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Immunoregulatory Effect and Mechanism of Epigallocatechin-3-Gallate in A Mouse Oral Cancer Model

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Abstract: *Objective:* This investigation delineates the anti-cancer potency of epigallocatechin-3-gallate (EGCG) in an oral cancer mouse model, with a focus on its effect on T-cell activation. *Methods:* An oral cancer model was established in male Balb/c mice using 4-nitroquinoline 1-oxide (4-NQO). The mice were systematically grouped and administered graded concentrations of EGCG. Key parameters such as body weight, hydration levels, tumor volume, and mass were meticulously tracked. T-cell activity and cytokine expression profiles, focusing on interleukin-2 (IL-2), interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α), were quantified using ELISA. A comprehensive statistical evaluation included one-way ANOVA, Tukey's HSD multiple comparison test, and the Kruskal-Wallis non-parametric assessment. *Results:* EGCG-administered cohorts exhibited a pronounced reduction in tumor size and mass, with the high-dose group showing the greatest efficacy. ELISA findings corroborated a significant increase in T-cell activity and concomitant upregulation of key cytokines, including IL-2, IFN-γ, and TNF-α ($P < 0.05$). *Conclusion:* This investigation confirms the tumor-suppressive efficacy of EGCG in a murine oral squamous cell carcinoma model. The therapeutic effects of EGCG are mediated through T-cell activation and the upregulation of pivotal cytokine expression, highlighting its potential immunomodulatory role in oral cancer treatment.

Keywords: Epigallocatechin-3-gallate (EGCG); Oral squamous cell carcinoma (OSCC); 4-nitroquinoline 1-oxide (4-NQO); Peripheral blood mononuclear cell (PBMC); Enzyme-linked immunosorbent assay (ELISA)

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1. Introduction

Head and neck malignancies, particularly oral cancer, have emerged as a significant public health challenge on the global health agenda. According to the Global Cancer Observatory (GLOBOCAN) 2020 data on global cancer statistics [1], over 377,713 new cases of oral cancer were diagnosed worldwide, with more than 177,757 deaths, underscoring its substantial impact on public health. The incidence and mortality rates of oral cancer are steadily rising globally, a trend particularly pronounced in developing countries such as China. For instance, in Hunan Province, the number of new cases annually has reached approximately 51,600, with around 23,200

deaths [2]. This increasing trend is closely associated with factors such as lifestyle changes, increased tobacco and alcohol consumption, and population aging.

Green tea, a distinctive type of tea in China, contains epigallocatechin-3-gallate (EGCG) as its primary active component, which is responsible for its anti-inflammatory, antioxidant, and anti-tumor properties. In recent years, research on EGCG has expanded, and clinical studies have found that EGCG, when combined with standard radiotherapy and chemotherapy, can enhance the treatment efficacy for oral cancer. However, the specific roles of EGCG in treatment and the mechanisms through which it exerts its effects remain unclear, and its broader application potential is still being explored.

In terms of treatment, traditional methods such as surgery, radiotherapy, and chemotherapy have achieved some success, but they remain limited in improving patients' long-term survival rates and quality of life. Therefore, there is an urgent need to explore and develop new therapeutic strategies [3]. In this context, EGCG has garnered widespread attention in the scientific community due to its significant antioxidant, anti-inflammatory, and anti-tumor effects [4].

2. Materials and methods

2.1. Experimental materials

Male Balb/c mice, aged 6–8 weeks and weighing 20–22 grams, were provided by Changsha Tianqin Biotechnology Co., Ltd. 4-Nitroquinoline-1-oxide (4-NQO, Sigma-Aldrich) was used to establish the oral cancer model, while epigallocatechin-3-gallate (EGCG, Sigma-Aldrich) was employed to test its inhibitory effects on the model. Ficoll solution (GE Healthcare) was utilized to isolate peripheral blood mononuclear cells (PBMCs) from whole blood samples collected from the mice. Enzyme-linked immunosorbent assay (ELISA) kits for interleukin-2 (IL-2), interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) [BD Biosciences] were used to measure cytokine levels. Anti-CD3 and anti-CD28 antibodies (Abcam) were applied to activate T cells *in vitro*, and an ELISA microplate reader (Bio-Rad) was used to read the results.

