Dose-dependent Epigallocatechin Gallate Treatment in HeLa cells Suppressed the Pro-inflammatory Cytokine *IL1A*

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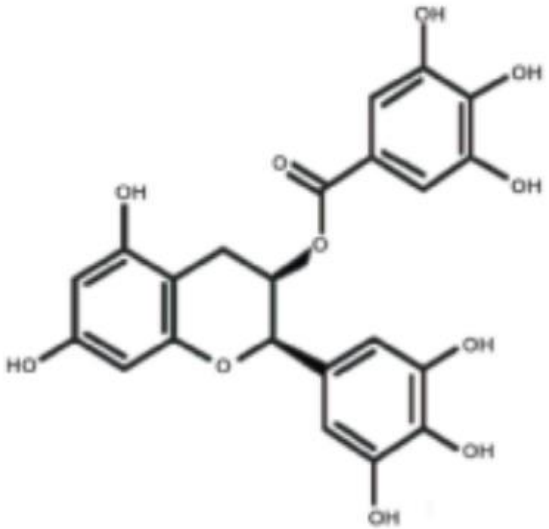
BACKGROUND

- Epigallocatechin gallate (EGCG) is a polyphenol, found mainly in green tea
- It is known for its antioxidant, anti-inflammatory, and potential anticancer properties
- Despite promising results in earlier studies, EGCG's role in regulating gene expression related to inflammation and tumor suppression remains unclear, especially in HeLa cervical cancer cells



The chemical structure of EGCG.

RATIONALE

- Interleukin-1-alpha (*IL1A*) and Interleukin-6 (*IL6*) are cytokines involved in inflammation and immune responses. In cancer, they can promote tumor growth and tumor survival

- *TP53* is a tumor suppressor that regulates the cell cycle and triggers apoptosis in damaged cells

Thus, these pro-inflammatory cytokines and tumor suppressor gene were chosen to investigate their expression changes in response to EGCG treatment in HeLa cells

OBJECTIVES

- To investigate how EGCG affects the expression of *IL1A*, *IL6*, and *TP53* genes in HeLa cells
- To determine the optimal concentration of EGCG that causes gene expression changes without reducing cell viability
- To identify whether EGCG has anti-inflammatory or tumor-suppressive effects at optimal concentrations

MATERIALS AND METHODS

❑ CELL CULTURE:

- HeLa cells were cultured in minimum essential medium and treated with EGCG at concentrations of 5, 10, 20, and 40 µg/ml during the optimization experiment. For gene expression analysis, 5 and 10 µg/ml concentrations were selected based on maintained cell viability. DMSO was used as a negative control

❑ CELLULAR MORPHOLOGY:

- It was assessed under a light microscope after 48 hours of EGCG treatment to evaluate cell viability

❑ QUANTITATIVE PCR (qPCR):

- Total RNA was extracted and converted into cDNA. qPCR was then performed to measure the expression levels of *IL1A*, *IL6*, and *TP53*. Expression was normalized to the housekeeping gene *PPIG*, due to its stable expression across all samples

❑ DATA ANALYSES:

