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Original Research

The Dual Mechanistic Actions of Natural Compounds CAPE and EGCG Targeting VEGF and PI3K/Akt/mTOR Pathways in Lung Cancer

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ABSTRACT

Objective: Lung cancer is characterized by uncontrolled proliferation, loss of apoptosis, angiogenesis, and metastatic potential. Caffeic acid phenethyl ester (CAPE), the major bioactive molecule of propolis, and epigallocatechin gallate (EGCG), a essential polyphenol of green tea, have documented antioxidant, anti-proliferative, and anti-angiogenic effects. This study aimed to investigate the effects of EGCG and CAPE on VEGF and the PI3K/Akt/mTOR signaling pathway, key regulators of lung cancer progression, using in silico and in vitro models.

Methods: This study Lung cancer cells (DSMZ/ACC-234) was conducted to demonstrate the inhibition of cell proliferation by the MTT method, molecular binding via autodock vina and protein expression by immunohistochemistry analysis, targeting the VEGF and PI3K/Akt/mTOR pathways with CAPE and EGCG treatment.

Results: EGCG and CAPE treatments significantly reduced cell proliferation in lung cancer cells. Untreated cells exhibited strong to very strong immunoreactivity for VEGF and PI3K/Akt/mTOR pathway markers, whereas EGCG reduced these signals to mild–moderate levels and to CAPE moderate–strong levels. Since both treatments resulted in a statistically significant increase in mTORC1 immunoreactivity, molecular docking targeting was performed via it.

Conclusion: EGCG and CAPE exhibit significant anti-proliferative and anti-angiogenic effects by adjusting the VEGF and PI3K/Akt/mTOR signaling pathways in lung cancer cells. mTORC1 activation molecular binding affinity is higher in the CAPE-treated group compared to the EGCG group. These findings highlight the therapeutic importance of targeting these pathways in developing novel treatment strategies for lung cancer.

Keywords: EGCG, CAPE, VEGF, PI3K/Akt/mTOR, signaling pathway

INTRODUCTION

Lung cancer (LC) continues to pose a significant global health challenge, with notable geographical and demographic differences in its incidence. Recent epidemiological reports indicate that, in some countries such as the United States, adenocarcinoma incidence among young women has reached or exceeded that of their male counterparts [1].

Accounting for nearly 85% of all LC diagnoses, non-small cell lung cancer (NSCLC) is the largest component and is mainly composed of adenocarcinoma and squamous cell carcinoma [2]. Although radiotherapy is a corner stone of lung cancer management, the inherent radioresistance of lung tumors often requires dose escalation, thereby heightening the risk of injury to adjacent healthy lung tissue [3, 4].

Current pharmacological approaches are similarly limited in their effectiveness. Chemotherapeutic regimens are frequently associated with considerable systemic toxicities, including neurological, gastrointestinal, and cardiovascular complications [5]. Targeted drugs, while effective in selected molecularly defined patient groups, may cause severe adverse events such as irreversible pulmonary fibrosis [6]. Immunotherapies benefit only a subset of individuals and often encounter the challenge of secondary resistance [7]. These limitations highlight the critical need for safer, more effective, and broadly applicable therapeutic alternatives for LC. This has amplified the interest in natural compounds with potential anticancer activity.

Caffeic acid phenethyl ester (CAPE), the principal bioactive phenolic constituent of propolis, exhibits cytotoxic effects in multiple cancer types, including breast, ovarian, prostate, and colon cancers [8]. In addition to its antitumor activity, CAPE has demonstrated radioprotective properties by downregulating inflammatory pathways such as Nrf2 and NF-κB in both cellular and animal models [9-11].

Similarly, epigallocatechin gallate (EGCG), a major polyphenol derived from green tea, exerts pleiotropic anti-cancer effects. It suppresses tumor cell proliferation, induces apoptosis, modulates autophagy, and enhances the efficacy of chemotherapeutic agents in a wide spectrum of cancers including lung, ovary, pancreatic, stomach, lymphoma, and myeloma [12, 13]. It suppresses cancer cell growth and proliferation, induces apoptotic cell death, and inhibits the expression of multiple proteins essential for tumor progression. Furthermore, EGCG has been reported to enhance the efficacy of chemotherapeutic agents by stimulating cell signaling initiators such as AMPK, and to act by limiting cancer cell growth and metastasis through autophagic processes [14]. According to earlier research, EGCG suppresses the activation of the PI3K/Akt signaling pathway, which causes apoptosis in human pancreatic and colon cancer cells [15, 16].

The PI3K signaling pathway is a key regulator of cancer progression, as it enhances cell survival, inhibits apoptotic processes, and promotes angiogenesis within the tumor microenvironment. Vascular endothelial growth factor (VEGF), which promotes neovascularization and triggers PI3K/Akt signaling to boost endothelial cell proliferation and vasculogenic mimicry, is a crucial mediator of this process [17]. This reciprocal interaction between VEGF and PI3K creates a reinforcing loop that supports tumor growth, metastasis, and the metabolic demands of rapidly dividing cancer cells. Because VEGF-mediated activation of the PI3K/Akt/mTOR pathway is a critical driver of angiogenesis, therapeutic approaches targeting this signaling axis have emerged as a central focus in the development of more effective anticancer strategies [18].

