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

BACKGROUND

Epigallocatechin gallate (EGCG) is a green tea polyphenol with antioxidant, anti-inflammatory, and anti-cancer properties (1,2). While previous studies have shown promising effects (3,4), its influence on gene expression related to inflammation and tumor suppression in HeLa cells, a human cervical cancer cell line, remains unclear.

To investigate this, I focused on three gene targets to assess EGCG's potential anti-inflammatory and tumor-suppressive effects:

- Interleukin-1-alpha (IL1A)
- Interleukin 6 (*IL6)*

IL1A and *IL6* pro-inflammatory cytokines involved in immune responses and cancer progression (5–7)

- *TP53,* tumor suppressor gene, a tumor suppressor gene that regulates cell cycle arrest and apoptosis (8).

METHODOLOGY

Cell culture: HeLa cells were cultured in minimum essential medium and treated with EGCG at 5, 10, 20, and 40 µg/ml. Based on cell viability, 5 and 10 µg/ml were used for gene expression analysis. DMSO served 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 analysis: 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

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) visible cell stress and shrinkage as seen in Figure 1.

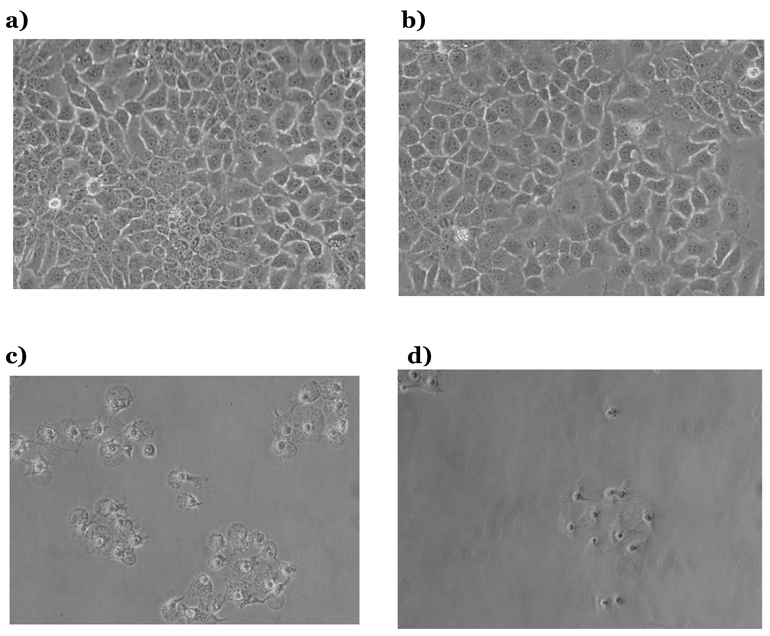

Figure 1: Day 2 morphology pictures of HeLa cells that were cultured in the **(a)** 5µg/ml **(b)** 10µg/ml **(c)** 20µg/ml and **(d)** 40µg/ml EGCG concentrated media