2.2. Methods

2.2.1. Establishment of mouse oral cancer models

A total of 60 male Balb/c mice were acclimatized for one week in the animal facility of the Life Sciences Building at Changsha Medical University. The mice were housed with free access to food and water under a 12-hour light/dark cycle, with the temperature maintained between 23°C and 26°C, and humidity controlled at 50 ± 5%. A 1% aqueous solution of 4-NQO was prepared with sterile distilled water and stored at 4°C in a light-protected refrigerator. For administration, it was diluted to 200 mg/L in tap water and provided in light-protected bottles for ad libitum drinking. The mice were randomly divided into five groups, with 12 mice per group. Throughout the experiment, the activity of each group was observed daily. Every two days, the remaining water in the bottles was measured using a graduated cylinder, and weekly records of water consumption, drug intake, and body weight were maintained. Starting from the 12th week, the oral mucosa of the mice was examined weekly under mild anesthesia for any changes. The appearance of grayish-white exophytic masses was considered indicative of a successful oral cancer model establishment.

2.2.2. Administration of EGCG in the mouse oral cancer model

Mice with successfully induced oral cancer were divided into four groups: a model group, a low-dose group, a medium-dose group, and a high-dose group. EGCG was prepared in aqueous solutions of 30 mg/100 mL, 50

mg/100 mL, and 100 mg/100 mL using sterile distilled water. The mice were administered these solutions via gavage at a dosage of 30 mL per day, and their conditions were monitored throughout the experiment. At the end of the study, the mice were euthanized via cervical dislocation, and the tumor tissues were collected and weighed. The tumor inhibition rate (IR) was calculated using the formula: IR = (1 - average tumor weight of the experimental group/average tumor weight of the control group) × 100%.

2.2.3. Isolation of peripheral blood mononuclear cells

A total of 10 mL of whole blood was drawn from the mice and heparinized to prevent coagulation. Two 15 mL centrifuge tubes containing 5 mL of Ficoll solution were prepared. The blood was diluted with an equal volume of phosphate-buffered saline (PBS) and gently layered over the Ficoll solution to prevent mixing. The tubes were centrifuged at 2,000 rpm for 20 minutes with the brake disengaged to ensure a gradual deceleration. After centrifugation, a white layer containing PBMCs was visible and carefully transferred into a clean 15 mL centrifuge tube using a pipette. Five volumes of PBS were added to achieve a final volume of 10–15 mL, followed by centrifugation at 1,500 rpm for 10 minutes. This washing step was repeated twice. After discarding the supernatant, 10 mL of 1640 medium was added, and the solution was thoroughly mixed before counting and preparing the cells for further use.

2.2.4. Detection of T cell activity and cytokine expression of IL-2, IFN-γ, and TNF-α by ELISA

The isolated PBMCs were cultured in a medium containing anti-CD3 antibodies (1 μg/mL) and anti-CD28 antibodies (2 μg/mL). The PBMCs were incubated at 37°C in a 5% CO_2 incubator for 2–3 days to activate T cells. After incubation, the culture medium was gently mixed and centrifuged at 1,500 rpm for 10 minutes to pellet the cells. The supernatant was collected, and ELISA kits were used to measure IL-2, IFN-γ, and TNF-α levels. The absorbance was measured at 450 nm using a microplate reader.

2.3. Statistical methods

Statistical analyses were performed using SPSS version 26. Quantitative data are presented as mean ± standard deviation (SD), while categorical data are expressed as rates or proportions. Analysis of variance (ANOVA) was conducted, followed by Tukey's Honestly Significant Difference (HSD) multiple comparison test and the Kruskal-Wallis non-parametric test. A *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Establishment of the oral cancer model and effects of EGCG treatment

After 17 weeks of 4-NQO induction, 44 mice across the five groups successfully developed oral cancer, yielding a success rate of approximately 73.3% (**Figure 1**). Following 12 weeks of EGCG treatment, a dose-dependent anti-tumor effect was observed in the EGCG-treated groups. The average tumor volume in the control (untreated) group was 500 mm^3, while the average tumor volumes in the low, medium, and high-dose EGCG groups decreased to 375 mm^3, 297 mm^3, and 196 mm^3, respectively. Correspondingly, the average tumor weights were reduced from 1.514 g in the control group to 0.775 g, 0.523 g, and 0.315 g in the low, medium, and high-dose EGCG groups. The calculated tumor inhibition rates were 13.3%, 26.7%, and 46.4%, respectively.

