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Molecular mechanisms underlying the epigallocatechin-3-gallate-mediated inhibition of oral squamous cell carcinogenesis

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ABSTRACT

Objectives: To reveal the mechanisms underlying the epigallocatechin-3-gallate (EGCG)-mediated inhibition of carcinogenesis and the related regulatory signaling pathways.
Design: The effect of EGCG on the proliferation of OSCC cells was examined. SuperPred, ChEMBL, Swiss TargetPrediction, DisGeNET, GeneCards, and National Center for Biotechnology Information databases were used to predict the EGCG target genes and oral leukoplakia (OL)-related, oral submucosal fibrosis (OSF)-related, and OSCC-related genes. The binding of EGCG to the target proteins was simulated using AutoDock and PyMOL. The Cancer Genome Atlas (TCGA) dataset was subjected to consensus clustering analysis to predict the downstream molecules associated with these targets, as well as their potential functions and pathways.
Results: EGCG significantly inhibited OSCC cell proliferation ($p < 0.001$). By comparing EGCG target genes with genes linked to oral potentially malignant disorder (OPMD) and OSCC, a total of eleven potential EGCG target genes were identified. Furthermore, EGCG has the capacity to bind to eleven proteins. Based on consensus clustering and enrichment analysis, it is suggested that EGCG may hinder the progression of cancer by altering the cell cycle and invasive properties in precancerous lesions of the oral cavity. Some possible strategies for modifying the cell cycle and invasive properties may include EGCG-mediated suppression of specific genes and proteins, which are associated with cancer development.
Conclusions: This study investigated the molecular mechanisms and signaling pathways associated with the EGCG-induced suppression of OSCC. The identification of specific pharmacological targets of EGCG during carcinogenesis is crucial for the development of innovative combination therapies involving EGCG.

1. Introduction

Oral cancer is a common malignant tumor of the oral cavity (Parris et al., 2014; Sand & Jalouli, 2014). The most frequent malignancy of the oral cavity is oral squamous cell carcinoma (OSCC), accounting for more than 90 % of oral malignancies (Islam et al., 2014; Güneri & Epstein, 2014). The development of oral cancer involves epigenetic alterations and discrete molecular genetic changes that are acquired from the loss of genomic integrity after prolonged exposure to environmental or dietary risk factors (Papagerakis et al., 2014; Wang et al., 2014). Thus, the development of oral cancer is a chronic process and mostly involves the transformation of oral potentially malignant disorder (OPMD).

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OPMDs are a major group of mucosal disorders that may precede the diagnosis of OSCC (Warnakulasuriya et al., 2007). Oral leukoplakia (OL) is the most frequently encountered representative OPMD (Aguirre-Urizar et al., 2021). The previously reported rates of malignant transformation for OL are highly variable (0.13–34.0 %) (Warnakulasuriya & Ariyawardana, 2016). Additionally, oral submucosal fibrosis (OSF) is a type of OPMD, which leads to stiffness in oral mucosa, fibrous banding, limitation in mouth opening, and the development of OSCC (in some cases) (Cox & Walker, 1996; Warnakulasuriya, 2018). Iocca et al. recently reported that the overall global estimate of the malignant transformation rate for OSF was 5.2 % (99 % confidence interval = 2.9–8.0 %) based on four studies (Iocca et al., 2020). Thus, there is an urgent need to prevent the transformation of potentially malignant diseases into cancer. Despite advancements in preventing and treating oral diseases, there remain numerous obstacles, such as the unfavorable reactions caused by medication and the inconsistent effectiveness of treatment. As a result, the study of natural products has become a prominent area of research.

In recent decades, numerous preclinical and epidemiological investigations have demonstrated the pivotal function of green tea in averting various age-related ailments, such as cardiovascular disease, diabetes, and even cancer (Filippini et al., 2020; Khan & Mukhtar, 2019; Liu et al., 2008; Mu et al., 2003; Nakachi et al., 2000). Intriguingly, consuming significant quantities of green tea can reduce the likelihood of developing gastric cancer (Hou et al., 2013), liver cancer (Ni et al., 2017), oral cancer (Wang et al., 2014), breast cancer (Sun et al., 2006), and prostate cancer (Johnson et al., 2010). Epigallocatechin-3-gallate (EGCG) is the most abundant polyphenol in green tea. One cup of 1.25 % w/v green tea (250 mL) comprises approximately 177 mg EGCG (Lambert & Yang, 2003; Yang et al., 2007). Several studies have demonstrated the preventive effects of EGCG against cancers at various sites, including the skin, lung, breast, colon, liver, stomach, and prostate (Mukhtar & Ahmad, 2000; Yang et al., 2002). At present, there are limited studies on the inhibitory effects of EGCG on oral cancer.Previous reports have demonstrated that EGCG can impede the growth and movement of oral cancer cells by reducing the phosphorylation of EGFR (Belobrov et al., 2019). Furthermore, EGCG can induce G1 phase arrest of tumor cells, which affects their ability to proliferate (Yoshimura et al., 2019). Additionally, EGCG can impact the apoptosis and invasion of oral cancer cells by modulating classical molecules and signaling pathways such as CASP8, MYC, and Hippo pathway (Li et al., 2018; Irimie et al., 2015). Nonetheless, there are still few studies regarding the specific binding targets of EGCG in oral cancer.

Based on clinical trials examining EGCG intervention in cervical cancer (Garcia et al., 2014), breast cancer (Arikawa et al., 2017; Dostal, Arikawa et al., 2016; Dostal, Samavat et al., 2016; Samavat et al., 2016; Samavat et al., 2019), and bladder cancer (Kumar et al., 2017; Kumar, Pow-Sang, Spiess, Park, Salup et al., 2016; Kumar, Pow-Sang, Spiess, Park, Chornokur et al., 2016; Zhang et al., 2016), it has been revealed that EGCG is well-tolerated and safe when administered during the advanced stages of cancer or following cancer surgery, but it is not effective in treating tumors. However, in a clinical trial focusing on precancerous lesions of prostate cancer, researchers discovered that EGCG has the potential to inhibit tumor growth and recommended early intervention during cancer cell transformation (Bettuzzi et al., 2006; Brausi et al., 2008; Aggarwal et al., 2022). This suggests that EGCG has substantial potential as a preventive treatment for potential malignancies, but there are currently no reports on its preventive treatment effect in suppressing the progression of potential malignant diseases in the oral cavity to cancer.

