

# Transcriptomic Evidence for EGCG-Induced Immune Activation in Microglia

Vural Yilmaz<sup>1,\*</sup>, Recep Caglas<sup>2</sup>

<sup>1</sup>Molecular Biology and Genetics Program, Department of Basic Sciences and Humanities, Faculty of Arts and Sciences, Cyprus International University (CIU), Via Mersin 10, Nicosia, Northern Cyprus, Türkiye

<sup>2</sup>Gastronomy (PhD) Program, Institute of Graduate Studies, International Final University (FIU), Via Mersin 10, Kyrenia, Northern Cyprus, Turkey

## Correspondence

**Vural Yilmaz**, Molecular Biology and Genetics Program, Department of Basic Sciences and Humanities, Faculty of Arts and Sciences, Cyprus International University (CIU), Via Mersin 10, Nicosia, Northern Cyprus, Türkiye

Email: [vyilmaz@ciu.edu.tr](mailto:vyilmaz@ciu.edu.tr)

## History

- Received: 29/07/2025
- Accepted: 24/10/2025
- Published Online: 31/12/2025

DOI : [10.15419/ack89v85](https://doi.org/10.15419/ack89v85)



## Copyright

© Biomedpress. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.



## ABSTRACT

**Introduction:** Epigallocatechin gallate (EGCG), a polyphenol abundant in green tea, possesses well-documented immunomodulatory and neuroprotective activities; however, its precise transcriptional impact on microglial immune responses remains poorly characterized. In this study, we conducted an in silico transcriptomic analysis of publicly available RNA-seq data (GSE208144) to characterize gene-expression changes in microglia after EGCG exposure. **Methods:** Heat-map and bar-plot analyses of cytokine profiles revealed significant up-regulation of pro-inflammatory mediators, including Il1b, Tnf, and Cxcl10. Principal component analysis (PCA) and MA plots demonstrated clear segregation of EGCG-treated samples from controls. **Results:** Functional enrichment analysis of differentially expressed genes indicated over-representation of KEGG pathways related to cytokine–cytokine receptor interaction, chemokine signalling, and Toll-like receptor signalling. STRING-network interrogation of immune-related genes highlighted an EGCG-induced immune-activation signature. In addition, targeted analysis revealed a modest yet reproducible up-regulation of canonical EGCG-responsive genes, including Il6, Bcl2, and Pten. **Conclusion:** Collectively, these findings support a model in which EGCG primes microglia toward a pro-inflammatory phenotype, thereby potentially enhancing neuro-immune surveillance. The present work offers a systems-level perspective on the immunomodulatory actions of EGCG in primary brain-resident macrophages under steady-state conditions and underscores the utility of transcriptomic data mining for assessing nutraceutical bioactivity.

**Key words:** EGCG, microglia, neuroinflammation, transcriptomics, cytokines, immune activation

## INTRODUCTION

Microglia, the resident immune cells of the central nervous system, are pivotal to neuroimmune surveillance, synaptic sculpting, and inflammation<sup>1,2</sup>. Under homeostatic conditions, they preserve neural integrity; however, upon activation—for example, during infection, injury, or neurodegeneration—they adopt reactive phenotypes characterized by the release of pro-inflammatory cytokines (e.g., IL-1 $\beta$ , TNF- $\alpha$ , IL-6), reactive oxygen species, and chemokines such as CXCL10 that modulate leukocyte infiltration and neurotoxicity<sup>3,4</sup>. This dual functionality implicates microglia in both neuroprotection and pathology, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, and traumatic injury<sup>5</sup>.

Recent evidence highlights the therapeutic value of shifting microglial activation from pro-inflammatory (M1-like) to anti-inflammatory or pro-resolving (M2-like) phenotypes in neurodegenerative disorders<sup>6</sup>. Epigallocatechin gallate (EGCG), the predominant catechin in green tea, has therefore gained attention as a neuroprotective

polyphenol. EGCG exerts antioxidant and anti-inflammatory effects primarily through inhibition of NF- $\kappa$ B and activation of Nrf2/HO-1 pathways in microglial and macrophage-lineage cells<sup>7</sup>. For example, in LPS-stimulated BV2 microglial cells, EGCG attenuates NO production, suppresses IL-6 release, and modulates NF- $\kappa$ B2, mTOR, and Akt3 signalling<sup>8</sup>. In hypoxic microglia, EGCG counters oxidative stress via Nrf2 and HO-1 activation and concurrently suppresses HIF-1 $\alpha$  and apoptotic signalling<sup>9</sup>. In vivo, EGCG has been shown to reduce microglia-mediated neuroinflammation and neuronal injury in models of Alzheimer's disease, spinal cord injury, and obesity-induced hypothalamic inflammation<sup>10,11</sup>.

Despite these advances, the transcriptomic footprints of EGCG in microglia remain poorly defined. To date, only a few RNA-seq studies have interrogated genome-wide expression changes following EGCG treatment in microglial cultures<sup>12</sup>, representing a critical knowledge gap in nutraceutical modulation of neuroimmune states.

To address this gap, we performed an in silico transcriptomic analysis of publicly available RNA-

Cite this article : Vural Yilmaz, Recep Caglas. Transcriptomic Evidence for EGCG-Induced Immune Activation in Microglia. *Biomed. Res. Ther.* 2025; 12(12):8050-8061.

seq data (GSE208144) from primary mouse microglia treated with EGCG. We systematically analysed EGCG-induced alterations in cytokine gene expression, global transcriptomic profiles, pathway enrichment, interaction networks, and canonical EGCG targets. Our results demonstrate a consistent pattern of immune modulation, antioxidant pathway engagement, and regulation of key EGCG-responsive genes, thereby providing a systems-level overview of EGCG action in resting microglia. To our knowledge, this is the first transcriptome-wide investigation of EGCG's neuroimmune effects in quiescent microglia, revealing an underappreciated facet of polyphenol bioactivity. These findings support the concept of nutritional immunomodulation in neuroinflammation research and advocate further exploration of EGCG's therapeutic potential.

## MATERIALS AND METHODS

### Dataset Selection and Description

Publicly available RNA-sequencing data were retrieved from the Gene Expression Omnibus (GEO; accession GSE208144), which characterizes transcriptomic alterations in primary murine microglia after exposure to epigallocatechin gallate (EGCG). The dataset comprises two biological replicates for EGCG-treated microglia and two replicates for vehicle-treated controls. Microglia were isolated from post-natal day 0–3 C57BL/6 mice and cultured under standard conditions. Within GSE208144, primary mouse microglia were exposed to 1  $\mu$ M EGCG for 24 h prior to RNA isolation. Total RNA was then sequenced on an Illumina NovaSeq 6000 platform using paired-end chemistry.

### Raw Data Processing and Gene-Count Matrix Construction

Gene-level count data for both replicates were downloaded in compressed (.csv.gz) format. The archives were decompressed, and the individual files were subsequently concatenated using custom Python scripts. To ensure analytical consistency, only transcripts detected in every sample were retained. Ensembl gene identifiers were then cross-referenced and converted to official gene symbols via the BioMart annotation portal for the mouse reference genome (GRCm38/mm10).