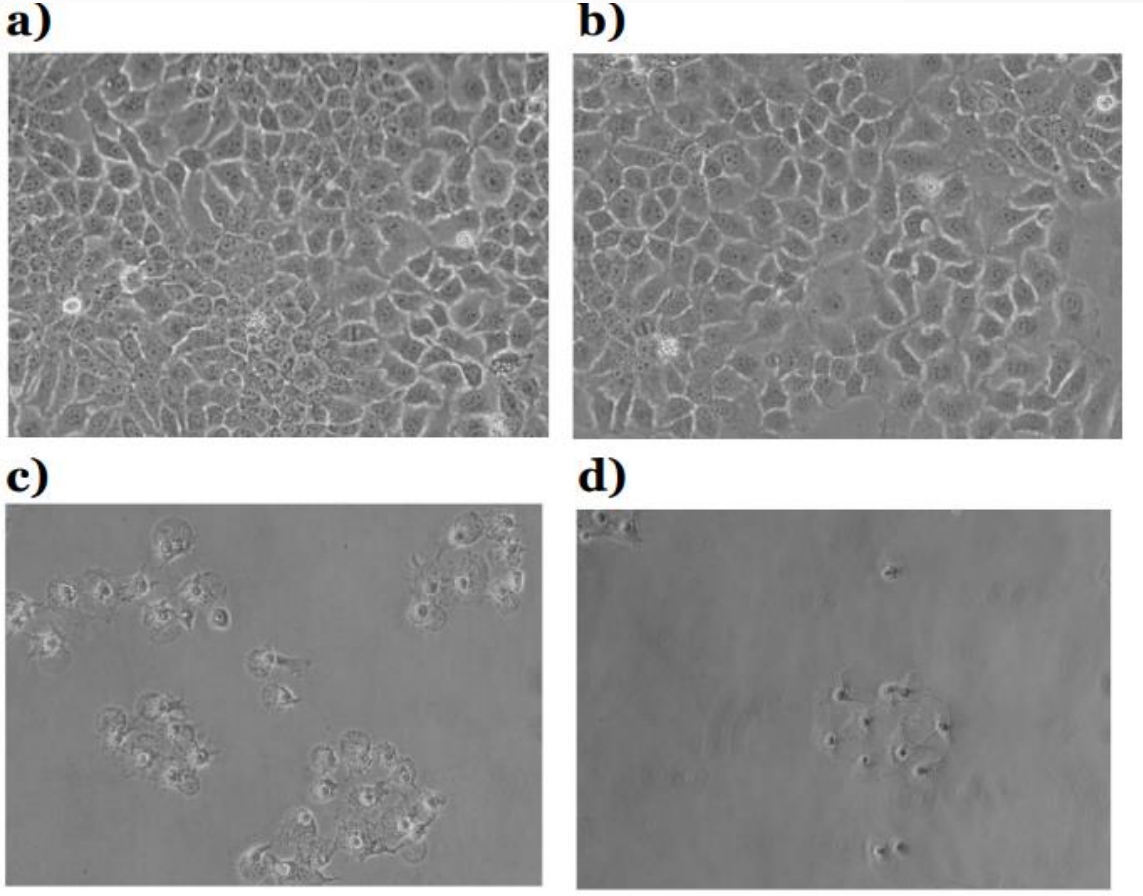
- Relative gene expression changes were calculated using the ΔΔCt method. All statistical analyses - relative fold change values of qPCR data and barplot visualizations were performed using R software

RESULTS

Optimization experiment

- The optimization experiment was performed to identify EGCG concentrations suitable for maintaining cell viability during subsequent gene expression analyses.
- Cell morphology images confirmed that 5 µg/ml and 10 µg/ml EGCG preserved cell viability, while higher concentrations (20 µg/ml and 40 µg/ml) caused visible cell stress and shrinkage.

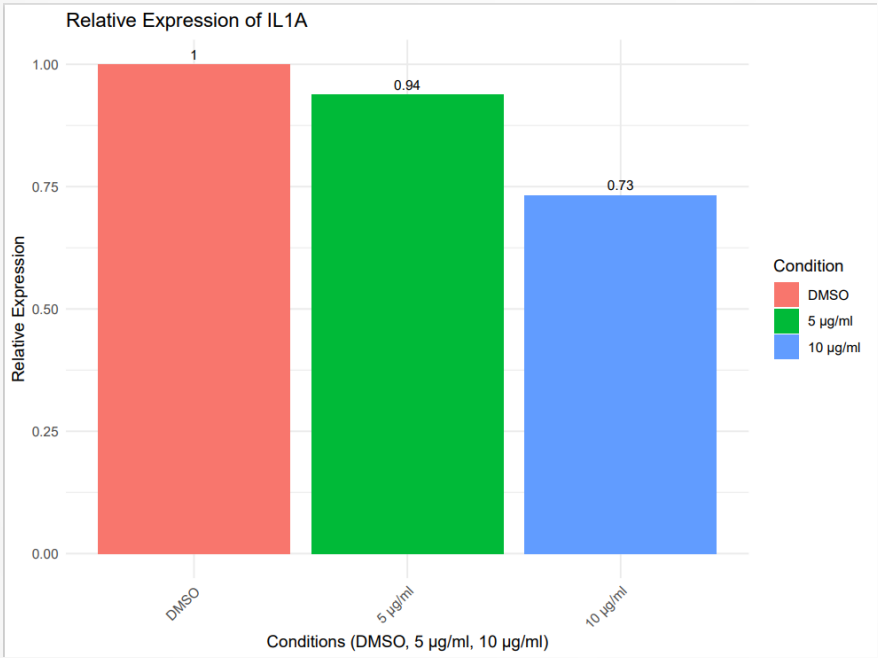
Therefore, concentrations 5 µg/ml and 10 µg/ml EGCG were chosen for further experiments.