This study validates the growth-suppressing effects of EGCG on OSCC and predicts, for the first time, its possible direct binding targets and related signaling pathways that could obstruct the transformation process of oral cancer. This study utilized bioinformatics analysis of available data and molecular docking techniques to explicate the potential molecular targets and mechanisms of EGCG in hindering the transformation of oral cancer (Fig. 1). Hence, it establishes a theoretical basis for translating the preventative efficacy of EGCG in precancerous lesions into clinical applications and potentially serves as a natural remedy for early preventive treatment of potential malignant diseases in the oral cavity.

2. Materials and methods

2.1. Cell culture

HN6 and CAL-27 cells were cultured in Dulbecco's modified Eagle's medium (Gibco, USA) supplemented with 10 % fetal bovine serum (Gibco, USA) in a humidified cell culture incubator at 37 ℃ and 5 % CO_2.

2.2. Cell proliferation

To determine the optimal concentration of EGCG, the HN6 cells seeded in 6-well plates at a density of 2.0×10^5 cells/ well were treated with 50, 100, and 200 μM EGCG (Selleck, USA, S2250). The treated cells were trypsinized every 24 h, and the number of cells was quantified using the CountStar automated cell counter. To evaluate cell proliferation, HN6 and CAL-27 cells were seeded in 6-well plates at a density of 2.0×10^5 cells/well. After the cells were completely adherent, 200 μM EGCG was added. The images were captured from the central region at 24 and 48 h at a magnification of 20 ×. The cells were digested for counting after capturing representative images of the morphology of each specimen.

2.3. Cell migration (Wound healing assay)

HN6 and CAL-27 cells (2×10^6 cells/well) were seeded in 6-well plates. After the complete adherence of cells, the cells in the experimental and control groups were incubated with 200 μM of EGCG and an equal volume of dimethyl sulfoxide, respectively, for 24 h in a cell incubator. A pipette tip was used to scratch the surface of the cell monolayer in a straight line. The images of the monolayer were captured at 24 h post-scratching.

2.4. Predicting the potential targets of EGCG

The following three databases were used to predict the targets of EGCG: SuperPred Database (https://prediction.charite.de/) (Nickel et al., 2014), ChEMBL (https://www.ebi.ac.uk/chembl/) (Gaulton et al., 2017), and Swiss TargetPrediction (http://www.swisstargetprediction.ch/) (Daina et al., 2019).

2.5. Disease-related gene sets

The following three databases were used to retrieve genes related to OL, OSF, and OSCC: DisGeNET (http://www.disgenet.org/home/) (Piñero et al., 2015), GeneCards (http://www.genecards.org/) (Safran et al., 2010), and National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/gene/) (Sayers et al., 2022).

2.6. Molecular Docking

The compound name, molecular weight, and three-dimensional (3D) structure were determined using the PubChem database (https://pubchemdocs.ncbi.nlm.nih.gov/) (Kim et al., 2021). The 3D structure corresponding to the active components was downloaded from the RCSB PDB database (http://www.rcsb.org/) (Berman et al., 2003). AutoDock and PyMOL were used to prepare the ligands and proteins for molecular docking. The crystal structure of the target proteins required pretreatment, including removal of water molecules, hydrogenation, modifying amino acids, optimizing energy, and adjusting the force field

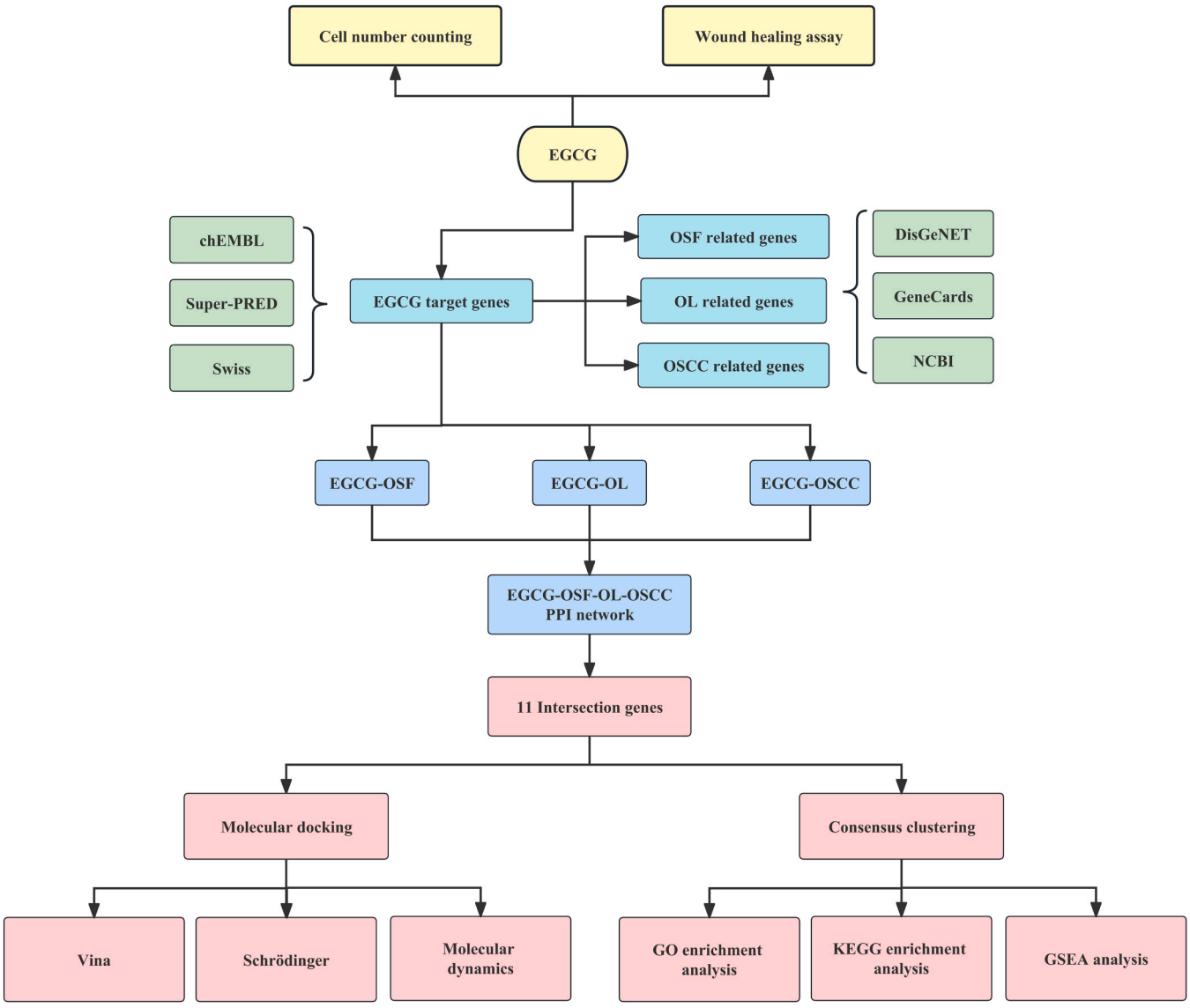

Fig. 1. Flowchart for elucidating the molecular mechanisms of epigallocatechin-gallate (EGCG).

parameters, to satisfy the low-energy conformation of the ligand structure. Finally, the target structure and the active component structure were docked using vina implemented in PyRx software (Eberhardt et al., 2021). To verify the accuracy of our docking results, we performed redocking using Schrödinger as well (Bhachoo & Beuming, 2017). The affinity (kcal/mol) value, which represents the binding ability of the two substances, is inversely proportional to the binding between the ligand and the receptor. The results were analyzed and visualized using the Discovery Studio software.