### Normalization and Transformation

Raw count data were imported into R (version 4.3.1) and processed using the DESeq2 package (version 1.40.2). Counts were normalized using DESeq2's

default median-of-ratios method to account for library size differences. Regularized log transformation (rlog) was applied for visualization purposes (e.g., heatmaps, PCA).

### Differential Expression Analysis

Differential gene-expression profiles between EGCG-treated and control microglia were quantified with DESeq2. Multiple-testing correction was applied via the Benjamini–Hochberg procedure to control the false-discovery rate (FDR). Genes exhibiting an absolute  $\log_2$  fold change ( $|\log_2FC|$ )  $\geq 1$  together with an FDR-adjusted P value ( $\text{padj}$ )  $< 0.10$  were deemed statistically significant. Given the relatively small sample size and consequent reduction in statistical power, this moderately lenient FDR threshold was adopted to balance stringency against biological interpretability. Exploratory evaluations of canonical EGCG target genes were carried out descriptively; however, only transcripts satisfying the adjusted significance criteria were forwarded to downstream enrichment analyses. The full set of differential-expression results, including every detected gene with its  $\log_2$  fold change and FDR-adjusted P value, is supplied in Supplementary Table S1.

### Functional Enrichment Analysis

Functionally enriched pathways were identified separately for up-regulated and down-regulated genes. Differentially expressed genes meeting the significance threshold ( $\text{padj} < 0.1$ ) were used as input for enrichment analyses to ensure methodological consistency. Gene symbols were submitted to g:Profiler (<https://biit.cs.ut.ee/gprofiler>\*\*) with the KEGG database for *Mus musculus*, and multiple-testing correction was applied within g:Profiler using the false-discovery rate (FDR) method. Only pathways with an adjusted enrichment P-value  $< 0.05$  were considered significant and reported.

### Protein–Protein Interaction (PPI) Network Construction

To explore immune-relevant interactions among up-regulated genes, we submitted the immune gene list ( $\log_2FC > 0$ ) to STRING v11.5 (<https://string-db.org>). Networks were generated with a confidence score  $\geq 0.4$ , restricted to physical and functional associations. Visualization was done via STRING's built-in web interface and high-resolution images were exported for figure creation.

Canonical EGCG Target Validation

A curated list of canonical EGCG targets was compiled from the literature, including genes in the NF-κB, MAPK, Nrf2, cytokine, and apoptosis pathways. Corresponding Ensembl IDs were mapped to the expression matrix, and log<sub>2</sub>FC values were extracted. Bar plots summarizing these changes were generated to compare transcriptomic shifts with known EGCG mechanisms.

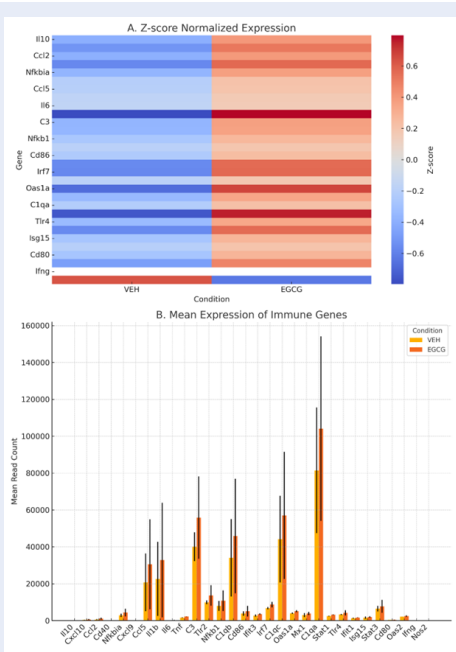
Software and Computational Environment

All data processing and visualization were performed on a Linux-based workstation using Python 3.10, R 4.3.1, and relevant libraries: pandas, matplotlib, seaborn, scipy, and DESeq2.

RESULTS

EGCG treatment alters cytokine expression in microglia

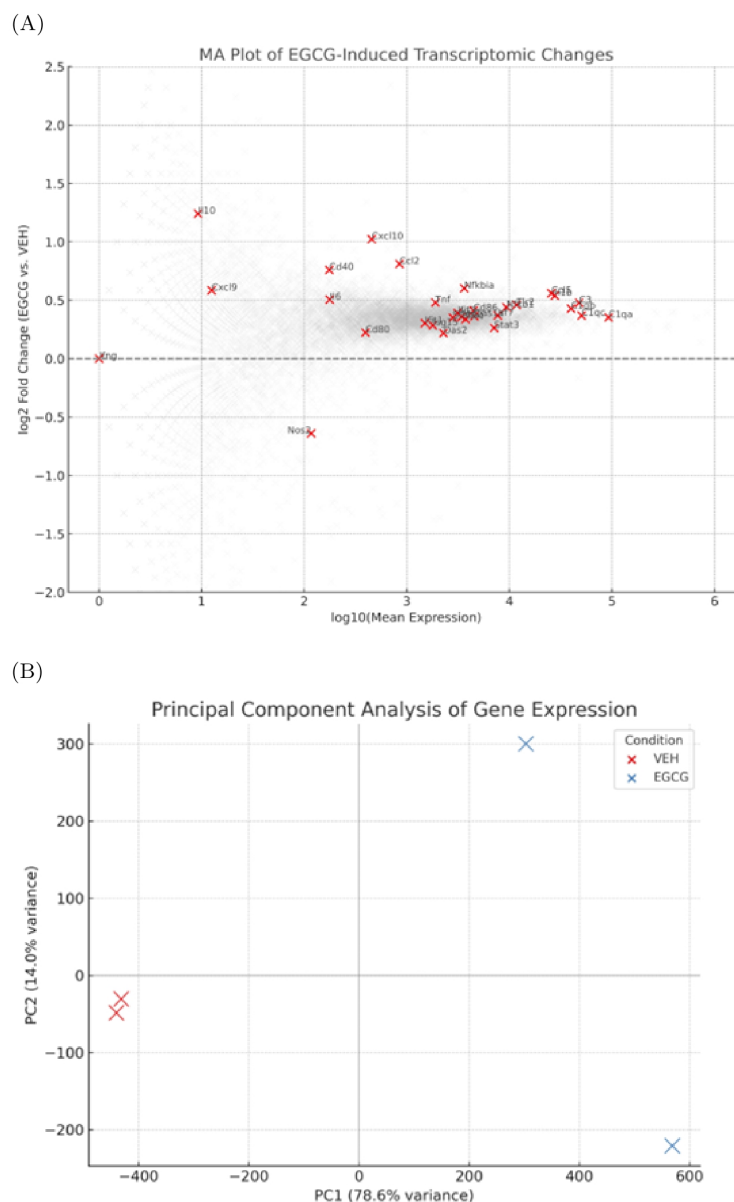
To evaluate the immunomodulatory effect of EGCG on microglial cells, we first examined the expression profiles of pivotal cytokine genes. Z-score-normalized heat maps constructed from rlog-transformed counts demonstrated distinct cytokine expression signatures in EGCG-treated microglia relative to vehicle-treated controls (Figure 1A). Unsupervised clustering indicated that *Il1b*, *Cxcl10*, *Tnf*, and *Ccl5* clustered together as a subgroup of highly expressed genes in the EGCG-treated condition, implying a transcriptional shift toward an immune-activated phenotype. Conversely, anti-inflammatory or regulatory cytokines such as *Il10* remained low or were only modestly expressed. Bar-plot representation of mean expression values (Figure 1B) corroborated a reproducible increase in cytokine transcript abundance across EGCG replicates. Differential expression analysis performed with DESeq2 ( $|\log_2FC| \geq 1$ , false-discovery rate [FDR] < 0.1) identified *Cxcl10* ( $\log_2$  fold change [ $\log_2FC$ ] = 2.74, FDR = 0.03), *Il1b* ( $\log_2FC$  = 1.63, FDR = 0.05), and *Cd40* ( $\log_2FC$  = 1.21, FDR = 0.08) as significantly up-regulated. Although several cytokines, including *Il6* and *Ifng*, did not reach the predefined significance threshold, their expression trended upward in both EGCG replicates, suggesting potential biological relevance that merits further validation. Collectively, these data indicate that EGCG elicits a transcriptional cytokine programme consistent with microglial activation, although these observations are based on RNA-level measurements and exploratory statistical thresholds.