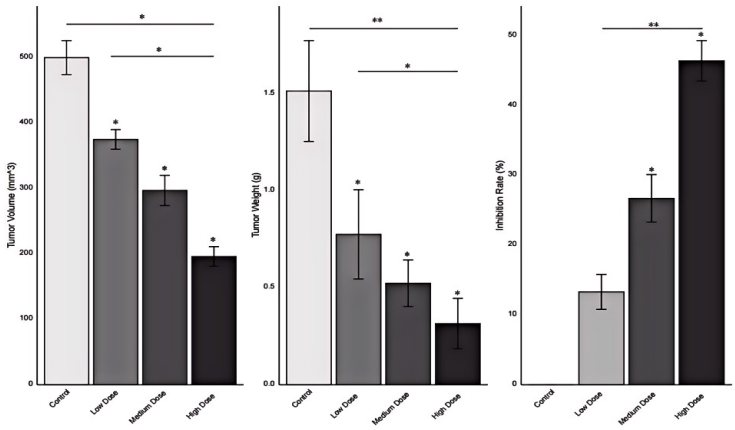

Figure 1. (A) Tumor volume; **(B)** Tumor weight; **(C)** Tumor inhibition rate (*$P < 0.05$, **$P < 0.01$)

3.2. Changes in T cell activity and cytokine expression

ELISA results demonstrated a significant increase in T-cell activity in the EGCG-treated groups compared to the control group (**Figure 2**). The PBMC counts in the low, medium, and high-dose EGCG groups were ($1.10 \times 10^6 \pm 0.03 \times 10^6$), ($1.20 \times 10^6 \pm 0.03 \times 10^6$), and ($1.30 \times 10^6 \pm 0.02 \times 10^6$), respectively, which were significantly higher than the control group's ($1.01 \times 10^6 \pm 0.05 \times 10^6$). Additionally, the levels of IL-2, IFN-γ, and TNF-α in the control group were 6.18 ± 0.23, 5.07 ± 0.34, and 4.03 ± 0.28, respectively. These levels increased to 76.97 ± 3.27, 91.25 ± 2.92, and 69.25 ± 2.92 in the high-dose EGCG group.