2.7. Molecular Dynamics (MD) simulation

We utilized the Gromacs 2022 software along with the AMBER 19 force field to simulate the complex (Pronk et al., 2013). Our initial step involved implementing a periodic boundary condition and surrounding all atoms with a cubic box that had a distance of 10 Å. To solvate the system, TIP3P water molecules were introduced with a density of 0.10 g/mL. Throughout the simulation, we maintained a temperature of 298 K and a pressure of 1 bar. To calculate long-range electrostatic interactions, we used the particle mesh Ewald method with an 8 Å cutoff.

Throughout the MD simulation, we used a 2 fs time step and saved simulation snapshots at 25 ps intervals. We conducted a 100 ns simulation for each system and analyzed the complex RMSD and protein RMSF by scrutinizing the MD trajectories. To analyze the binding energy, we employed gmx_MMPBSA (Valdés-Tresanco et al., 2021).

2.8. Consensus clustering analysis

Consensus clustering analysis was performed with the TCGA dataset based on eleven target genes using the R package DESeq2 (Love et al., 2014) for differential analysis according to the subgroup. The criteria for the differentially upregulated genes were as follows: log fold change (FC) > 1 and adjusted P-value < 0.05. Meanwhile, the criteria for the differentially downregulated genes were as follows: log FC < -1 and adjusted P-value < 0.05. Heatmap was constructed for targeted and differential genes using the pheatmap package, and the differential gene results were saved for subsequent enrichment analysis.

2.9. Enrichment analyses

Gene ontology (GO) analysis (Gene Ontology Consortium, 2015) is commonly used to perform large-scale functional enrichment analyses based on the enrichment of genes in the terms biological process (BP), cellular components (CC), and molecular functions (MF). Meanwhile,

Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa & Goto, 2000) is a widely used database of genomes, biological pathways, diseases, and drugs. The R package clusterProfiler was used to perform GO annotation analysis and KEGG pathway enrichment analysis with the potential target genes of EGCG involved in OSCC carcinogenesis. Differences were considered significant at a false discovery rate of < 0.05.

Gene set enrichment analysis (GSEA), which is a computational method to analyze if a particular gene set is significantly different between two biological states, is commonly used to estimate changes in biological process and pathway in the expression datasets of samples.

2.10. Statistical analysis

All statistical analyses were performed using the R program (https://www.r-project.org/ Magi version 4.0.2). For continuous variables, the means of normally distributed variables were estimated using independent-samples t-test. Meanwhile, the means of non-normally distributed variables were analyzed using Mann-Whitney U-test (or