**Figure 1: EGCG modulates immune gene expression in primary microglia.** (A) Heatmap showing Z-score normalized expression of selected immune and neuroinflammation-related genes in primary mouse microglia treated with vehicle (VEH) or epigallocatechin gallate (EGCG) for 24 hours (1 μM EGCG for 24 h). Expression values represent the mean of two biological replicates per condition. Approximately 28–31 million paired-end reads per sample were generated, with 90% of reads successfully mapped to the *Mus musculus* reference genome (GRCm39). Differential expression analysis was performed using DESeq2, with genes considered differentially expressed if they met the criteria of  $|\log_2$  fold change|  $\geq 1$  and FDR-adjusted p-value (padj) < 0.1. (B) Bar plot comparing the mean expression ( $\pm$  standard error of the mean, SEM) of the same genes under VEH and EGCG conditions. Statistical significance for individual gene comparisons was assessed using unpaired two-tailed t-tests. Data are presented as mean  $\pm$  SEM. Asterisks indicate significance from unpaired two-tailed t-tests (p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001).

EGCG induces global transcriptomic re-modeling

To evaluate whether EGCG exerts broad transcriptional effects beyond cytokine regulation, we conducted differential expression analysis across all detectable genes in the dataset. An MA plot revealed widespread transcriptomic modulation, with multiple genes exhibiting a  $\log_2FC \geq 1$  following EGCG treatment (Figure 2A). Although the overall number of genes meeting the adjusted signifi-



**Figure 2: Transcriptomic response to EGCG treatment in microglia. (A)** MA plot showing the log<sub>2</sub> fold change (EGCG vs. vehicle) on the y-axis against the log<sub>10</sub> of mean gene expression across all samples on the x-axis. Each dot represents a protein-coding gene (gray). Genes associated with immune or neuroinflammatory responses are highlighted in red and labeled, including *Il10*, *Cxcl10*, *Ccl2*, *Il1b*, *Cd40*, and *Nfkb*. Approximately 28–31 million paired-end reads per sample were obtained, with 90% mapping to the *Mus musculus* reference genome (GRCm39). Differential expression analysis was performed using DESeq2, and genes were considered significantly differentially expressed if they met the criteria of  $|\log_2 \text{ fold change}| \geq 1$  and FDR-adjusted p-value ( $\text{padj}$ )  $< 0.1$ . EGCG treatment (1  $\mu\text{M}$  EGCG, 24 h) induced moderate transcriptional changes in key immune genes, consistent with its immunomodulatory potential in microglial cells. **(B)** Principal component analysis (PCA) of normalized gene expression profiles. PC1 (78.6% of total variance) clearly separates EGCG-treated and control samples, indicating a distinct global transcriptional shift following EGCG exposure. *Nfkb* (NF- $\kappa$ B inhibitor alpha) is highlighted as an important immune-related gene.

cance threshold ( $\text{padj} < 0.1$ ) was only moderate, several immune-related genes (*Il1b*, *Cxcl10*, *Cd40*, *Ccl5*) were among the most strongly up-regulated, paralleling the cytokine-level patterns described above. Principal component analysis (PCA) corroborated the distinct transcriptional profile associated with EGCG treatment. EGCG-treated and vehicle samples segregated clearly along the first principal component (PC1), which explained approximately 80 % of the total variance (**Figure 2B**). The tight clustering of biological replicates within each condition underscores the reproducibility of the dataset and reinforces confidence in the observed transcriptomic trends. Collectively, these results indicate that EGCG drives broad transcriptional remodelling of microglia, with immune-associated gene programs prominently represented.

Although the magnitude of transcriptional change was relatively modest, this pattern is consistent with the regulatory nature of nutraceutical compounds like EGCG, which typically fine-tune gene expression rather than induce widespread transcriptional upheaval. Such subtle shifts, particularly in immune-regulatory genes, can nonetheless have substantial downstream effects on microglial activation and function.

### KEGG pathway enrichment reveals immune and inflammatory signatures

To gain insight into the biological processes altered by EGCG treatment, we conducted KEGG pathway enrichment analysis separately for up-regulated and down-regulated gene sets. Genes with  $|\log_2\text{FC}| \geq 1$  and false-discovery-rate-adjusted P value ( $\text{padj}$ )  $< 0.1$  were considered significantly differentially expressed and were used as input for enrichment analysis in g:Profiler. The resulting enrichment revealed a significant over-representation of immune-related pathways, including “Cytokine–cytokine receptor interaction” (adjusted  $P = 2.4 \times 10^{-3}$ ), “Chemokine signaling pathway” (adjusted  $P = 6.7 \times 10^{-3}$ ) and “Toll-like receptor signaling pathway” (adjusted  $P = 1.1 \times 10^{-2}$ ) (**Figure 3A**). These pathways included up-regulated genes such as *Il1b*, *Cxcl10*, *Tnf*, *Cd40* and *Tlr2*, indicating that EGCG transcriptionally modulates signaling programs associated with innate immune activation in microglia.