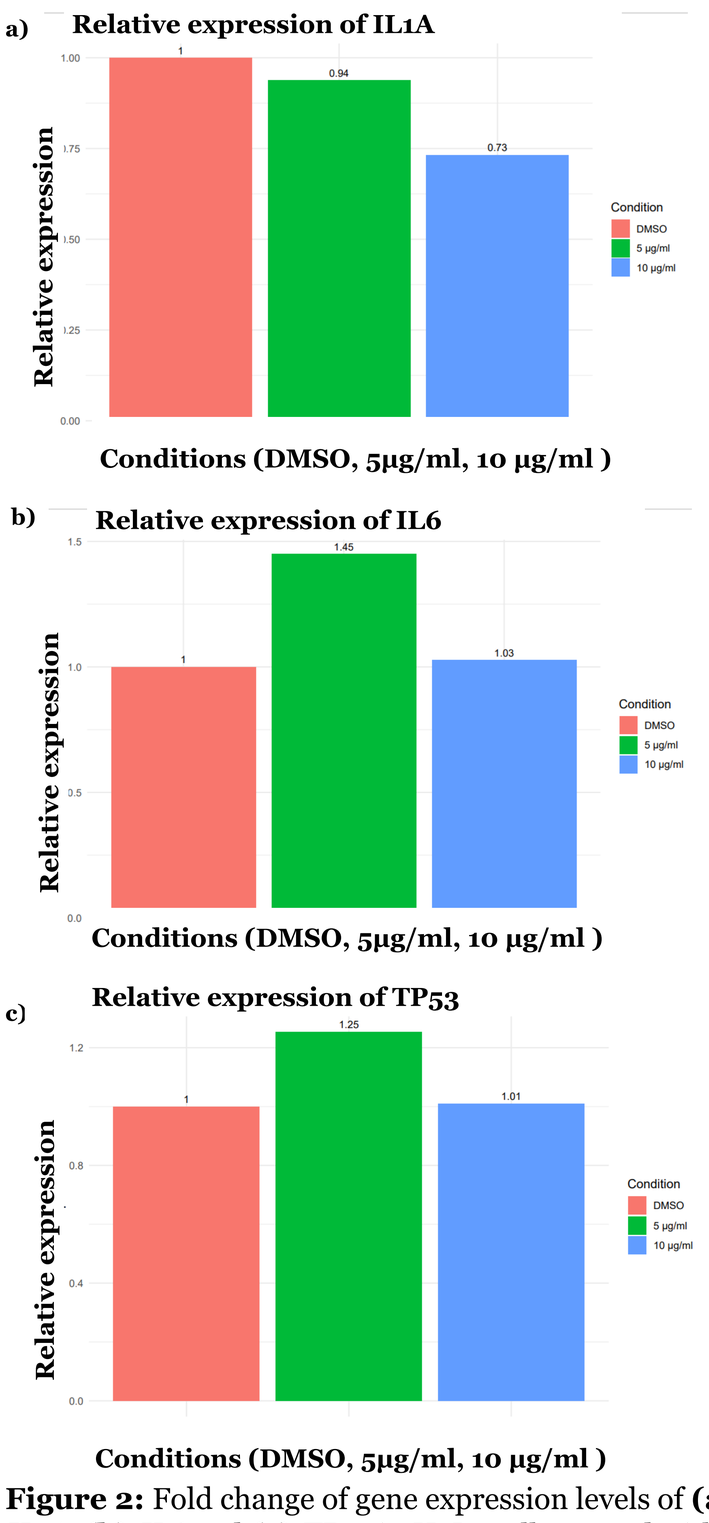

Figure 2: Fold change of gene expression levels of **(a)** *IL1A*, **(b)** *IL6* and **(c)** *TP53* in HeLa cells treated with different EGCG concentrations along with DMSO.

qPCR analysis revealed gene-specific, dose-dependent effects of EGCG on *IL1A*, *IL6*, and *TP53* expression in HeLa cells:

- ***IL1A*** was slightly downregulated at 5 µg/ml (0.94-fold) and more clearly suppressed at 10 µg/ml (0.73-fold), suggesting potential anti-inflammatory effects at higher doses.
- ***IL6*** was upregulated at 5 µg/ml (1.49-fold), possibly reflecting early stress signaling, but returned to baseline at 10 µg/ml (1.03-fold).
- ***TP53*** was upregulated at 5 µg/ml (1.25-fold), indicating activation of tumor-suppressive mechanisms, while expression plateaued at 10 µg/ml (1.01-fold).

These findings suggest that low-dose EGCG (5 µg/ml) elicits more pronounced biological effects in HeLa cells, particularly through modulation of inflammation- and tumor-related gene expression.

CONCLUSIONS

- EGCG treatment altered gene expression in a concentration-dependent manner.
- At 5 µg/ml, notable effects were observed:
  - *TP53* and *IL6* expression increased
  - *IL1A* expression decreased
- At 10 µg/ml, gene expression changes were weaker or absent.
- These findings suggest that low-dose EGCG may have anti-inflammatory and tumor-suppressive effects by modulating gene expression.
- Further studies are needed to explore its molecular mechanisms and therapeutic potential.

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