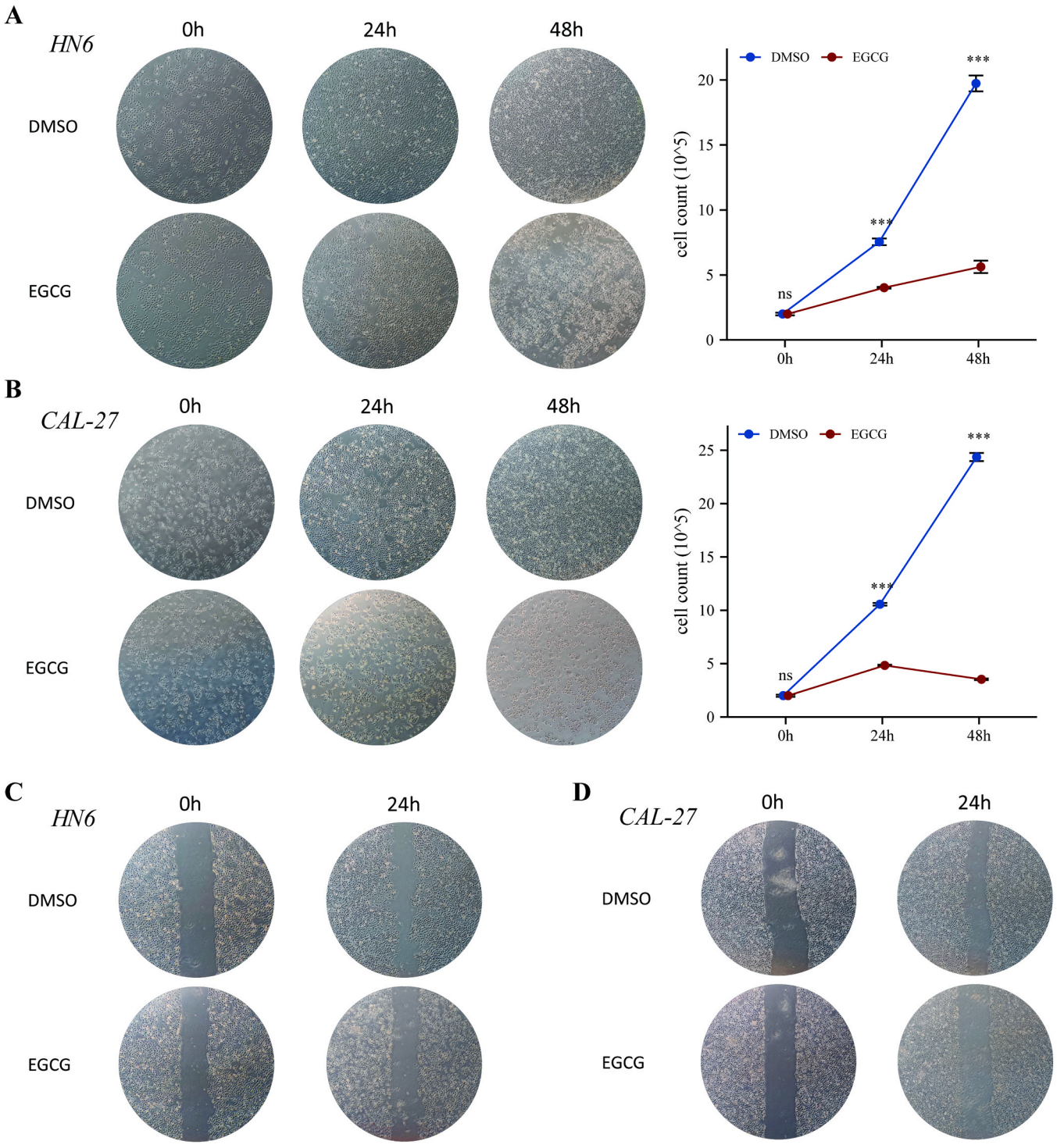

Fig. 2. Epigallocatechin-3-gallate (EGCG) inhibited oral squamous cancer cell proliferation and migration. A-B. The representative images of cell proliferation assays with HN6 and CAL-27 cells. C-D.The representative images of wound healing assays with HN6 and CAL-27. (magnification = ×20).

Wilcoxon rank sum test). All statistical tests were two-sided. Differences were considered at $P < 0.05$.

3. Results

3.1. EGCG inhibited the proliferation and migration of OSCC cells

HN6 cells were treated with different concentrations of EGCG to define the optimal concentration that exerts growth-inhibitory effects on OSCC. At a concentration of 50 μM, EGCG markedly inhibited the proliferation of OSCC cells. Additionally, EGCG dose-dependently inhibited the proliferation of OSCC cells (Supplementary Fig. 1), and 200 μM was chosen for further experiments. The results of experiments with HN6 and CAL-27 cells treated with 200 μM EGCG were similar to those of the preliminary experiments (Fig. 2A–B). In addition to the inhibition of cell proliferation, EGCG significantly altered cell morphology. This indicated that EGCG also regulated the relevant functions of the cytoskeleton.

To examine the effect of EGCG on the migration of OSCC cells, wound healing assays were performed with EGCG-treated HN6 and CAL-27 cells. The cells in the control group migrated to the wound area. In contrast, EGCG-treated cells did not migrate to the wound area (Fig. 2C–D). This indicates that EGCG significantly inhibited the migration of oral tumor cells.

3.2. Molecular mechanism of EGCG

Next, the molecular mechanism underlying the tumor suppressor effects of EGCG was evaluated. SuperPred, ChEMBL, and Swiss Target-Prediction databases were used to predict the potential target genes of EGCG. Simultaneously, genes related to OSF, OL, and OSCC were retrieved from DisGeNET, GeneCards, and NCBI databases. The potential target genes of EGCG were intersected with the disease-related genes. A Venn diagram was used for visualizing the intersecting genes (Fig. 3A–C). Finally, the target genes of EGCG related to OSF, OL, and OSCC were intersected to obtain the key genes targeted by EGCG that may play a role in carcinogenesis (Fig. 3D). All target genes of EGCG were visualized using Cytoscape software (Fig. 3E).

3.3. Molecular docking and molecular dynamics

To examine the binding of EGCG to the target proteins, simulated molecular docking and visualization were performed using the AutoDock, PyMOL, and Discovery Studio software. EGCG exhibited differential binding capacities with MMP2 (Fig. 4A), HIF1A (Fig. 4B), MMP9 (Fig. 4C), VEGFA (Fig. 4D), NOS2 (Fig. 4E), CA9 (Fig. 4F), ITGB1 (Fig. 4G), CDK1 (Fig. 4H), BCL2 (Fig. 4I), TERT (Fig. 4J), and APEX (Fig. 4K) (Supplementary Table S1). The 3D models of EGCG in the active sites of proteins are shown in the ray tracing diagram.

