO₂ contributes to the preservation of cell activity and stemness in CD8⁺ T cells. This study provides solid metabolomic evidence for the immunomodulatory mechanism of MnO₂ nanomaterials.

Effects of MnO₂ nanomaterials on the expression of TCF7 protein in CD8⁺ T cells

To explore the effect of MnO₂ nanomaterials on the expression of stemness-related protein TCF7 in CD8⁺T cells under high potassium stress, Western Blot method was used to detect the protein levels of TCF7 under the treatment conditions of Control, α -MnO₂, α -K and K- α -MnO₂, and GAPDH was used as the internal reference protein for correction. Figure 5a shows the results of Western Blot, which intuitively showed the protein expression bands of TCF7 and GAPDH in each group. The signal intensity of TCF7 band in the α -K group was significantly higher than that in the Control group, while the band signal in the K- α -MnO₂ group was significantly weaker than that in the α -K group. There was no significant difference in band intensity between the α -MnO₂ group and the Control group. Figure 5b is a histogram of TCF7 relative protein expression after gray-scale quantitative analysis by ImageJ software and normalized by GAPDH. The quantitative results show that compared with the Control group, the relative expression of TCF7 in the α -K group was significantly up-regulated ($p < 0.01$), while the expression level of TCF7 in the K- α -MnO₂ group was significantly lower than that in the α -K group. At the same time, there was no significant difference in TCF7 expression between the α -MnO₂ group and the control group (ns). The above results showed that the high potassium microenvironment could significantly induce the up-regulation of the expression of the stemness transcription factor TCF7 in CD8⁺ T cells, while MnO₂ nanomaterials could effectively reverse this process, suggesting that it could affect the stemness characteristics of CD8⁺ T cells by regulating the high potassium stress state.

MnO₂ effectively prolongs the survival time of melanoma-bearing mice

To further understand the effect of nanomaterials on T cells in tumors, we constructed a mouse melanoma mouse model, and then injected nanomaterials to intervene. The results showed that the survival time of tumor mice injected with MnO₂ was the longest, and the survival time of mice after K intervention with adsorbed MnO₂ was longer than that of K intervention, and shorter than that of MnO₂ intervention (Fig. 6).

Expression characteristics of CD8⁺ T cell stem phenotype under different intervention conditions

Four experimental groups were established in this study, including the control group, K- α -MnO₂ group, α -MnO₂ group, and α -K group. Cells in each group were cultured with the corresponding treatments for 10 days, while cells in the control group were maintained in routine culture medium. Microscopic observations (Fig. 4a) revealed that the α -K group exhibited the lowest number of viable cells. Combined with the CCK-8 assay results (Fig. 4b), these findings confirmed that K⁺ induces autophagy in CD8⁺ T

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To further elucidate the regulatory effects of different treatments on the stem-like phenotype of tumor-infiltrating CD8⁺ T cells, multiplex immunofluorescence staining and flow cytometry were performed on samples from four groups: PBS control, α -K⁺, α -MnO₂, and K⁺- α -MnO₂. Representative immunofluorescence images (Fig. 7a) illustrate the staining of cell nuclei (DAPI, blue), CD8⁺ T cells (green), and the stemness marker TCF-7 (red), with merged images displaying double-positive CD8⁺TCF-7⁺ stem-like T cells. In the PBS and α -K⁺ groups, both CD8⁺ T cell infiltration and TCF-7 fluorescence signals were weak, with negligible CD8⁺TCF-7⁺ cell populations observed. The α -MnO₂ group exhibited a

slight increase in CD8⁺ T cell infiltration but no obvious upregulation of TCF-7 expression. In contrast, the K⁺-α-MnO₂ combined treatment markedly enhanced both CD8⁺ T cell infiltration and TCF-7 positivity, yielding abundant CD8⁺TCF-7⁺ stem-like T cells in tumor tissues.

Flow cytometry was further applied to quantify the proportion of CD8⁺TCF-7⁺ cells in each group (Fig. 7b). The statistical results showed that the proportions of CD8⁺TCF-7⁺ cells in the PBS, α-K⁺, and α-MnO₂ groups were 2.74%, 2.44%, and 7.38%, respectively. Notably, the K⁺-α-MnO₂ group achieved a prominently higher proportion of CD8⁺TCF-7⁺ cells at 14.8%, which was significantly superior to that of the other three groups.