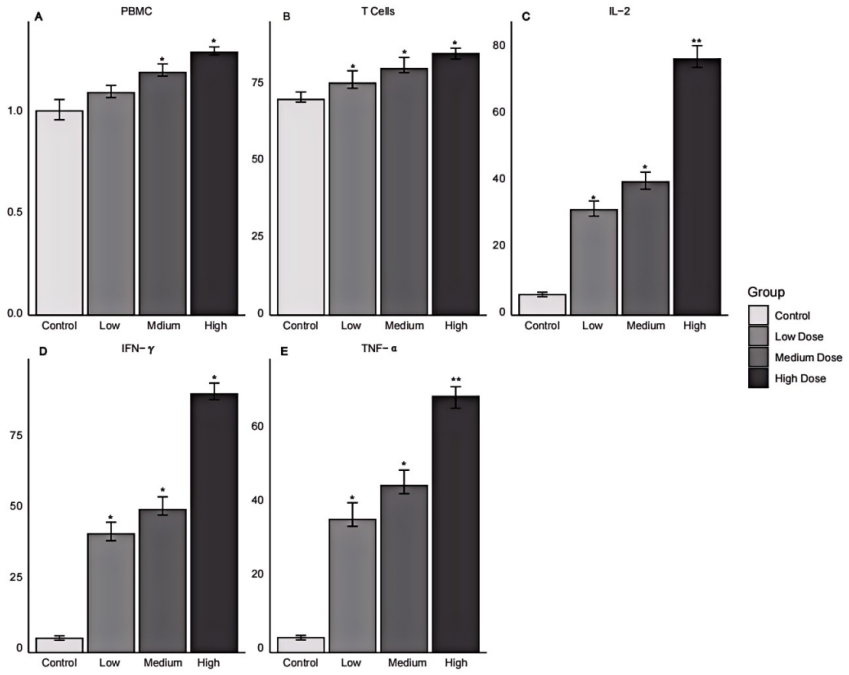

Figure 2. (A) PBMC; **(B)** T cell ratio; **(C)** IL-2; **(D)** IFN-γ; **(E)** TNF-α (*$P < 0.05$, **$P < 0.01$)

3.3. Statistical analysis

One-way ANOVA revealed that different EGCG doses significantly affected gene expression levels, with a *P*-value < 0.01. ANOVA results also showed significant differences between groups for PBMC count, T cell proportion, and levels of IL-2, IFN-γ, and TNF-α ($P < 0.05$). Tukey's HSD multiple comparisons test further highlighted significant differences in IL-2, IFN-γ, and TNF-α levels between the high-dose EGCG group and the control, low-dose, and medium-dose groups. The Kruskal-Wallis test also indicated significant differences between groups for all variables.

4. Discussion

This study conducted an in-depth investigation into the effects of EGCG, a major active component of green tea, in a mouse oral cancer model, with a focus on its anti-tumor mechanisms. EGCG, a naturally occurring polyphenol, demonstrates significant biological activity, particularly in antioxidation, anti-inflammation, and modulation of cellular signaling pathways [5]. In terms of EGCG's tumor-suppressive effects, a dose-dependent reduction in tumor volume and weight was observed in mice as the EGCG dosage increased. This effect may be associated with EGCG's regulatory influence on the cell cycle. EGCG can inhibit critical checkpoints in the tumor cell cycle, such as the G1/S or G2/M transitions, thereby blocking tumor cell proliferation [6].

Regarding T-cell activity and cytokine production, ELISA analysis revealed that EGCG significantly increased both the proportion and activity of T cells, as well as the levels of key cytokines such as IL-2, IFN-γ, and TNF-α. These cytokines are essential for activating and regulating T-cell immune responses [7]. IL-2 promotes T cell growth and differentiation, IFN-γ plays a role in antiviral and anti-tumor immunity, and TNF-α contributes to inflammation and cell death [8]. By elevating the levels of these cytokines, EGCG may enhance the cytotoxicity of T cells, enabling them to more effectively target and eliminate tumor cells [9]. This finding underscores EGCG's potential to strengthen the immune system, particularly through T cell-mediated immune responses.

In conclusion, EGCG exerts significant anti-tumor activity via multiple mechanisms affecting both tumor cells and the immune system. First, EGCG directly inhibits tumor cell proliferation and induces apoptosis [10]. Second, through its antioxidant and anti-inflammatory effects, EGCG may improve the tumor microenvironment, reducing the survival and metastatic potential of tumor cells [11].

As the focus on healthy lifestyles grows, natural medicines and dietary therapies have become increasingly popular topics of public interest. Green tea, a traditional beverage, has become deeply integrated into daily life, and its health benefits are progressively being validated by scientific research [12]. Against this backdrop, the current study not only highlights the anti-cancer potential of EGCG, a component of green tea, but also offers the public a simple and feasible method for promoting health. This approach, which combines traditional dietary culture with modern scientific research, is gradually becoming a part of global health management and disease prevention strategies [13].

Despite the positive results of this study, it is important to acknowledge the limitations of natural medicine therapies. Issues such as the bioavailability of natural compounds, potential side effects, and interactions with conventional treatments require further exploration. Additionally, while this experimental model provides robust evidence, there are inherent differences between human and animal models. Therefore, the clinical translation of EGCG still necessitates additional research to support and validate its use. Future studies should focus on preclinical research on EGCG, including pharmacokinetic and toxicological assessments, and clinical trial design, to provide a stronger scientific basis for its clinical application.

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Disclosure statement

The author declares no conflict of interest.