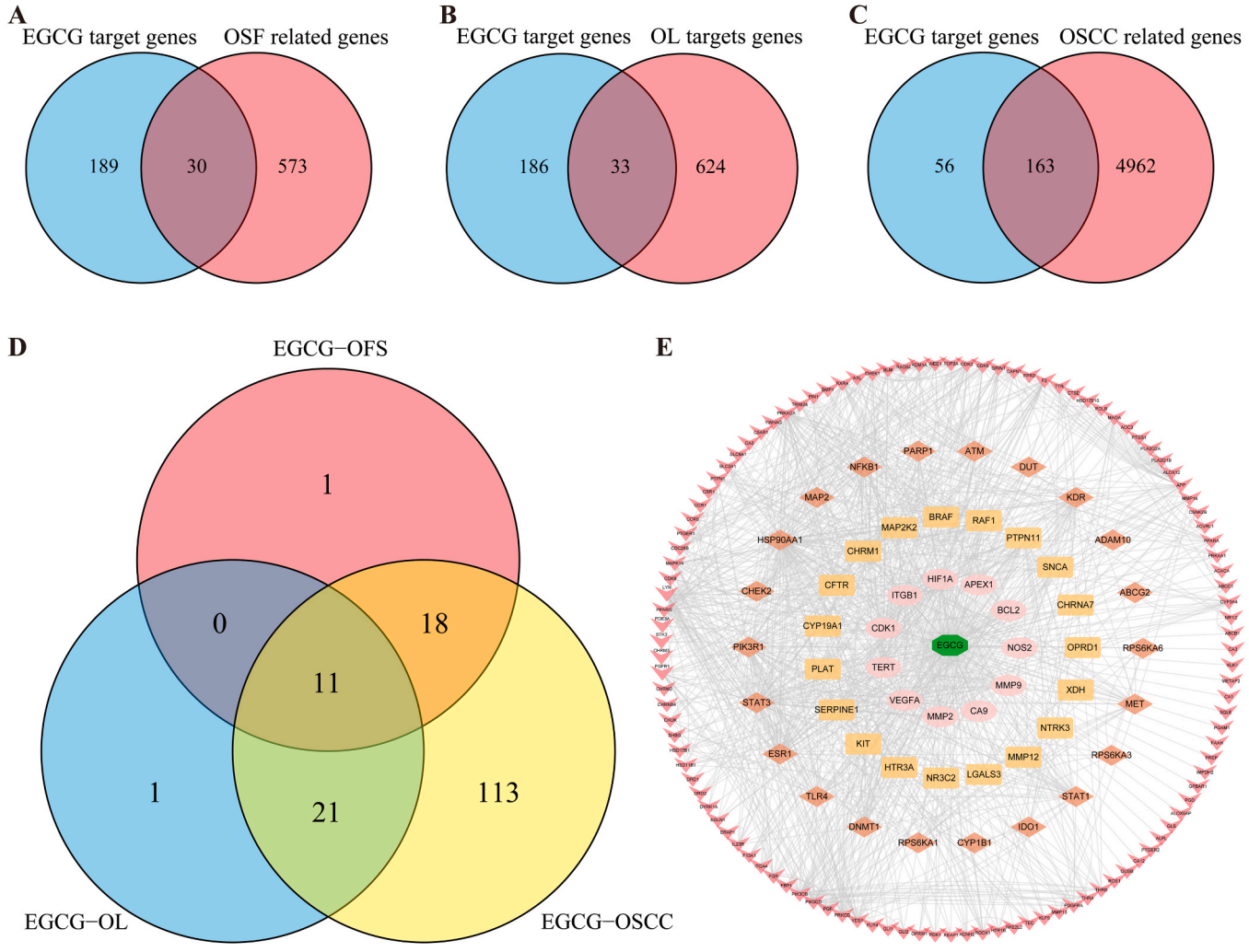

Fig. 3. Target genes of epigallocatechin-3-gallate (EGCG) involved in carcinogenesis. A-C. Venn diagram of EGCG target genes and oral submucosal fibrosis (OSF)-related, oral leukoplakia (OL)-related, and oral squamous cell carcinoma (OSCC)-related genes. D. The intersection of the EGCG target genes and the OSF-related, OL-related, and OSCC-related genes. E. Target genes of EGCG involved in carcinogenesis.

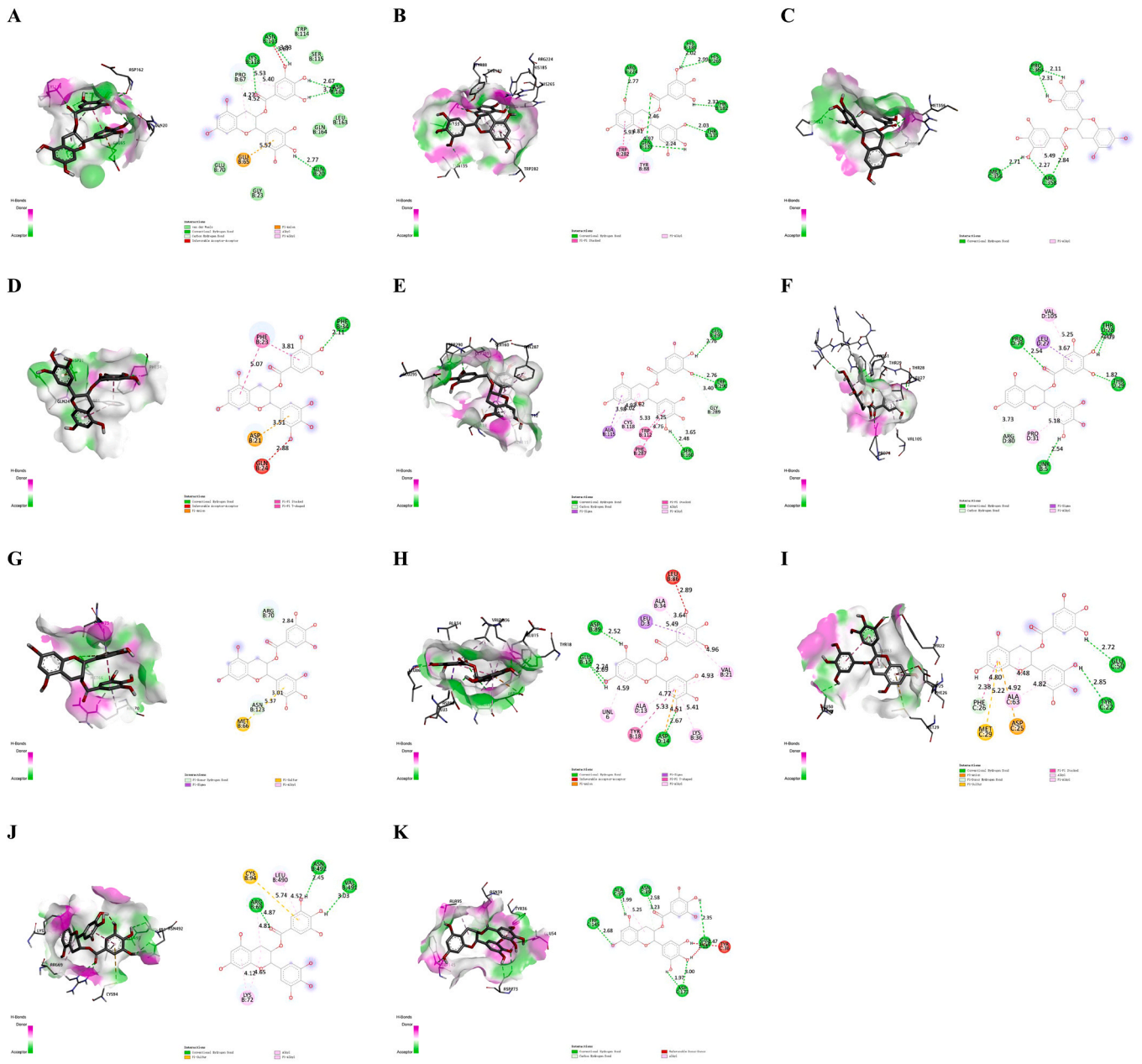

Fig. 4. Molecular docking using vina of epigallocatechin-3-gallate (EGCG) with target proteins. A-K. Binding of EGCG with MMP2 (A), HIF1A (B), MMP9 (C), VEGFA (D), NOS2 (E), CA9 (F), ITGB1 (G), CDK1 (H), BCL2 (I), TERT (J), and APEX (K).

We employed the Schrödinger software to re-dock the molecules (Supplementary Fig. 2), which uncovered that EGCG could bind to eleven proteins (Supplementary Table S2). To authenticate this discovery, we screened for two proteins, namely CDK1 and NOS2, with good binding affinity, as well as two proteins, ITGB1 and VEGFA, with poor binding affinity, based on the molecular docking free energy. Subsequently, we conducted a molecular dynamics analysis to investigate the binding of EGCG with these four proteins. We extracted the root mean square deviation (RMSD), root mean square fluctuation (RMSF) of the small molecule and protein complexes, as well as the number of hydrogen bonds between the protein and small molecule over time from the 100 ns trajectory (Supplementary Fig. 3). Following 50 ns, the RMSD of the CDK1-EGCG complex, NOS2-EGCG complex, and ITGB1-EGCG complex achieved a steady state of around 0.2 nm, indicating that these three proteins and the small molecule reached equilibrium. However, the RMSD of the VEGFA-EGCG complex exhibited slight fluctuations, suggesting a less stable binding between VEGFA and EGCG. Additionally, the RMSF of VEGFA-EGCG was slightly larger due to its poor binding affinity. The number of hydrogen bonds between CDK1-EGCG and NOS2-EGCG reached a stable state of 4 and 3, respectively, after 50 ns, while the hydrogen bonds of ITGB1-EGCG and VEGFA-EGCG fluctuated during the 100 ns trajectory.