Consistent with the above phenotypic results, the high-potassium microenvironment alone failed to effectively induce the stemness phenotype of CD8⁺ T cells. Single α-MnO₂ treatment only mildly improved CD8⁺ T cell infiltration and could not efficiently restore T cell stemness. In comparison, the synergistic intervention of K⁺ and α-MnO₂ significantly promoted intratumoral CD8⁺ T cell infiltration and the generation of stem-like CD8⁺ T cells. These findings demonstrate that the combined treatment efficiently sustains the stemness characteristics of CD8⁺ T cells, which supports the persistent activation and anti-tumor function of T cells. Importantly, these phenotypic observations are highly consistent with our previous results regarding cell viability and metabolic reprogramming.

Discussion

In this study, we successfully constructed a research system in which MnO₂ nanomaterials regulate the extracellular K⁺ concentration to activate CD8⁺ T cells. From the aspects of material characterization, cell level, metabolomics and animal models, it was confirmed that MnO₂ can alleviate the metabolic inhibition and functional exhaustion of CD8⁺ T cells by chelating the abnormally elevated K⁺ in the tumor microenvironment, and then restore its stemness characteristics and anti-tumor immunity. The continuous release of K⁺ from necrotic cells in the tumor microenvironment leads to a significant increase in extracellular K⁺ concentration, which has been proved to be a key inducement to induce metabolic disorder, autophagy activation, amino acid deficiency and abnormal stemness of CD8⁺T cells. Although these stem-like CD8⁺T cells highly express TCF-1/TCF-7, they are accompanied by decreased cytotoxicity and decreased proliferation ability, which makes it difficult to effectively perform anti-tumor immune response and eventually leads to tumor immune escape. The results of this study are highly consistent with the above mechanism. High potassium intervention(α-K group)can significantly reduce the activity of CD8⁺ T cells, reduce the content of essential amino acids such as serine, lysine and methionine, and inhibit the functional generation of TCF-7⁺ dry subsets, suggesting that high potassium-mediated metabolic disorder is the core link of T cell dysfunction.

With its unique physical and chemical properties, α-MnO₂ nanomaterials have shown a dual key role in this study. α-MnO₂ nanomaterials were synthesized via a hydrothermal method. Potassium permanganate and manganese sulfate monohydrate were dissolved in deionized water until complete dissolution, followed by the dropwise addition of concentrated sulfuric acid. The resulting mixture was

transferred into a Teflon-lined stainless-steel autoclave, sealed, and heated. After the reaction, the obtained brownish-black precipitate was collected, yielding the α -MnO₂ product.

The crystal structure of the sample was characterized by X-ray diffraction (XRD). In the recorded XRD pattern, all diffraction peaks matched well with those of tetragonal α -MnO₂ (JCPDS standard card 44-0141). The characteristic peaks located at 12.7°, 18.1°, 28.8°, 37.5°, 42.0°, 49.9°, 56.7°, and 60.3° corresponded to the (110), (200), (310), (211), (301), (411), (600), and (521) crystal planes, respectively, with a space group of I4/m. No impurity peaks were detected, confirming that the product was pure-phase α -MnO₂.

To further elucidate the morphology, crystal structure, and growth orientation of the α -MnO₂ nanorods, systematic analyses including low-magnification morphology imaging, high-resolution lattice imaging, and selected area electron diffraction (SAED) were carried out using transmission electron microscopy (TEM). Low-magnification TEM images revealed that the product exhibited a uniform one-dimensional rod-like morphology with good dispersion and no evident agglomeration. The nanorods possessed smooth surfaces and well-defined contours, with lengths ranging from 50 to 150 nm and an average diameter of approximately 10 nm, showing typical characteristics of nanorods. High-resolution TEM (HRTEM) images further disclosed the atomic-scale structural details of individual nanorods. In the direction perpendicular to the nanorod axis, a set of lattice fringes parallel to the axis displayed a d-spacing of 0.28 nm, which agreed with the interplanar spacing of the (001) planes of tetragonal α -MnO₂. This lattice image indicated that the nanorods grew preferentially along the [001] crystallographic direction, with the side surfaces mainly enclosed by facets. SAED performed on a single nanorod along its length yielded a pattern composed of regular and sharp diffraction spots, further confirming the single-crystalline nature of the nanorod. This SAED pattern could be indexed to the diffraction pattern of tetragonal α -MnO₂ along the [1-10] zone axis, consistent with the HRTEM analysis.