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Writing – review & editing: all authors

References

[1] Sung H, Ferlay J, Siegel RL, et al., Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin, 71(3): 209–249. https://doi.org/10.3322/caac.21660

[2] Fang Y, Yu Y, Wu DW, et al., 2020, A Review of Immune-Related Adverse Events Associated with Immunotherapy. Zhonghua Zhong Liu Za Zhi, 42(1): 17–21. https://doi.org/10.3760/cma.j.issn.0253-3766.2020.01.002

[3] Ferrari E, Bettuzzi S, Naponelli V, 2022, The Potential of Epigallocatechin Gallate (EGCG) in Targeting Autophagy for Cancer Treatment: A Narrative Review. Int J Mol Sci, 23(11): 6075. https://doi.org/10.3390/ijms23116075

[4] Aggarwal V, Tuli HS, Tania M, et al., 2022, Molecular Mechanisms of Action of Epigallocatechin Gallate in Cancer: Recent Trends and Advancement. Semin Cancer Biol, 80: 256–275. https://doi.org/10.1016/j.semcancer.2020.05.011

[5] Rawangkan A, Wongsirisin P, Namiki K, et al., 2018, Green Tea Catechin Is an Alternative Immune Checkpoint Inhibitor that Inhibits PD-L1 Expression and Lung Tumor Growth. Molecules, 23(8): 2071. https://doi.org/10.3390/molecules23082071

[6] Xiao X, Jiang K, Xu Y, et al., 2019, (-)-Epigallocatechin-3-Gallate Induces Cell Apoptosis in Chronic Myeloid Leukaemia by Regulating Bcr/Abl-Mediated p38-MAPK/JNK and JAK2/STAT3/AKT Signalling Pathways. Clin Exp Pharmacol Physiol, 46(2): 126–136. https://doi.org/10.1111/1440-1681.13037

[7] Huang SC, Kao YH, Shih SF, et al., 2021, Epigallocatechin-3-Gallate Exhibits Immunomodulatory Effects in Human Primary T Cells. Biochem Biophys Res Commun, 550: 70–76. https://doi.org/10.1016/j.bbrc.2021.02.132

[8] Pan Y, Long X, Yi R, et al., 2018, Polyphenols in Liubao Tea Can Prevent CCl_4-Induced Hepatic Damage in Mice through Its Antioxidant Capacities. Nutrients, 10(9): 1280. https://doi.org/10.3390/nu10091280

[9] Shen H, Wu N, Liu Z, et al., 2017, Epigallocatechin-3-Gallate Alleviates Paraquat-Induced Acute Lung Injury and Inhibits Upregulation of Toll-Like Receptors. Life Sci, 170: 25–32. https://doi.org/10.1016/j.lfs.2016.11.021

[10] Luo KW, Lung WY, Chun X, et al., 2018, EGCG Inhibited Bladder Cancer T24 and 5637 Cell Proliferation and Migration via PI3K/AKT Pathway. Oncotarget, 9(15): 12261–12272. https://doi.org/10.18632/oncotarget.24301

[11] Zhou CG, Hui LM, Luo JM, 2018, Epigallocatechin Gallate Inhibits the Proliferation and Induces Apoptosis of Multiple Myeloma Cells via Inactivating EZH2. Eur Rev Med Pharmacol Sci, 22(7): 2093–2098. https://doi.org/10.26355/eurrev_201804_14742

[12] Luo KW, Wei C, Lung WY, et al., 2017, EGCG Inhibited Bladder Cancer SW780 Cell Proliferation and Migration Both *In Vitro* and *In Vivo* via Down-Regulation of NF-κB and MMP-9. J Nutr Biochem, 41: 56–64. https://doi.org/10.1016/j.jnutbio.2016.12.004. Erratum in J Nutr Biochem, 123: 109530. https://doi.org/10.1016/j.jnutbio.2023.109530

[13] Zhao J, Blayney A, Liu X, et al., 2021, EGCG Binds Intrinsically Disordered N-Terminal Domain of p53 and Disrupts p53-MDM2 Interaction. Nat Commun, 12(1): 986. https://doi.org/10.1038/s41467-021-21258-5