Day 2 morphology pictures of HeLa cells that were cultured in the (a) 5µg/ml (b) 10µg/ml (c) 20µg/ml and (d) 40µg/ml EGCG concentrated media.

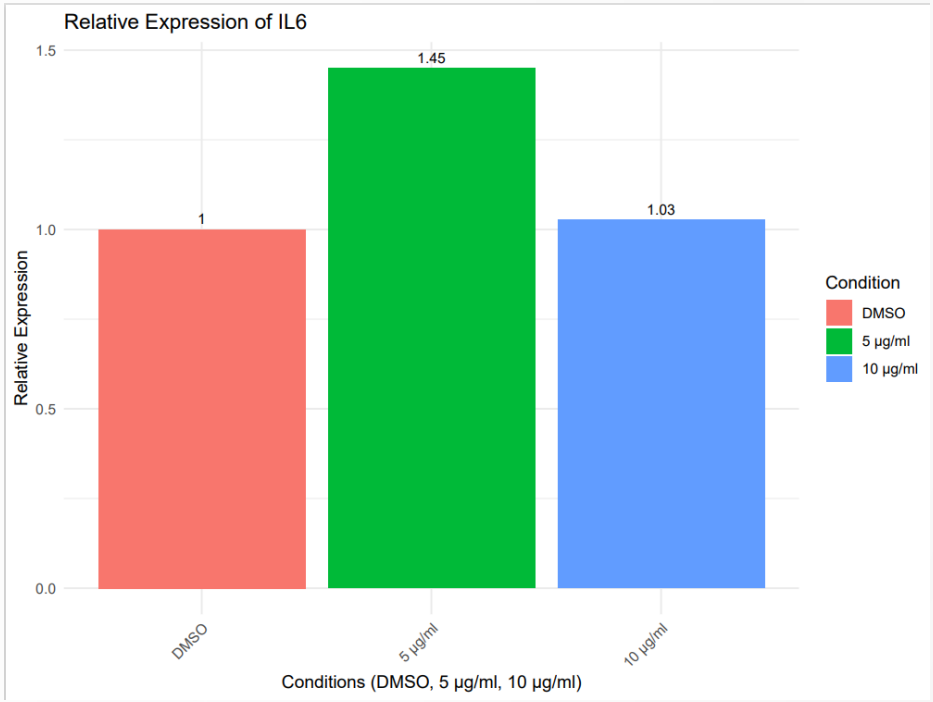
EGCG modulates the expression of cytokines *IL6* and *IL1A*

IL1A was slightly downregulated at 5 µg/ml (0.94-fold) and more clearly suppressed at 10 µg/ml (0.73-fold) relative to DMSO control



Fold change of gene expression levels of *IL1A* in HeLa cells treated with different EGCG concentrations in triplicates along with DMSO.

IL6 was notably upregulated at 5 µg/ml (1.49- fold) but returned to baseline at 10 µg/ml (1.03- fold)