On the one hand, EDX elemental analysis clearly confirmed that α -MnO₂ can effectively adsorb and chelate K⁺, directly reducing the extracellular K⁺ concentration, thereby reversing the electrochemical gradient imbalance and nutrient uptake disorder caused by high potassium. Compared with the high potassium group alone, K- α -MnO₂ combined intervention significantly increased the activity of CD8⁺ T cells and restored the metabolic balance of various amino acids, especially promoted the accumulation of arginine, citrulline, histidine, valine and other amino acids closely related to immune function, and reduced the content of lysine, hydroxyproline and other amino acids related to structural synthesis, suggesting that the focus of cell metabolism shifted from structural synthesis to immune function maintenance. The results of amino acid metabolomics further showed that α -MnO₂ could provide a material basis for the activation and stemness maintenance of CD8⁺ T cells by regulating nitrogen metabolism, arginine-citrulline cycle and branched-chain amino acid metabolism pathways, which was consistent with the results of CCK-8 cell activity assay.

At the level of stemness regulation, this study confirmed by multiple immunofluorescence and flow cytometry that the high potassium environment itself could not effectively generate functional stem cell-

like CD8⁺ T cells, while the combined intervention of α -MnO₂ could significantly increase the proportion of CD8⁺TCF-7⁺ subsets and promote the normalization of T cell stemness phenotype rather than abnormal depletion of stemness. As a core transcription factor regulating the self-renewal and anti-tumor persistence of stem cell-like T cells, the high expression of TCF-7 means that CD8⁺ T cells have stronger proliferation potential, reactivation ability and long-term immune surveillance function. The above results indicate that α -MnO₂ does not simply induce stemness, but reprograms abnormally dry and low-functional CD8⁺ T cells into effector cells with normal stemness and anti-tumor activity by correcting K⁺ disorder. This finding provides a new theoretical basis for the immune regulation of targeted ion microenvironment.

The results of animal experiments further support the conclusions at the cellular and molecular levels. In the melanoma mouse model, the survival time of α -MnO₂ group and K- α -MnO₂ group was significantly better than that of α -K group, and the survival benefit of α -MnO₂ group was the most obvious, suggesting that α -MnO₂ can effectively remove the high potassium in the tumor microenvironment in vivo, improve the local immunosuppressive state of the tumor, and enhance the anti-tumor immunity mediated by endogenous CD8⁺ T cells. At the same time, immunofluorescence results showed that α -MnO₂ could significantly increase the co-localization level of CD8⁺ T cell infiltration and TCF-7 in tumor tissues, confirming that its mechanism of action in vivo was consistent with that in vitro, that is, T cell metabolism and stemness function were restored by regulating K⁺ concentration.

This study has several limitations. Firstly, the specific molecular signaling mechanisms, including downstream calcium ion signaling, the mTOR pathway and methylation modification following the regulation of K⁺ by α -MnO₂, were not thoroughly elucidated. Secondly, we did not evaluate the therapeutic synergy by combining clinical agents such as immune checkpoint inhibitors. Finally, the broad applicability of this strategy was not validated in additional tumor models. Nevertheless, this study clearly elucidates a novel immunotherapeutic pathway of “ionic microenvironment regulation-metabolic remodeling-T cell stemness activation”, which breaks through the traditional immunotherapy strategies dominated by antibodies and cytokines.

Conclusion

This study confirmed that the successfully synthesized α -MnO₂ nanomaterials had good K⁺ chelating ability and no obvious toxicity to cells. At the cellular level, high potassium stress can significantly induce the high expression of stem core protein TCF7 in CD8⁺ T cells, and lead to abnormal activation of T cell stemness. The intervention of α -MnO₂ nanomaterials can effectively reverse this phenomenon, significantly down-regulate the abnormal high expression of TCF7 protein in high potassium environment, and inhibit the aberrant stem-like phenotype of CD8⁺ T cells of CD8⁺ T cells^[40–44]. Meanwhile, the material can effectively reduce the extracellular high K⁺ concentration, alleviate the inhibition of glucose metabolism and the deficiency of essential amino acids (serine, lysine, methionine, etc.) in CD8⁺ T cells caused by high K⁺, reduce cell autophagy, enhance cell activity and promote stemness recovery. The cell activity and amino acid content of K- α -MnO₂ group were significantly higher

than those of high K⁺ intervention group. At the animal level, the intervention of α-MnO₂ nanomaterials could significantly prolong the survival time of melanoma model mice. The survival time of α-MnO₂ group was the longest, and the survival time of K-α-MnO₂ group was better than that of high K⁺ intervention group, which confirmed that it could also improve T cell function and exert anti-tumor effect by regulating K⁺ concentration in vivo. In summary, α-MnO₂ nanomaterials can activate the anti-tumor function of stem-like CD8⁺ T cells by chelating K⁺, improving the metabolic state of CD8⁺ T cells and inhibiting the high expression of TCF7/TCF-1 in tumor microenvironment^[45], which breaks through the limitations of traditional tumor immunotherapy and provides a new strategy and experimental basis for tumor immunotherapy. It has important theoretical significance and potential clinical application value.