After analyzing the trajectories and using the gmx_mmpbsa program to calculate the molecular mechanics generalized Born surface area (MM/GBSA) binding free energy (Supplementary Fig. 4), we determined that EGCG could bind to four proteins, with binding free energy values of −23.34 kcal/mol for CDK1, −35.38 kcal/mol for NOS2, −20.52 kcal/mol for ITGB1, and −14.62 kcal/mol for VEGFA. We used the MM/GBSA binding free energy decomposition to ascertain the contribution of amino acid residues to the binding energy. Our results indicated that VEGFA had significantly fewer active sites that promoted molecular binding. These findings corroborated the molecular docking results, demonstrating that EGCG could bind to all four proteins, but with stronger binding affinity observed for CDK1 and NOS2, whereas the

binding with ITGB1 and VEGFA was less stable.

3.4. Consensus clustering analysis of eleven target genes

To analyze the downstream molecules potentially affected by EGCG target genes and the related biological functions, consensus clustering analysis was performed based on the expression of the target genes using TCGA datasets. The classification of the data into two subtypes could best reflect the characteristics of the data. Increasing the number of subtypes increased the outlier data (Fig. 5A). This grouping information was used to generate the relevant heatmap of the target genes (Fig. 5B). The gene expression trends were not consistent although they exhibited characteristic expression patterns according to the grouping information. Next, the heatmap of typical differentially expressed genes (Fig. 5C), which are potential downstream genes that are regulated after the binding of EGCG to the target genes, was constructed.

3.5. Enrichment analysis

The functional enrichment of the differentially expressed downstream genes of the eleven target genes was examined using GO and KEGG analyses. EGCG potentially modulated the functions of extracellular matrix (ECM) organization and collagen metabolic process (Fig. 6A), which was consistent with the altered cell morphology observed in the proliferation experiment. KEGG analysis (Fig. 6B) revealed the enrichment of the signaling pathways, such as focal adhesion, regulation of actin cytoskeleton, and ECM-receptor interaction. This study focused on the focal adhesion (Supplementary Fig. 5) and ECM-receptor interaction (Supplementary Fig. 6) pathways. The proteins related to these pathways modulated by EGCG functioned upstream, suggesting that EGCG is an upstream regulatory factor.

GSEA was performed to obtain the comprehensive downstream pathways and the modulated functions. The results of GSEA indicated that EGCG may affect processes, such as epithelial-mesenchymal transition and inflammatory response (Fig. 7A) and pathways, such as ECM-receptor interaction and focal adhesion (Fig. 7B).

These findings demonstrated that the target genes have key roles in cancer progression and that EGCG exerts growth-inhibitory effects against tumors by modulating these regulatory processes.

4. Discussion

Current therapies for oral tumors are mainly aimed at the targets of tumor tissues. Limited studies have examined potential therapeutics that target the carcinogenesis of OPMDs. Therefore, there is an urgent need to identify drugs that can inhibit both carcinogenesis and cancers of OPMDs, as well as the tumorigenesis and progression of these tumors. Previous studies have suggested that flavonoid-rich fruits, teas, or herbs