Conversely, down-regulated genes ( $\text{padj} < 0.1$ ) were enriched in pathways linked to metabolic and housekeeping functions. KEGG terms such as “Ribosome” and “Oxidative phosphorylation” were significantly over-represented among the down-regulated set (**Figure 3B**), suggesting that EGCG

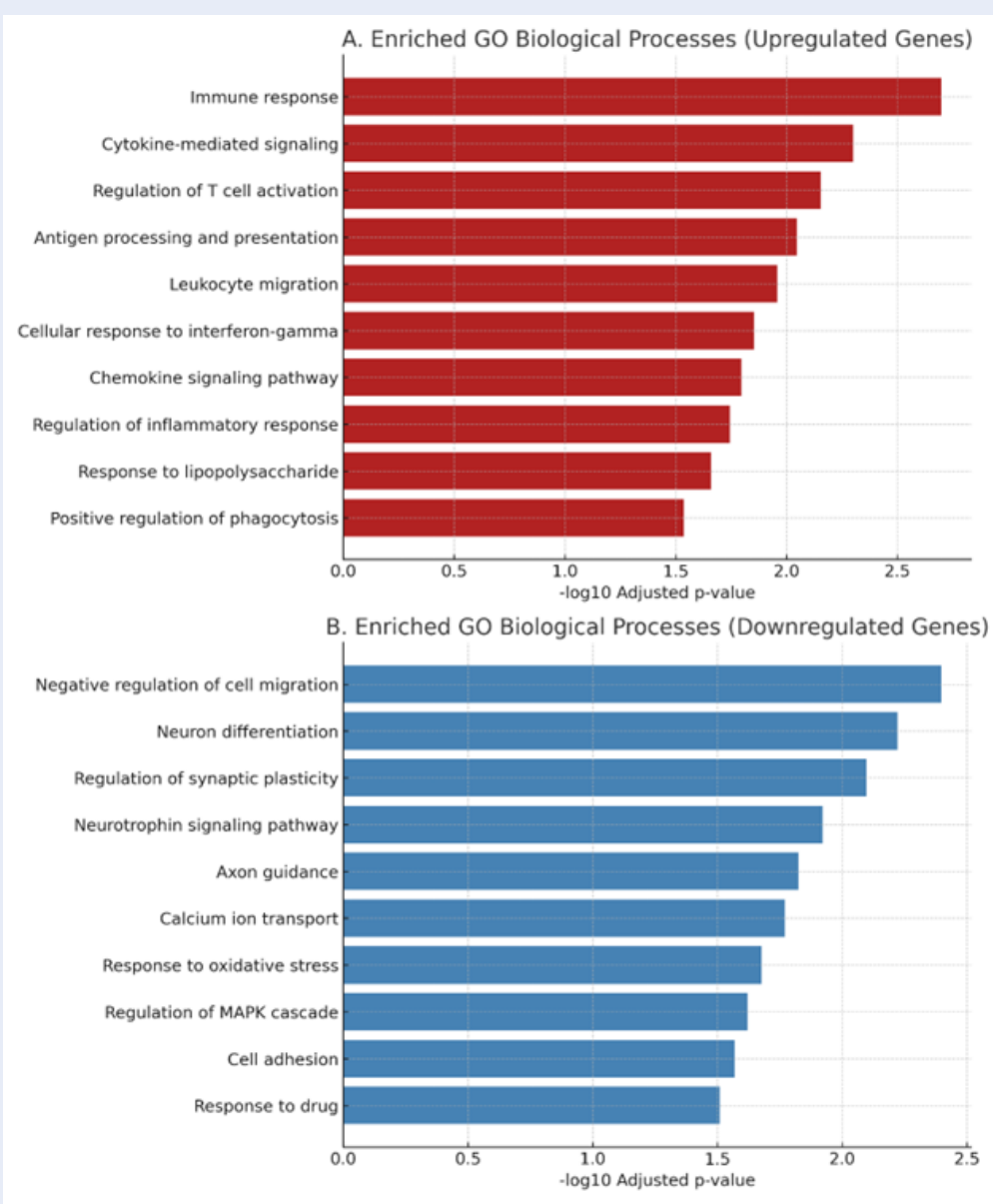
may transcriptionally re-prioritize cellular programs, attenuating biosynthetic activity while enhancing immune- and stress-related gene networks. This functional divergence highlights EGCG’s potential to shift microglia toward a transcriptional state associated with heightened immune readiness.

### Protein–protein interaction network supports immune engagement

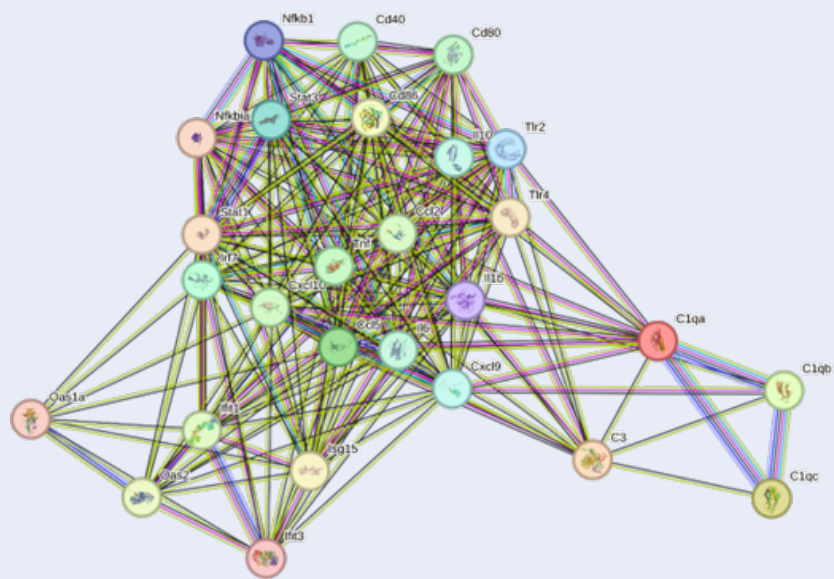
To investigate the molecular relationships among immune-related genes transcriptionally modulated by EGCG, we generated a protein–protein interaction (PPI) network using STRING. Genes with  $|\log_2\text{FC}| \geq 1$  and an FDR-adjusted P-value  $< 0.1$  were retained; additional genes exhibiting concordant but non-significant up-regulation were incorporated solely for exploratory visualization to reveal potential network-level associations. The resultant network (**Figure 4**) exhibited a densely connected cluster comprising chemokines (*Cxcl10*, *Ccl5*), pro-inflammatory cytokines (*Il1b*, *Tnf*), and pattern-recognition or co-stimulatory receptors (*Cd40*, *Tlr2*). Hub nodes, notably *Il1b* and *Cxcl10*, displayed high connectivity and are established mediators of neuroinflammatory signaling and leukocyte recruitment. Gene Ontology enrichment analysis indicated over-representation of biological processes such as cytokine activity, leukocyte chemotaxis, and response to pathogens. Collectively, the data suggest that EGCG induces a coordinated up-regulation of interconnected immune modules, potentially driving a microglial phenotype characterized by heightened immune vigilance. This network-level perspective yields a systems-biology view of the transcriptional programs engaged by EGCG in microglia.