Declarations

Acknowledgment

This work was supported by grants from the Zhejiang Medical Health Science & Technology Project (2022KY1403), Taizhou Science and Technology Plan Project (25ywa18) and Shanghai Wu Mengchao Medical Foundation (JJHXM-2025023).

Competing interest

All authors declare that there are no conflicts of interest.

Funding

No funding.

CRedit authorship contribution statement

Conception and design of the work: Wenrui Hu, Youhan Liu and Shengpeng Ying; acquisition and analysis of data: Wenrui Hu, Mengran Cao, Ganghui Ye and Xiaowei Li; writing and preparing the original draft: Wenrui Hu, Qinglu Wang and Ganghui Ye; writing, reviewing and editing the paper: Xiaowei Li and Shengpeng Ying. All authors read and approved the final manuscript.

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Figures

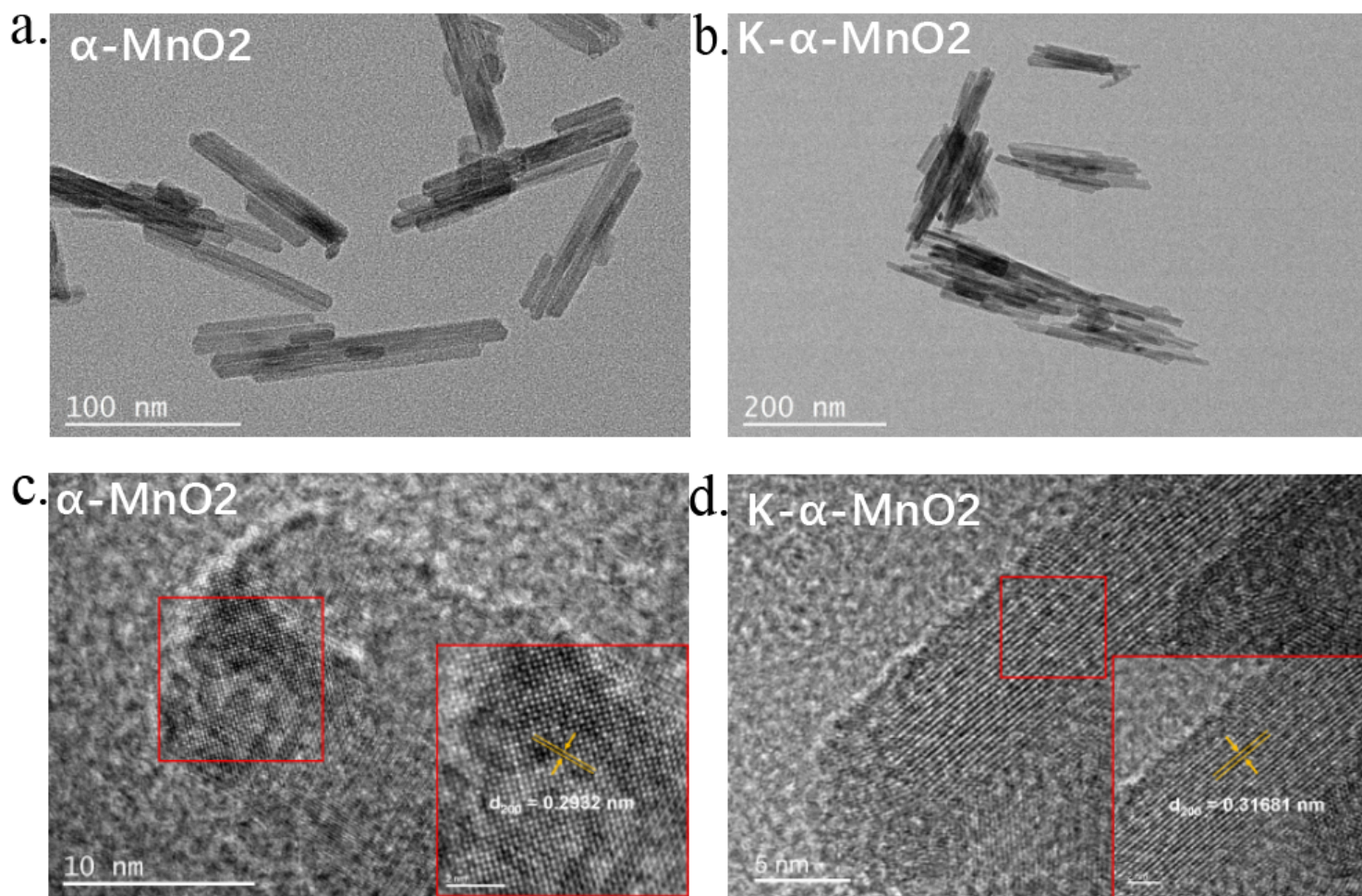


Figure 1

TEM and HRTEM characterization of MnO₂ and K⁺-adsorbed MnO₂ nanomaterials

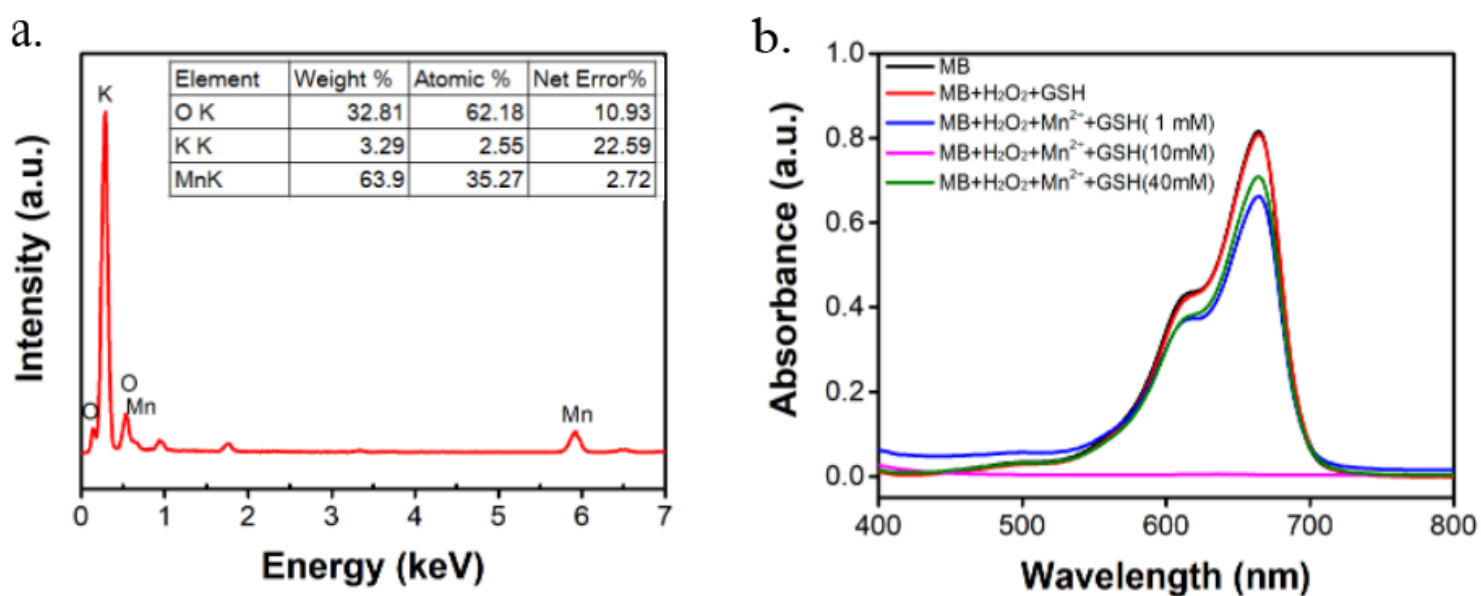


Figure 2

EDX analysis of K^+-MnO_2 and UV-visible spectra of K^+-MnO_2 under different GSH concentrations