This study aims to evaluate the effects of varying concentrations of CAPE and EGCG on the proliferative capacity and angiogenic potential of ACC-234 lung cancer cells, while elucidating the underlying molecular mechanisms. The findings are intended to provide a theoretical basis for the potential therapeutic application of CAPE and EGCG in lung cancer management.

Main Points

- CAPE and EGCG markedly suppress cell proliferation in lung cancer cells.
- Both compounds effectively modulate the VEGF and PI3K/Akt/mTOR signaling pathways that drive tumor progression.
- Molecular docking analyses revealed a higher binding affinity of CAPE to mTORC1 compared with EGCG.
- These results underscore the therapeutic potential of natural compounds as targeted strategies for lung cancer treatment.

MATERIALS AND METHODS

Cell Culture

Cell lines for lung cancer (ACC 234) were acquired from DSMZ (Braunschweig, Germany). RPMI-1640(Capricorn, Germany) liquid raw medium was prepared by adding fetal bovine serum (FBS; Gibco, Life Technologies Corporation, Paisley, UK) at a final concentration of 10% (v/v), penicillin-streptomycin (Gibco, Life Technologies, Grand Island, USA) at a concentration of 1%, and 100 µl of amphotericin-B (Gibco, Life Technologies Limited, Paisley, UK) to a volume of 500 ml. This prepared mixture was sterilized by filtering it through a 0.22 µm bottle-top filter and stored at +4°C. ACC 234 cell culture was maintained at 37°C, 5% carbon dioxide, and 95% humidity in an incubator. Once the cells reached 70–80% confluency, they were transferred to new flasks after being detached from the flask surface using trypsin (0.05%)-EDTA (0.02%) solution (Gibco).

CAPE and EGCG Treatment

Caffeic acid phenethyl ester (CAPE) (Santa Cruz, CA, USA) was mixed in dimethyl sulfoxide (DMSO) to create a 1 mM stock solution, which was used to control cell growth. The final working concentrations of 50, 100, and 150 µM were obtained by diluting the stock solution with full culture media [19]. The final working concentrations of 10, 30, and 50 µM were obtained by diluting the 1 mM stock solution of epigallocatechin gallate (EGCG) (Sigma-Aldrich, Germany) with complete culture media after it had been dissolved in dimethyl sulfoxide (DMSO)[20]. The ideal dosage and duration of CAPE and EGCG treatment for ACC 234 cells were determined in part by the MTT assay results.

Cell Viability

An MTT assay (3-(4,5 - dimethylthiazol - 2 - yl) - 2,5 - diphenyltetrazolium bromide) was performed to analyze cell viability. MTT (Gold Biotechnology Inc., St. Louis, MO, USA) was dissolved in a PBS solution (5 mg/mL) and filtered via a 0.22 µm pore size filter. ACC-234 cells was seeded in sterile 96-well plates at a density of 5 × 103 cells per 100 µL of medium. After culturing the cells overnight to ensure adhesion, they were treated with cell culture medium containing 1 µL/mL DMSO, CAPE (50, 100 and 150 µM) or EGCG (10, 30 and 50 µM) and then incubated for 48 hours.The incubation medium was removed, and 90 µL of the fresh medium along with 10 µL of MTT were added to each well. Following 2 h incubation at 37 °C, the purple formazan crystals that had formed were dissolved by the addition of 100 µL of DMSO. Absorbance was measured at 570 and 690 nm using a MicroQuant plate reader (Bio-Tek Instruments Inc., Charlotte, VT, USA). The absorbance from the control group was considered as 100% cell viability, and cell viability (%) was calculated using the formula: Cell viability (%) = (Abs sample/Abs control) × 100.

Five different experimental groups were created according to the MTT cell viability results: (1) Control (only cell+ medium for 48 h); (2) DMSO (cells incubated with 1 µL/mL DMSO for 24 h); (3) CAPE (150 µM, 48 h), and (4) EGCG (50 µM, 48 h).

Immunostaining

Eight-well cell culture slides were seeded with approximately 1 x 10^5 cells per well and incubated overnight at 37°C in a 5% CO_2 environment. After the cells reached approximately 70% density, CAPE and EGCG were added to the culture medium, and the cells were incubated for 48 hours. After incubation, the cells were washed with cold PBS and fixed with 4% paraformaldehyde for ten minutes. Following further washes with PBS, 5% normal goat serum was used for blocking after permeabilization with 0.2% Triton X-100 for 30 minutes. Cells were then incubated overnight at 4°C with primary antibodies (all diluted 1:200) against VEGF (Sc-9013, Santa Cruz Biotech, Heidelberg, Germany), PI3K (BT-AP07136, BT Bio Tech. Lab, China), phospho-Akt (649001, BioLegend Discovery), mTORC1 (Sc-27744, Santa Cruz Biotech, Germany), and mTORC2 (Sc-130865, Santa Cruz Biotech, Germany). After PBS washes, appropriate biotinylated secondary antibodies and streptavidin-peroxidase were applied, each for 30 minutes. Diaminobenzidine was used as the chromogen, and Mayer's hematoxylin as the counterstain. Slides were prepared and examined under a light microscope.