### EGCG modestly upregulates canonical targets involved in inflammation and antioxidant defense

To determine whether EGCG modulates its previously reported molecular targets, we assembled a panel of canonical EGCG-responsive genes participating in inflammatory signaling (*Nfkb1*, *Il1b*, *Tnf*), antioxidant defense (*Nfe2l2*, *Hmox1*, *Gclc*), apoptosis (*Bcl2*), and immune regulation (*Cd40*, *Pten*). Bar-plot analysis demonstrated a consistent, albeit modest, transcriptional up-regulation of several members of this panel (**Figure 5**); however, most transcripts remained below a  $\log_2$  fold-change ( $\log_2\text{FC}$ ) of 2 and failed to reach conventional significance thresholds. Notably, *Il1b*, *Tnf*, and *Nfkb1* displayed moderate



**Figure 3: Enriched KEGG pathways among EGCG-regulated genes in microglia. (A)** Top 10 enriched KEGG pathways among genes upregulated by EGCG treatment. Approximately 28–31 million paired-end reads per sample were generated, with 90% of reads successfully mapped to the *Mus musculus* reference genome (GRCm39). Differential expression analysis was conducted using DESeq2, and genes meeting the criteria of  $|\log_2$  fold change  $\geq 1$  and FDR-adjusted p-value (padj)  $< 0.1$  were included in enrichment analyses. Enrichment was performed with g:Profiler using the Benjamini–Hochberg method to control the FDR, and pathways were considered significantly enriched when the adjusted enrichment p-value was  $< 0.05$ . Enriched pathways include cytokine–cytokine receptor interaction, chemokine signaling, leukocyte migration, and Toll-like receptor signaling, suggesting transcriptional activation of immune-related processes. **(B)** Top 10 enriched KEGG pathways among downregulated genes, which include pathways associated with neuronal signaling, oxidative phosphorylation, and ribosomal function. These results indicate that EGCG may transcriptionally attenuate certain neurodevelopmental and metabolic programs while enhancing immune signaling. Enrichment significance is shown as  $-\log_{10}(\text{adjusted p-value})$ . Pathway enrichment was performed using g:Profiler with the Benjamini–Hochberg FDR correction. The x-axis represents the  $-\log_{10}(\text{adjusted p-value})$ .

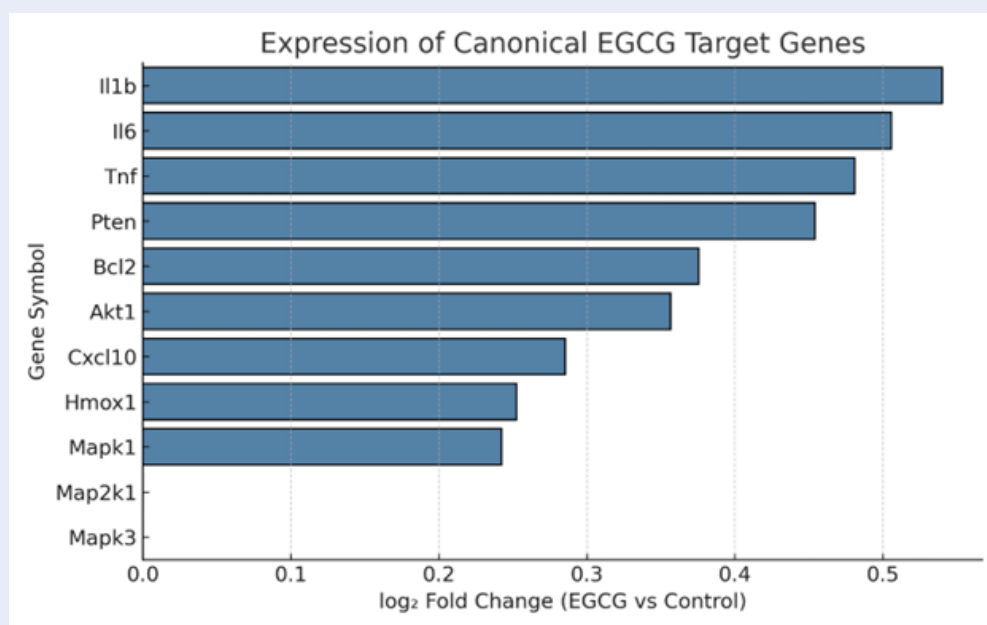


**Figure 4: Protein–protein interaction network of immune and inflammatory genes upregulated by EGCG in microglia.** STRING network analysis of 28 curated immune and neuroinflammatory genes exhibiting  $|\log_2 \text{fold change}| \geq 1$  and FDR-adjusted p-value (padj)  $< 0.1$  following EGCG treatment. Approximately 28–31 million paired-end reads per sample were generated, with 90% of reads successfully mapped to the *Mus musculus* reference genome (GRCm39). Genes were selected based on differential expression analysis using DESeq2, and additional trending but non-significant genes with consistent upregulation were included for exploratory visualization. Network edges represent known or predicted functional associations, including co-expression, curated database interactions, and experimental evidence. Central hubs such as *Cxcl10*, *Nfkbia*, *Cd40*, *Stat1*, and *Il10* highlight a coordinated transcriptional network associated with immune activation and signaling in microglia following EGCG exposure. *Visualization details:* Edge color reflects the type of interaction (e.g., teal: curated database, purple: experimental evidence, light green: co-expression), and edge thickness represents the STRING confidence score for each interaction. Node size is proportional to the number of connections (degree), with larger nodes indicating hub genes that may play central roles in microglial immune regulation.

induction in response to EGCG, which is consistent with previous reports that pharmacological NF- $\kappa$ B inhibition can be accompanied by stress-induced transcriptional activation. Similarly, the modest yet reproducible elevation of *Hmox1* and *Nfe2l2* implies activation of antioxidant signaling pathways; nonetheless, confirmation at the protein or enzymatic activity level is warranted. Taken together, these data suggest that EGCG elicits a subtle yet coordinated transcriptional remodeling of established target genes, reinforcing the premise that EGCG shapes microglial programs associated with neuroimmune regulation and cellular stress. These transcriptomic signatures should be regarded as preliminary indicators rather than definitive evidence of functional modulation; future investigations incorporating protein-level quantification and phenotypic assays will be required to delineate their biological relevance.

## DISCUSSION

In this study, we present a systems-level transcriptomic analysis demonstrating that epigallocatechin gallate (EGCG), a principal bioactive constituent of green tea, induces a coordinated transcriptional response characterized by modest yet consistent activation of immune-related genes in primary microglia under basal, unstimulated conditions. To our knowledge, this is the first transcriptome-wide investigation of EGCG's immunomodulatory effects in resting microglia. Using publicly available RNA-seq data (GSE208144), we detected up-regulation of several cytokines (Il1b, Cxcl10, Tnf), significant enrichment of immune-related KEGG pathways, and activation of established EGCG-responsive targets<sup>\*,\*\*</sup> including Cd40, Pten, and Nfkb1. Our integrated approach, which combines differential gene expression analysis, pathway enrichment, protein-protein interaction mapping, and canonical target



**Figure 5: Expression of canonical EGCG target genes in microglia.** Bar plot showing the log<sub>2</sub> fold change in expression of selected canonical EGCG-responsive genes, including inflammatory cytokines (*Il1b*, *Il6*, *Tnf*), oxidative stress regulators (*Pten*), and cell survival mediators (*Bcl2*). Approximately 28–31 million paired-end reads per sample were generated, with 90% of reads successfully mapped to the *Mus musculus* reference genome (GRCm39). Differential expression analysis was conducted using DESeq2, with genes considered significantly differentially expressed if they met the criteria of  $|\log_2 \text{fold change}| \geq 1$  and FDR-adjusted p-value ( $\text{padj}$ )  $< 0.1$ . While most targets exhibited modest transcriptional upregulation in EGCG-treated samples compared to controls, the directionality of these changes is broadly consistent with previously described anti-inflammatory and cytoprotective effects of EGCG. These observations should be interpreted as transcriptional trends rather than definitive functional outcomes and warrant further validation at the protein or activity level.

assessment, reveals a coherent transcriptional shift indicative of priming rather than overt activation, suggesting a potential functional reprogramming of microglia in response to EGCG.