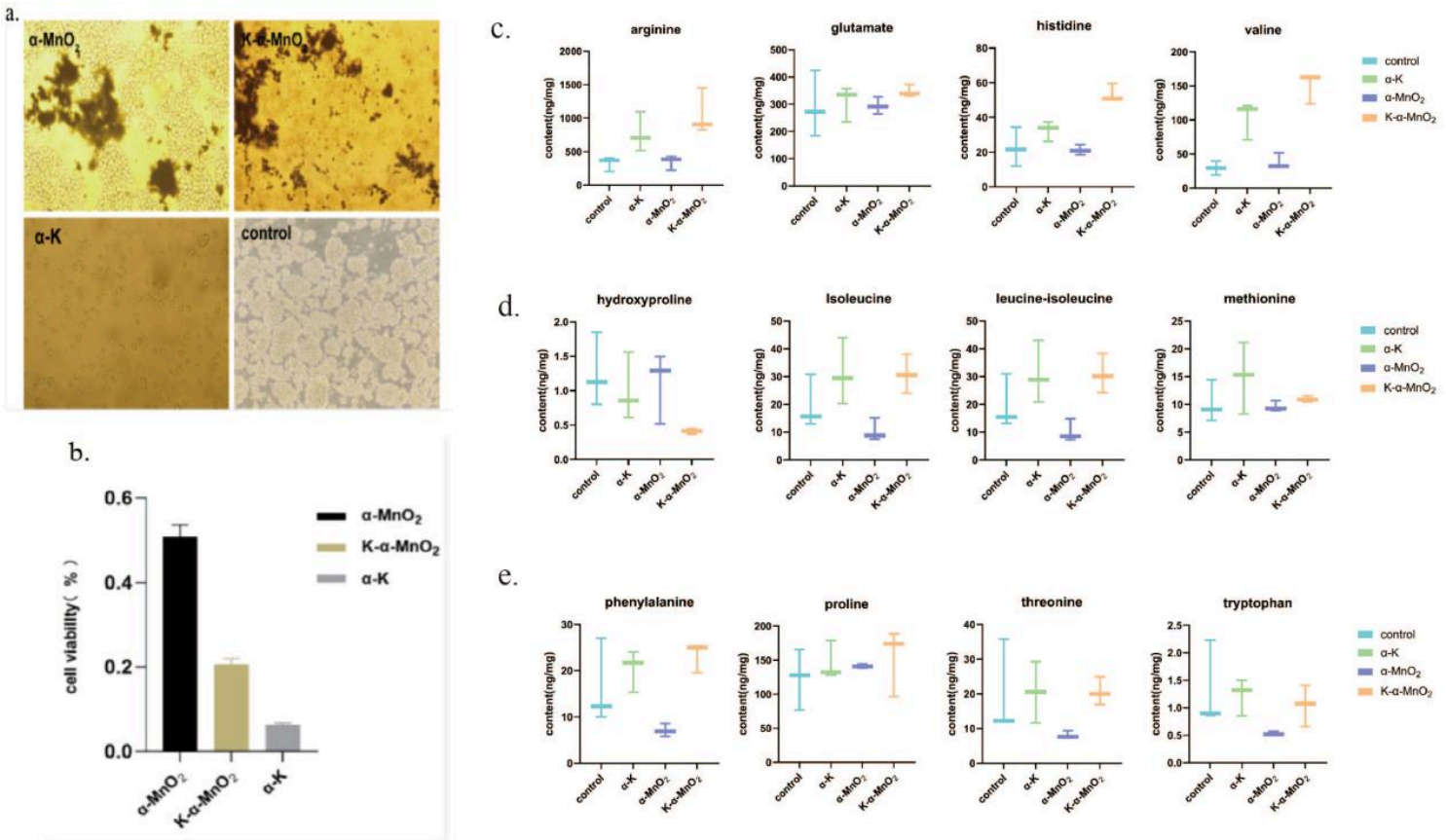


Figure 3

Fig.4 Morphological observation, cell activity and amino acid metabolomics analysis of CD8⁺T cells in different treatment groups.