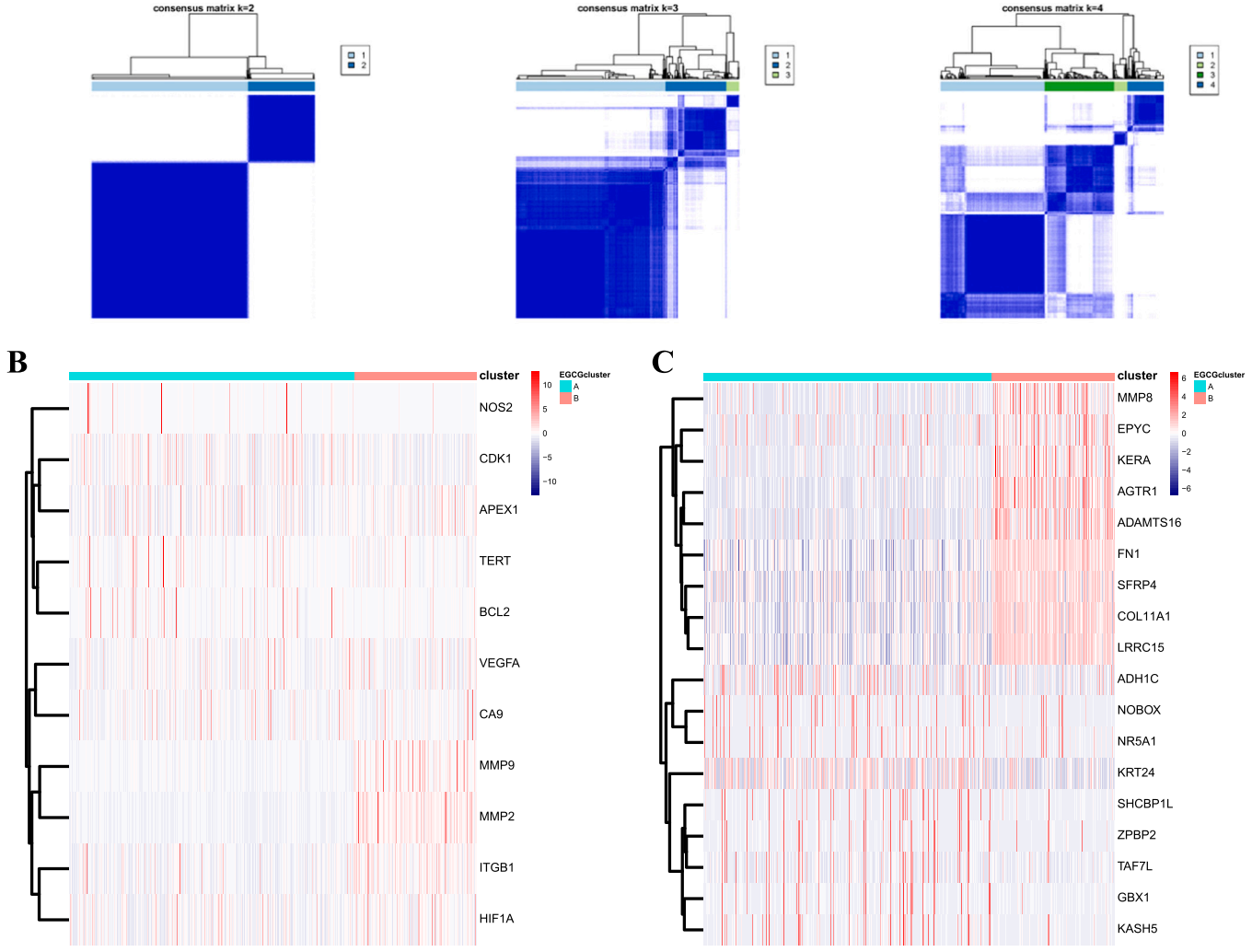

Fig. 5. Consensus clustering analysis. A. Consistency clustering determines the number of groups. B. Heatmap drawn by subtype grouping, showing the expression of the target genes. C. Heatmap drawn by subtype grouping, showing the representation differential expressed genes. The heatmap in red indicates upregulation and downregulation in blue.

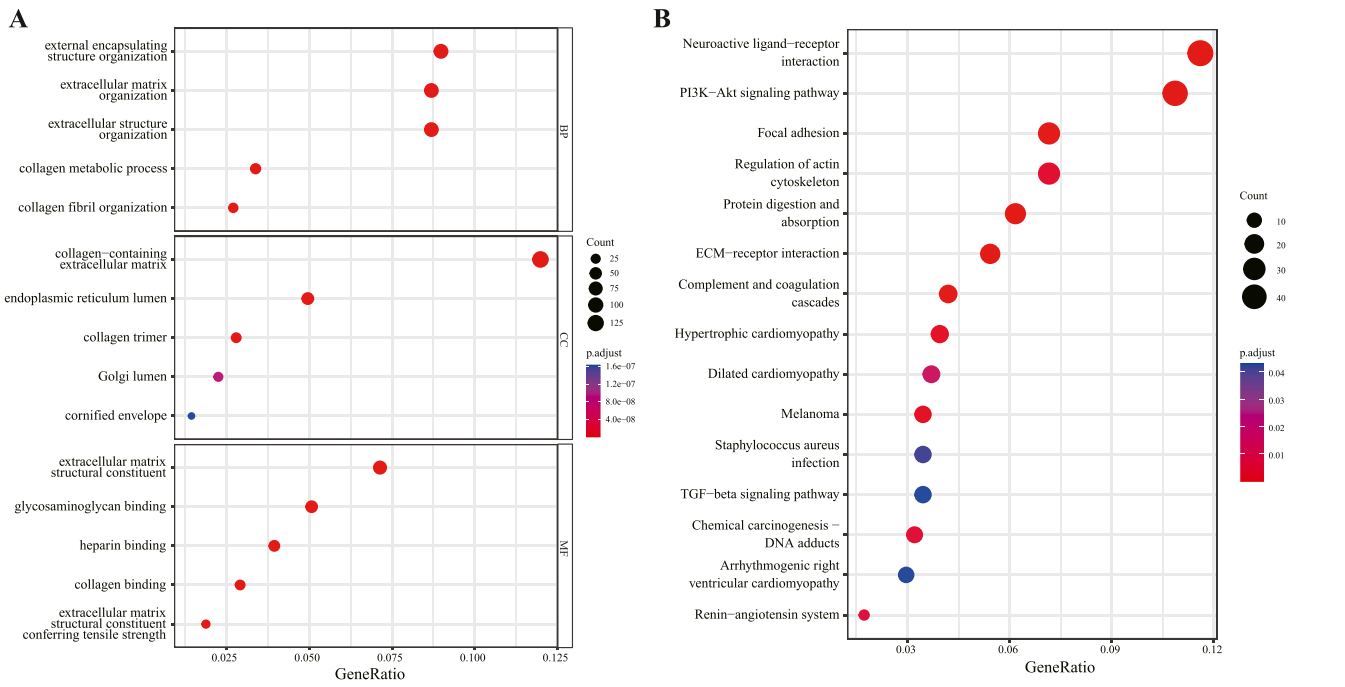

Fig. 6. Enrichment analysis. A. Genes enriched in the biological processes (BP), cellular components (CC), and molecular functions (MF). B. Genes enriched in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

can exert anticancer effects by regulating different molecules (Zhang et al., 2014). Recently, EGCG from green tea extract has attracted increasing attention. Previous studies have demonstrated the antioxidant, anti-inflammatory, and anticancer effects of EGCG. This study demonstrated the growth-inhibitory effect of EGCG on oral cancer and preliminarily analyzed the molecular targets and related signaling pathways of EGCG involved in inhibiting the carcinogenesis of OPMDs.

EGCG inhibits the proliferation of various cancers, such as breast carcinoma (Steed et al., 2020; Das et al., 2021; Gonzalez Suarez et al., 2022), cervical and endocervical cancers (Panji et al., 2021), non-small cell lung cancer (Minnelli et al., 2021), esophageal carcinoma (Taghvaei et al., 2021), head and neck squamous cell carcinoma (Amin et al., 2021; Agarwal et al., 2023), colorectal cancer (Md Nesran et al., 2020; Wu et al., 2021; Wu et al., 2022), Clear Cell Renal Cell Carcinoma (Lyu et al., 2022) and bladder urothelial carcinoma (Luo et al., 2020). Previous studies have reported the role and molecular mechanisms of EGCG in cancer inhibition. Limited studies have focused on the potential role of EGCG in suppressing the development of cancer. This study focused on exploring the same binding targets of EGCG in OPMDs and OSCC, performed molecular docking, and predicted the molecular regulatory pathways of these target genes. Several previous studies have reported that EGCG can affect various signaling pathways in cancers, including the VEGF/VEGFR (Kumar et al., 2018; Scandlyn et al., 2008; Shirakami et al., 2009; Shimizu et al., 2010), PI3K/AKT (Du et al., 2022; Hsieh et al., 2018; Yin et al., 2021), and p53 signaling pathways (Luo et al., 2020; Zhao et al., 2021; Guan et al., 2023). In this study, the target proteins of EGCG were enriched in these previously reported pathways and related functions, as well as in the focal adhesion signaling pathway and inflammatory cell migration-related pathways, which provided a new direction for further investigation.