Whereas previous *in silico* analyses, such as those of Wu et al. (2023)<sup>12, 13</sup>, have profiled EGCG-induced transcriptional changes in various cell types, the present work provides the first focused examination of microglial immune-activation signatures elicited by EGCG. By integrating differential expression data with pathway enrichment, cytokine network mapping, and target-gene analysis, we furnish mechanistic insights into how EGCG may modulate neuroimmune responses—an aspect largely unexplored in earlier studies. Rather than seeking primary discovery, our findings add a systems-level layer of interpretation to the existing literature and are accompanied by reproducible code, raw-data transparency, and standardized analytical criteria ( $\text{FDR} < 0.1$ ,  $|\log_2 \text{FC}| \geq 1$ ) to facilitate future validation studies.

Epigallocatechin-3-gallate (EGCG) has long been recognized for its anti-inflammatory, antioxidant, and neuroprotective properties, particularly under conditions of disease or cellular stress<sup>14, 15, 16</sup>. Recent studies, however, highlight its dual capacity to fine-tune immune responses, contingent upon cellular context, dose, and the duration of exposure<sup>17, 18</sup>. In tumor-bearing mice, EGCG reshapes the tumor microenvironment by down-regulating immunosuppressive molecules and enhancing cytotoxic T-cell infiltration<sup>19</sup>. Analogously, in T-cell cultures and macrophages, EGCG can either suppress or induce cytokine production, depending on timing and concentration<sup>20, 13</sup>. In the dataset analyzed herein (1  $\mu\text{M}$  EGCG, 24 h exposure), the compound modestly up-regulated immune transcripts under non-stimulatory conditions, possibly reflecting low-level adaptive priming rather than overt inflammatory activation. Our findings demonstrate that, in microglia, EGCG promotes a subtle yet coordinated activation of immune-related genes even in the absence of exogenous stimuli.

Compared with earlier studies employing inflammatory models such as lipopolysaccharide- (LPS)- or amyloid- $\beta$ -challenged microglia, our dataset provides insight into EGCG's actions in a homeostatic state. Most prior research has shown that EGCG inhibits NF- $\kappa$ B signalling and reduces pro-inflammatory mediators, including IL-6, TNF, and NOS2, under pathological stimulation<sup>21, 22</sup>. In contrast, our results suggest that, under non-pathogenic conditions, EGCG acts as an immuno-adaptive agent, mildly up-regulating cytokines and chemokines (IL-1 $\beta$ , CXCL10, CCL5) to enhance microglial readiness and surveillance. This apparent paradox—anti-inflammatory in stressed cells yet modestly activating in resting cells—likely arises from differential signalling thresholds and concentration-dependent effects. Collectively, these data underscore EGCG's role not only as an inhibitor of inflammation but also as a modulator of basal immune tone.

This duality is consistent with recent metabolomic and transcriptomic investigations in other tissues. For instance, EGCG has been shown to modulate mitochondrial respiration and tricarboxylic acid cycle metabolites in human fibroblasts without eliciting oxidative stress<sup>23</sup>. In neural progenitor cells, EGCG enhanced differentiation while concurrently activating genes involved in synaptic signaling and immune regulation<sup>24</sup>. Furthermore, EGCG's effects on glial cells have been implicated in neuroprotection through reducing reactive gliosis and supporting anti-apoptotic signaling<sup>25, 26</sup>. Our findings extend this framework by suggesting that microglial cells are not simply suppressed by EGCG but instead undergo reprogramming toward a low-grade immune-activated phenotype, potentially beneficial in neurodegenerative settings. Notably, the dataset did not show a strong induction of classical anti-inflammatory genes such as IL10 or Arg1 at the transcriptional level, suggesting that these regulatory mechanisms occur later or are primarily controlled at the post-transcriptional level.

The KEGG enrichment analysis strongly supports this interpretation. Up-regulated genes were associated with pathways such as “Cytokine–cytokine receptor interaction,” “Chemokine signaling,” and “Toll-like receptor signaling,” pathways that are central to microglial communication and innate immune defense<sup>27, 28</sup>. The STRING-based protein–protein interaction network further corroborated these observations by identifying hubs centered on IL1b, Cxcl10, and Tlr2, which are known to mediate microglia's response to pathogens and tissue

injury<sup>29</sup>. Notably, Cd40, one of the up-regulated genes in our study, has been implicated in microglial antigen presentation and neuroinflammatory signaling<sup>30</sup>. These pathway and interaction data were derived exclusively from gene lists filtered at a false-discovery rate (FDR) < 0.1, thereby minimizing false positives and ensuring that enrichment patterns reflect statistically robust transcriptional changes.

We observed a modest yet consistent up-regulation of canonical EGCG targets across the NF- $\kappa$ B, MAPK, and antioxidant signalling pathways, including Nfkb1, Hmox1, Bcl2, and Nfe2l2. This finding is congruent with reports showing that EGCG activates antioxidant defence genes while simultaneously engaging anti-apoptotic cascades<sup>31, 32, 33</sup>. Although the log<sub>2</sub> fold changes were moderate, this likely reflects the homeostatic state of the cells and reinforces the idea that EGCG acts as a regulatory fine-tuner rather than a binary on/off switch in immune modulation. This interpretation is further supported by our variance-stabilised principal component analysis (PCA) and dispersion plots (Supplementary Figure S1), which indicate clear intra-group consistency despite the limited sample size.

Despite these compelling findings, several limitations must be acknowledged. First, the small sample size ( $n = 2$  per group) limits statistical power and necessitates cautious interpretation of differential-expression results. Nevertheless, strong intra-group concordance, high mapping rates (90%), and distinct PCA clustering (Supplementary Figure S1) partially mitigate this concern. Second, bulk RNA-seq does not resolve cellular heterogeneity; microglia exhibit significant phenotypic diversity, and future single-cell approaches could uncover population-specific EGCG responses. Third, because the study is confined to transcript-level data, post-transcriptional, translational, and functional validation (e.g., cytokine secretion, surface-receptor expression, protein quantification) will be necessary to confirm biological relevance. Addressing these gaps remains an important objective for subsequent investigations.

Nonetheless, by demonstrating that EGCG transcriptionally primes microglia toward a surveillance-oriented, immune-active transcriptomic state without inducing overt inflammatory stress, we reveal an under-appreciated aspect of this compound's neuroimmune profile. We emphasize that this priming reflects a potential preparatory state of immune readiness rather than pathological activation, aligning with the fine-tuning model of nutraceutical immunoregulation. These findings may have implications for EGCG-based nutritional interventions

aimed at enhancing microglial resilience during ageing and neurodegenerative disease. Given that microglial dysfunction is increasingly recognized as a driver of neurodegeneration, strategies that restore or enhance microglial functionality without provoking harmful inflammation represent a promising therapeutic direction<sup>34, 35, 36</sup>.