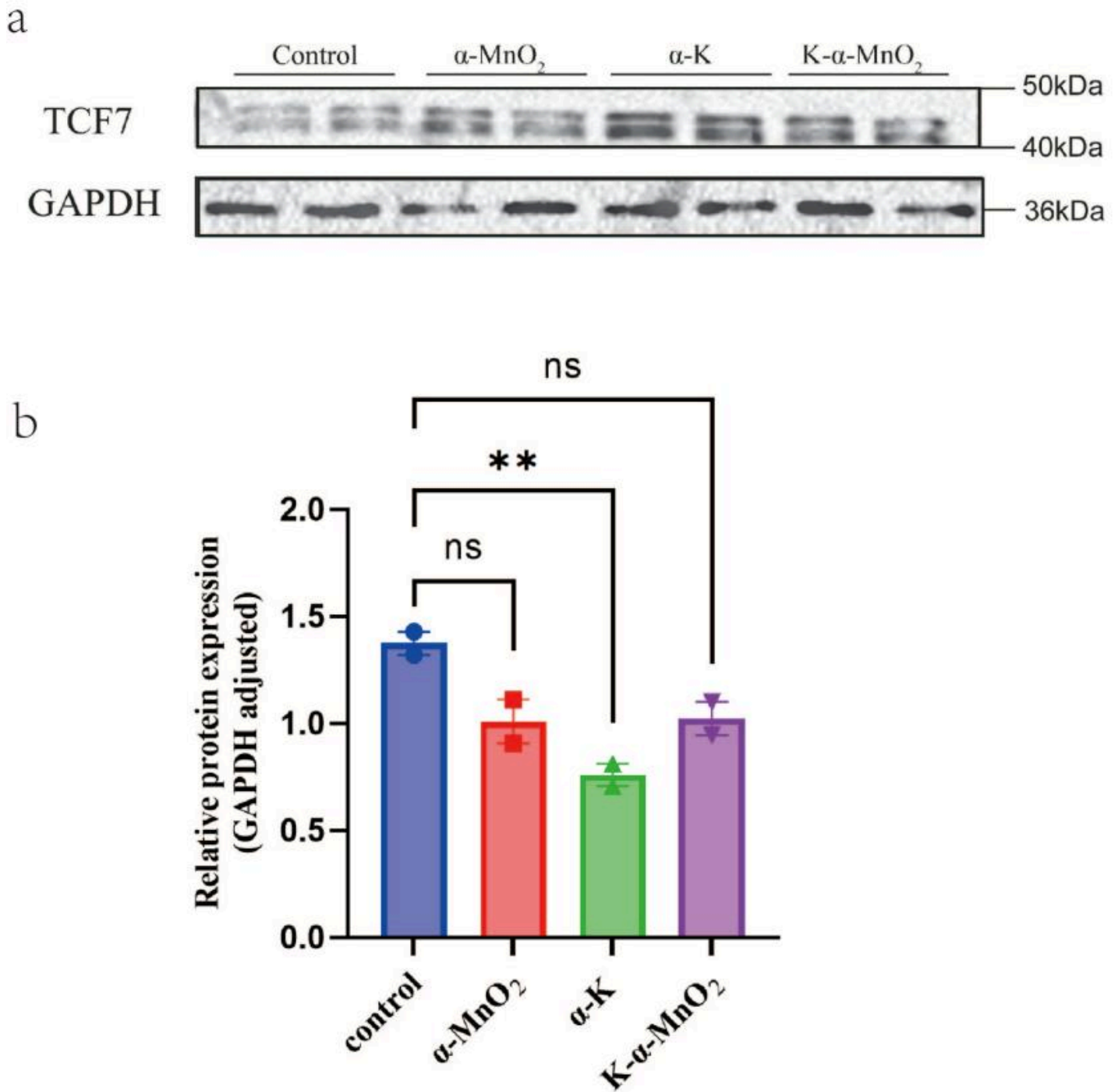


Figure 4

Fig.5 Effects of MnO₂ nanomaterials on the expression of TCF7 protein in CD8⁺T cells under high potassium stress. Note: Asterisks indicate statistically significant differences. *P < 0.05, **P < 0.01.