Although several studies have demonstrated the anticancer effects of EGCG, limited studies have examined the underlying molecular mechanisms, especially the molecular targets that directly bind to EGCG. In 1997, Sazuka et al. used affinity chromatography technology to demonstrate that EGCG-binding target proteins included MMP2 and MMP9 (Sazuka et al., 1997). In this study, we not only identified these target molecules reported in the past, but also predicted not previously reported targets such as CDK1、APEX、CA9. The findings of this study indicated that these targets may inhibit the progression of OPMDs into tumor through these molecular mechanisms.

Although this study explored the targets and potential molecular mechanisms of EGCG involved in suppressing the carcinogenesis of OPMDs from a new perspective, this study has several limitations. This study mainly analyzed two common OPMDs and did not include all OPMDs. The inhibitory effect of EGCG on carcinogenesis cannot be confirmed based on the results of this study. Furthermore, clinical trials were not performed to verify the inhibitory effects of EGCG on carcinogenesis. However, this study provides the potential mechanism underlying the EGCG-mediated suppression of carcinogenesis. Further basic theories and clinical evidence are needed to address these limitations.

While more extensive exploration and validation are still required for the preventive and therapeutic effects of EGCG on cancer, its potential efficacy and safety make it a promising area of research. Future studies should continue to investigate the inhibitory effects of EGCG on cancer transformation and delve deeply into its therapeutic effects when used in combination with other treatment methods. Additionally, more clinical trials are needed to confirm the application prospects of EGCG in cancer treatment.

5. Conclusion

This study revealed the potential therapeutic value of EGCG in suppressing the carcinogenesis of OPMDs, explored the underlying molecular mechanisms, and provided theoretical evidence for the translation of laboratory findings into clinical practice. The findings of this study suggest that EGCG is a potential therapeutic to prevent the carcinogenesis of OPMDs.

CRediT authorship contribution statement

Fengyang Jing: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft. **Lijing Zhu:** Conceptualization, Data curation, Formal analysis, Investigation, Software, Visualization, Writing - original draft. **Jiaying Bai:** Conceptualization, Data curation, Formal analysis, Investigation,

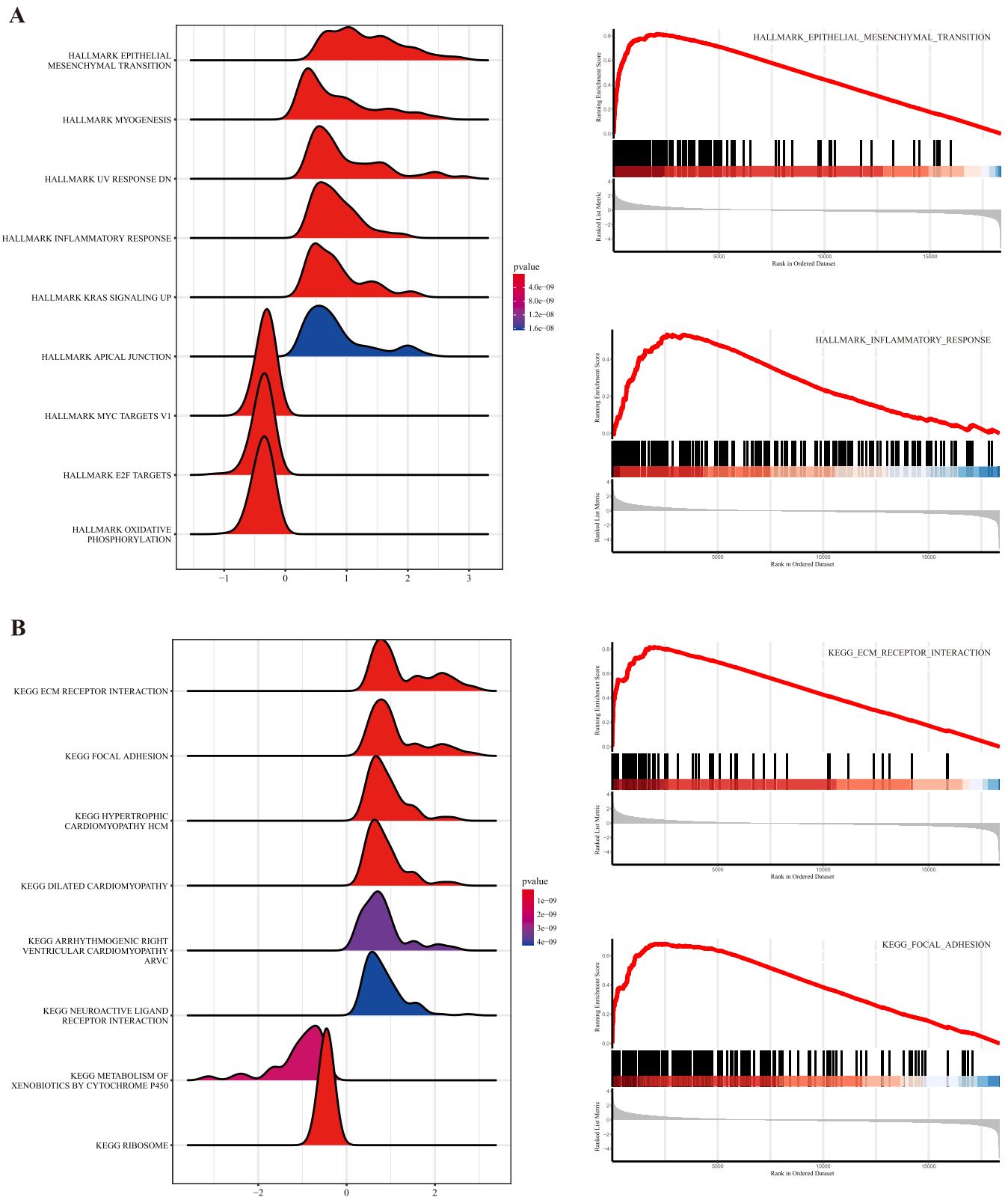

Fig. 7. Gene set enrichment analysis (GSEA). A. The mountain map and representative pictures showed the hallmark enrichment results. The abscissa was gene ratio, the ordinate was HALLMARK and the color represented p-value. B. The mountain map and representative pictures showed the KEGG enrichment results. The abscissa was gene ratio, the ordinate was KEGG and the color represented p-value.

Visualization, Writing - original draft. **Xinjia Cai:** Conceptualization, Data curation, Formal analysis, Investigation, Writing - original draft. **Xuan Zhou:** Conceptualization, Data curation, Formal analysis, Investigation, Writing - original draft. **Jianyun Zhang:** Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Heyu Zhang:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Tiejun Li:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

All authors declare that they have no competing interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.archoralbio.2023.105740.

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