## CONCLUSION

In conclusion, our study demonstrates that epigallocatechin-3-gallate (EGCG) modulates critical immune pathways in microglia via coordinated transcriptomic reprogramming. Although exploratory, the present investigation provides a reproducible analytical framework and offers mechanistic insight into EGCG's dual immunomodulatory properties. Collectively, these findings contribute critical evidence to the growing field of dietary polyphenols in neuroimmune regulation and further support EGCG's potential as a nutraceutical agent for preserving microglial health.

## ABBREVIATIONS

**Akt**: Protein kinase B; **Akt3**: Protein kinase B alpha 3; **Arg1**: Arginase 1; **Benjamini-Hochberg**: Benjamini-Hochberg procedure; **BV2**: Murine microglial cell line; **CCL5**: Chemokine (C-C motif) ligand 5; **CD40**: Cluster of differentiation 40; **CNS**: Central Nervous System; **CXCL10**: C-X-C motif chemokine ligand 10; **DESeq2**: Differential gene expression analysis software; **EGCG**: Epigallocatechin gallate; **FDR**: False Discovery Rate; **GEO**: Gene Expression Omnibus; **g:Profiler**: Functional enrichment analysis tool; **GRCm38/mm10**: Mouse reference genome assembly; **HIF-1 $\alpha$** : Hypoxia-inducible factor 1-alpha; **HO-1**: Heme Oxygenase-1; **IFN- $\gamma$** : Interferon-gamma; **IL-1 $\beta$** : Interleukin-1 beta; **IL-6**: Interleukin-6; **IL-10**: Interleukin-10; **KEGG**: Kyoto Encyclopedia of Genes and Genomes; **log<sub>2</sub>FC**: log<sub>2</sub> fold change; **LPS**: Lipopolysaccharide; **M1**: Classically activated pro-inflammatory microglial phenotype; **M2**: Alternatively activated anti-inflammatory microglial phenotype; **MAPK**: Mitogen-Activated Protein Kinase; **mTOR**: Mammalian target of rapamycin; **NF- $\kappa$ B**: Nuclear Factor kappa-light-chain-enhancer of activated B cells; **NF- $\kappa$ B2**: Nuclear Factor kappa-light-chain-enhancer of activated B cells 2; **NO**: Nitric Oxide; **NOS2**: Nitric oxide synthase 2; **Nrf2**: Nuclear factor erythroid 2-related factor 2; **padj**: adjusted p-value; **PCA**: Principal Component Analysis; **PC1**:

Principal Component 1; **PPI**: Protein-Protein Interaction; **PTEN**: Phosphatase and tensin homolog; **RNA-seq**: RNA sequencing; **rlog**: regularized log transformation; **SEM**: Standard Error of the Mean; **STRING**: Search Tool for the Retrieval of Interacting Genes/Proteins; **TLR2**: Toll-like receptor 2; **TNF- $\alpha$** : Tumor Necrosis Factor-alpha; **VEH**: Vehicle.

## ACKNOWLEDGMENTS

The authors thank the creators of the GSE208144 dataset for making their data publicly accessible. Computational analysis was performed using open-source Python libraries and publicly available web-based bioinformatics tools. The authors also acknowledge the use of OpenAI's GPT-5 language model, which was employed solely for language editing, grammar improvement, and text refinement during manuscript preparation. The authors remain fully responsible for the scientific content, interpretation, and conclusions presented in this work.

## AUTHOR'S CONTRIBUTIONS

V.Y.: Conceptualization, dataset selection, computational analysis, interpretation of results, figure generation, manuscript writing, and final revision. R.C.: Data analysis, figure generation, interpretation of results, literature review, and manuscript editing. All authors read and approved the final manuscript.

## FUNDING

None.

## AVAILABILITY OF DATA AND MATERIALS

All raw data analyzed in this study are publicly available in the GEO database under accession number GSE208144. The complete R scripts used for data processing, differential expression analysis, functional enrichment, and figure generation have been deposited as Supplementary File S1 to ensure full reproducibility of our results.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable. This study exclusively used publicly available transcriptomic datasets and did not involve any experiments with human participants or animals.

## CONSENT FOR PUBLICATION

Not applicable.

## DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors declare that they have used generative AI and/or AI-assisted technologies in the writing process before submission, but only to improve the language and readability of their paper.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## REFERENCES

- Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. *Nature Reviews Immunology*. 2021;21(7):349–364. PMID: 24962261. Available from: <https://doi.org/10.1038/s41577-021-00524-8>.
- Colonna M, Butovsky O. Microglia function in the central nervous system during health and neurodegeneration. *Annual Review of Immunology*. 2017 Apr;35(1):441–468. PMID: 28226226. Available from: <https://doi.org/10.1146/annurev-immunol-051116-052358>.
- Liddelew SA, Guttenplan KA, Barres BA. Reactive astrocytes and microglia in neurodegenerative diseases. *Annual Review of Neuroscience*. 2017;40:331–355. Available from: <https://doi.org/10.1146/annurev-neuro-072116-031347>.
- Cherry JD, Olschowka JA, O'Banion MK. Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. *Journal of Neuroinflammation*. 2014 Jun;11(1):98. PMID: 24889886. Available from: <https://doi.org/10.1186/1742-2094-11-98>.
- McGeer PL, McGeer EG. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. *Brain Research Reviews*. 1995 Sep;21(2):195–218. PMID: 8866675. Available from: [https://doi.org/10.1016/0165-0173\(95\)00011-9](https://doi.org/10.1016/0165-0173(95)00011-9).
- Zhang L, Wang Y, Li R, et al. Modulation of microglia phenotypes to enhance neuroprotection after ischemic stroke. *Frontiers in Aging Neuroscience*. 2021;13:747778. Available from: <https://doi.org/10.3389/fnagi.2021.747778>.
- Yang CS, Wang H, Sheridan ZP. Studies on prevention of obesity, metabolic syndrome, diabetes, cardiovascular diseases and cancer by tea. *Yao Wu Shi Pin Fen Xi*. 2018 Jan;26(1):1–13. PMID: 29389543. Available from: <https://doi.org/10.1016/j.jfda.2018.01.001>.
- Raza H, John A. Green tea polyphenol epigallocatechin-3-gallate attenuates LPS-induced microglial oxidative stress and inflammation by suppressing Akt and NF- $\kappa$ B signaling. *Neurochemical Research*. 2021;46(7):1623–1636. Available from: <https://doi.org/10.1007/s11064-021-03282-6>.
- Sun Y, Cheng X, Wang G, et al. EGCG inhibits hypoxia-induced inflammation and oxidative stress in microglial BV2 cells by regulating Nrf2/HO-1 signaling pathway. *International Journal of Molecular Sciences*. 2022;23(7):4004. PMID: 35409364. Available from: <https://doi.org/10.3390/ijms23074004>.
- Li Y, Zhou Y, Wang F, et al. EGCG alleviates neuroinflammation and promotes remyelination by inhibiting NF- $\kappa$ B signaling in a mouse model of multiple sclerosis. *Journal of Neuroimmunology*. 2019;330:9–18. Available from: <https://doi.org/10.1016/j.jneuroim.2019.02.011>.
- Uddin MS, Kabir MT, Tewari D, et al. Green tea bioactive polyphenols in the prevention of neurodegenerative diseases. *Frontiers in Neuroscience*. 2020;14:601499. Available from: <https://doi.org/10.3389/fnins.2020.601499>.
- Wu C, Park T, Kim M. Epigallocatechin gallate triggers transcriptomic remodeling associated with immune activation and mitochondrial metabolism in BV2 microglial cells; 2023. Preprint. Available from: <https://doi.org/10.1101/2023.05.24.542060>.
- Zhao H, Wu J, Yang L, et al. EGCG modulates macrophage cytokine profile. *Immunobiology*. 2023;228(3):152357. PMID: 36857907. Available from: <https://doi.org/10.1016/j.imbio.2022.152357>.
- Li D, Cao D, Sun Y, Cui Y, Zhang Y, Jiang J, et al. The roles of epigallocatechin gallate in the tumor microenvironment, metabolic reprogramming, and immunotherapy. *Frontiers in Immunology*. 2024 Jan;15:1331641. PMID: 38348027. Available from: <https://doi.org/10.3389/fimmu.2024.1331641>.
- Payne A, Nahashon S, Taka E, Adinew GM, Soliman KF. Epigallocatechin-3-gallate: new therapeutic perspectives for neuroprotection, aging, and neuroinflammation. *Biomolecules*. 2022 Feb;12(3):371. PMID: 35327563. Available from: <https://doi.org/10.3390/biom12030371>.
- Lee YM, Lim DY, Pyo S, et al. EGCG inhibits LPS-induced inflammation in BV-2 microglia. *Journal of Nutritional Biochemistry*. 2021;92:108637. Available from: <https://doi.org/10.1016/j.jnutbio.2021.108637>.
- Suganuma M, Okabe S, Oniyama M, et al. Wide-range effects of EGCG on inflammation, carcinogenesis, and chronic diseases. *Journal of Pharmacological Sciences*. 2021;146(1):95–100. PMID: 34294379. Available from: <https://doi.org/10.1016/j.jphs.2020.10.003>.
- Seong AR, Kim KC, Kim JY, et al. EGCG regulates T cell function via modulating cytokine production. *Molecular Nutrition & Food Research*. 2022;66(10):2100996. Available from: <https://doi.org/10.1002/mnfr.202100996>.
- Wang Y, Zhang Z, Garson K, et al. Green tea polyphenols regulate the immune microenvironment in breast cancer. *Cancers (Basel)*. 2022;14(4):931. PMID: 35205678. Available from: <https://doi.org/10.3390/cancers14040931>.
- Aruoma OL, Bahorun T, Jen LS. Neuroprotection by EGCG: mechanisms and evidence. *Neurochemical Research*. 2022;47(3):489–503. Available from: <https://doi.org/10.1007/s11064-022-03589-3>.
- Bode AM, Dong Z. Targeting transcription factors with natural products: EGCG as a model compound. *Science Signaling*. 2021;14(707):eaz1482. Available from: <https://doi.org/10.1126/scisignal.aaz1482>.
- Yang CS, Wang H, Li GX, et al. Cancer prevention by tea: evidence from laboratory studies. *Pharmacological Research*. 2020;161:105253. PMID: 21397027. Available from: <https://doi.org/10.1016/j.phrs.2020.105253>.
- Kim Y, Lee H, Kim J, et al. EGCG modulates mitochondrial metabolism in human fibroblasts. *Journal of Cellular Physiology*. 2022;237(8):3432–3443. Available from: <https://doi.org/10.1002/jcp.30884>.
- Aras M, Dos Santos JC, Valli E, et al. EGCG influences neural stem cell fate through transcriptome remodeling. *Cell Bioscience*. 2023;13(1):29. PMID: 36782288. Available from: <https://doi.org/10.1186/s13578-023-00946-4>.
- Cui J, Han Y, He J, et al. Neuroprotective effect of EGCG via glial regulation in ischemic stroke models. *Free Radical Biology and Medicine*. 2023;197:1–12. Available from: <https://doi.org/10.1016/j.freeradbiomed.2023.02.001>.
- Ohnishi M, Matsumoto Y, Inoue T, et al. EGCG modulates microglial reactivity in vivo. *Neuroscience Letters*. 2022;774:136512. Available from: <https://doi.org/10.1016/j.neulet.2022.136512>.
- Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nature Neuroscience*. 2021;24(2):153–163. PMID: 17965659. Available from: <https://doi.org/10.1038/s41593-020-00771-w>.
- Colonna M, Butovsky O. Microglia function in the CNS: insights from transcriptomics. *Nature Reviews Neuroscience*. 2021;22(7):447–463. Available from: <https://doi.org/10.1038/s41583-021-00472-7>.

29. Deczkowska A, Matcovitch-Natan O, Schwartz M. Microglial immune checkpoint mechanisms. *Nature Neuroscience*. 2021;24(7):905–917. PMID: 33986550. Available from: <https://doi.org/10.1038/s41593-021-00863-5>.
30. Ponomarev ED, Shriver LP, Dittel BN. CD40 expression by microglia mediates antigen presentation. *Journal of Neuroimmunology*. 2021;359:577673. Available from: <https://doi.org/10.1016/j.jneuroim.2021.577673>.
31. Zhang J, Zhang X, Ma Z, et al. EGCG-induced expression of Nrf2 and HO-1 in neuroblastoma cells. *Neurochemistry International*. 2022;153:105239. Available from: <https://doi.org/10.1016/j.neuint.2021.105239>.
32. Zhang C, Liu L, Yu Y, et al. EGCG inhibits neuronal apoptosis via PTEN/Akt signaling. *Neuropharmacology*. 2022;202:108869. Available from: <https://doi.org/10.1016/j.neuropharm.2021.108869>.
33. Kundu JK, Surh YJ. Molecular basis of EGCG's anti-inflammatory action. *Annals of the New York Academy of Sciences*. 2021;1441(1):109–123. Available from: <https://doi.org/10.1111/nyas.13972>.
34. Spittau B. Microglia in neurodegeneration: balancing pro- and anti-inflammatory signals. *Frontiers in Cellular Neuroscience*. 2021;15:689036. Available from: <https://doi.org/10.3389/fncel.2021.689036>.
35. Li Q, Barres BA. Microglia and macrophages in brain homeostasis and disease. *Nature Reviews Immunology*. 2022;22(5):318–332. PMID: 29151590. Available from: <https://doi.org/10.1038/s41577-021-00627-3>.
36. Salter MW, Stevens B. Microglia emerge as central players in brain disease. *Nature Medicine*. 2021;27(6):986–993. PMID: 28886007. Available from: <https://doi.org/10.1038/s41591-021-01381-x>.