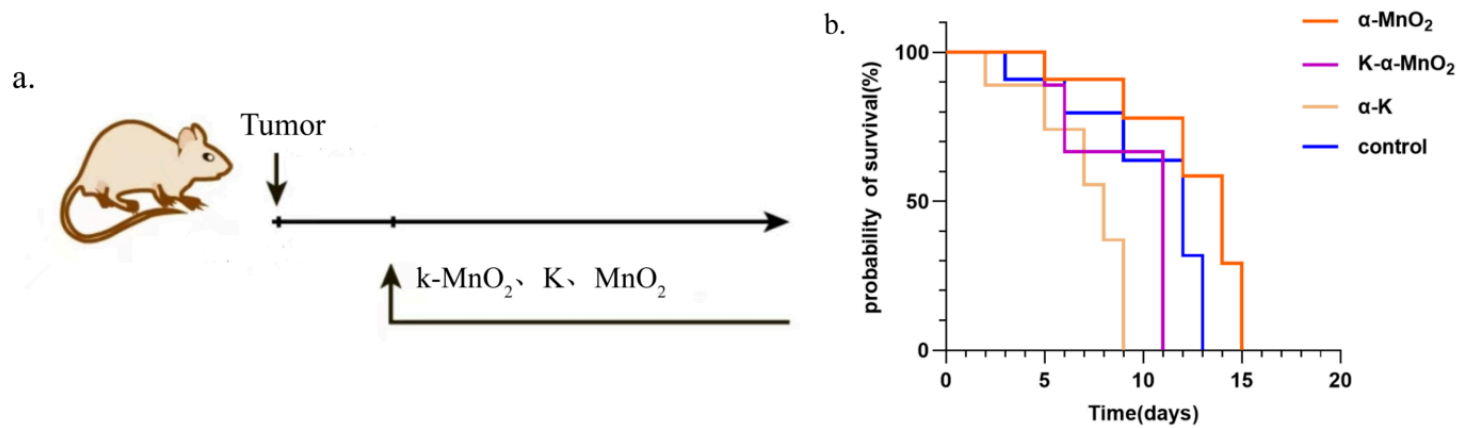


Figure 5

Fig. 6 Survival curves of melanoma-bearing mice after different interventions

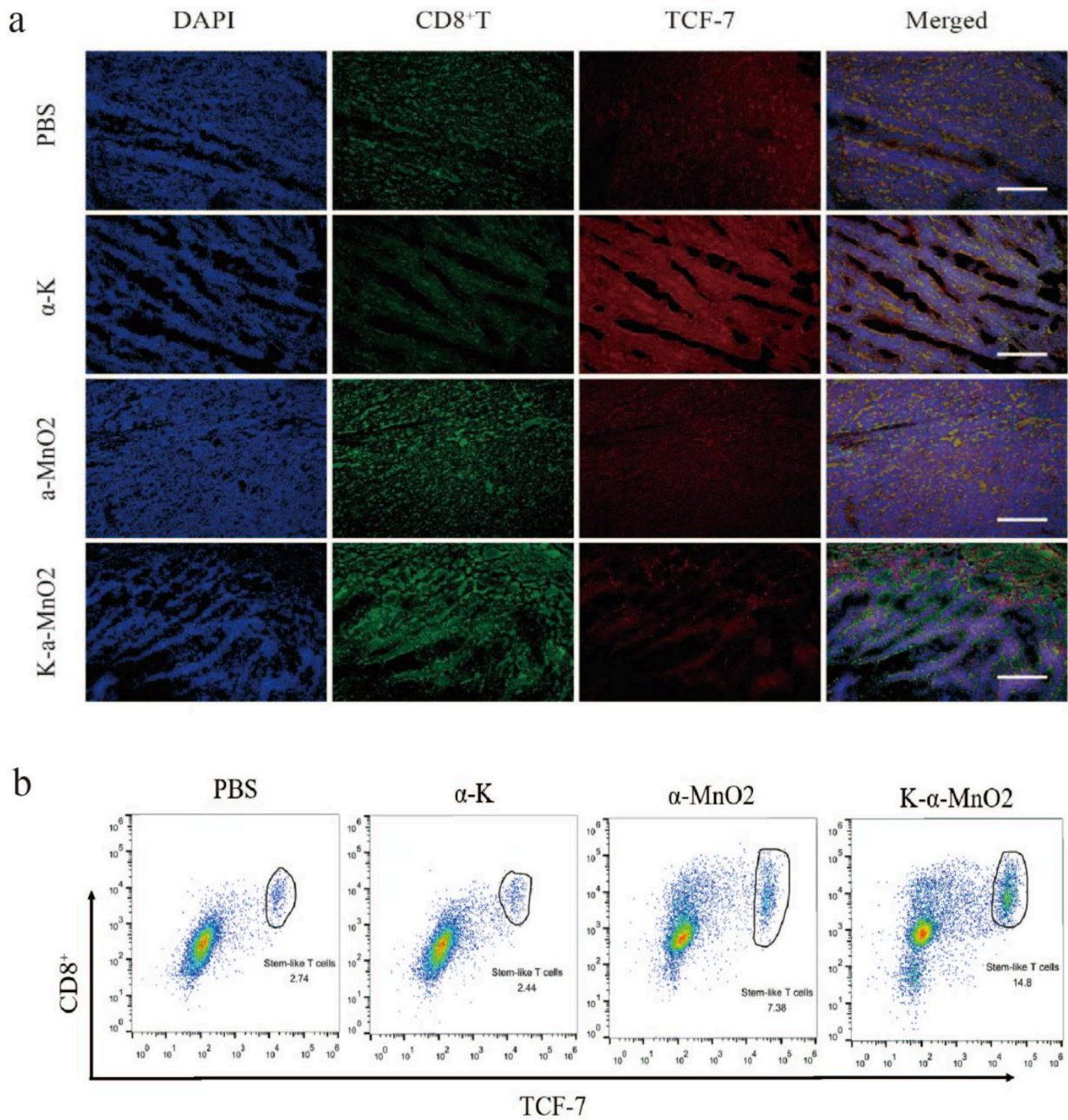


Figure 6

Fig.7 The expression characteristics of CD8⁺T cell stem phenotype under different intervention conditions