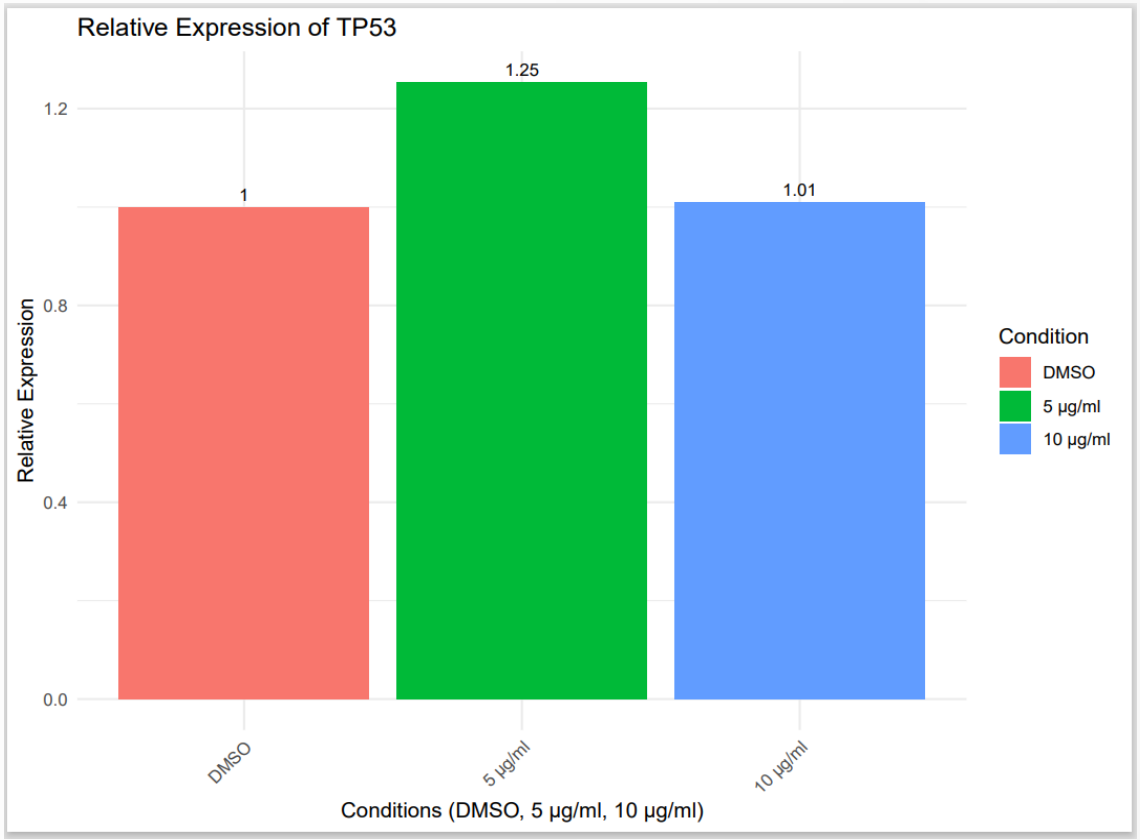


Fold change of gene expression levels of *IL6* in HeLa cells treated with different EGCG concentrations in triplicates along with DMSO.

EGCG upregulates the expression of *TP53*

To evaluate EGCG's anti-inflammatory and tumor-suppressive potential, qPCR was used to measure IL1A, IL6, and TP53 gene expression in HeLa cells.

- ***TP53***, a tumor suppressor gene, was upregulated at 5 µg/ml (1.25-fold), with minimal change at 10 µg/ml (1.01-fold).



Fold change of gene expression levels of *TP53* in HeLa cells treated with different EGCG concentrations in triplicates along with DMSO.

DISCUSSION

The results showed that EGCG regulates gene specific and dose-sensitive effects on *IL1A*, *IL6*, and *TP53* expression.

- *IL1A* suppression at higher dose suggests potential anti-inflammatory activity, though the effect at 5 µg/ml was mild.
- *IL6* upregulation at low dose may reflect activation of stress-related or signaling pathways that are no longer triggered at higher concentrations.
- *TP53* upregulation at 5 µg/ml indicates activation of tumor-suppressive mechanisms, possibly due to mild cellular stress. Lack of stronger response at 10 µg/ml suggests a plateau or feedback regulation in gene expression.

Results support the idea that low-dose EGCG has more pronounced biological effects in HeLa cells.

CONCLUSIONS

- EGCG treatment affected gene expression in a concentration-dependent way. At 5 µg/ml, the most notable changes were seen in gene expression, including increased *TP53* and *IL6* and decreased *IL1A* expression. Whereas, at 10 µg/ml, the effects were weaker or absent.

- These results suggest that low dose EGCG may exert anti-inflammatory and tumor-suppressive effects through gene expression modulation, warranting further investigation into its molecular mechanisms and therapeutic potential.