We calculated H-scores [21] using a semiquantitative assessment of both theintensity of staining and the percentage of positive cells. The intensity of staining was assessed semiquantitatively as: 0,no staining; 1, weak staining; 2, moderate staining; 3, strongstaining. Both the ratio of immunopositive cells to all cancercells in the selected fields and the respective H-scores werecalculated. More than 200 cancer cells were counted foreach group for each antibody and the H-scores werecalculated by adding the percentage of strongly stainednuclei (3), the percentage of moderately stained nuclei (2),and the percentage of weakly stained nuclei (1) fora possible range of 0–200.

Protein Preparation

The methods employed in this study integrate molecular docking with network-based systems analysis to prioritize targets and interpret binding-related pathways. The three-dimensional (3D) structure of mTORC1 (PDB ID: 7YRJ) was retrieved from the Protein Data Bank (PDB) and prepared prior to docking. B chain of the protein was left untouched.

Ligand Preparation and Optimization

The canonical SMILES representations of CAPE (PubChem ID: 5281787) and EGCG (PubChem ID: 65064) were retrieved from the PubChem(https://pubchem.ncbi.nlm.nih.gov/) (accessed on September 12, 2024) database. These molecular descriptors were used to define the chemical structures of the ligands in a standardized and reproducible format. Following retrieval, the SMILES strings were uploaded to the CB Dock platform, where they were automatically converted into three-dimensional ligand conformations suitable for molecular docking. This approach ensured consistent ligand preparation and enabled reliable evaluation of the binding interactions between CAPE, EGCG, and the target protein during the docking simulations.

Docking Protocol

Next, the ligand and protein were uploaded, and molecular docking was performed using CB-DOCK2[22]. We uploaded the mTORC1 protein PDB file and the ligand(CAPE and EGCG) SDF file to CB-DOCK2, selecting the "auto blind docking" mode to obtain the docking results. CB-DOCK2 conducts fully automatic protein–ligand blind docking calculations in four steps: (1) input and quality check of protein and ligand structural data; (2) preprocessing of structural data, including the repair of missing atoms and hydrogen atoms, as well as the removal of water molecules and other heteroatoms; (3) protein pocket detection, spatial parameter estimation, and molecular docking on the basis of Vina scoring and template matching with conformational transfer; and (4) comprehensive processing and visualization of the calculation results. The results were then visualized in two dimensions via Discovery Studio 2019 (https://www.3ds.com/products/) and Maestro 12.8 software (https://www.schrodinger.com/platform/products/maestro/). To further valida te the docking results, we also used AutoDock Tools 1.5.7 and AutoDock Vina for additional analysis.

Statistical Analysis

Immunohistochemical staining was evaluated using H-scores. The H-score results were analyzed using one-way ANOVA by Tukey-Kramer multiple comparisons testusing GraphPad Software (San Diego, CA). Values for $p \leq 0.05$ were considered statistically significant.The specific statistical tests applied to each measurement are described in detail in the corresponding figure and table legends. Prior to inter group comparisons, data distribution was assessed using normality tests. When the data did not meet the assumptions of normal distribution, appropriate nonparametric statistical tests were applied**.**

RESULTS

Cell Viability Analysis

Cell viability analysis was performed on ACC 234 lung cancer cells following 48 hours of CAPE (50, 100, 150 μM) and EGCG (10, 30, and 50 μM) treatment (Figure 1). Cell viability in lung cancer cells decreased significantly at 100 and 150 μM CAPE doses compared to the control and other groups at 48 hours. However, significant differences were also observed between the 100 and 150 μM doses (Figure 1-A).

The vitality values of lung cancer cells were considerably decreased by all EGCG doses given for 48 hours. When lung cancer cells were treated with 30 and 50 μM EGCG for 48 hours, their viability was much lower than that of the control group. Compared to the other groups, 50 μM EGCG administration for 48 hours dramatically decreased cell growth in ACC 234 cells (Figure 1-B).

VEGF, VEGFR1 and VEGFR2 Levels in ACC 234 lung cancer Cells

Figures 2-A show VEGF, VEGFR1 and VEGFR2 immunohistochemistry staining in ACC 234 lung cancer cell line. Immunohistochemical staining quantification (Figure 2-B) of VEGF, VEGFR1, and VEGFR2 proteins was significantly decreased in both CAPE (150 μM) and EGCG (50 μM) groups compared to the control group. VEGF stimulation was significantly decreased in the EGCG group compared to the CAPE group.

PI3K, p-AKT, mTORC1 and mTORC2 Levels in ACC 234 lung cancer Cells

Figure 3-A shows immunohistochemical staining of PI3K, p-AKT, mTORC1, and mTORC2 protein levels in lung cancer cells after CAPE and EGCG administration.

When comparing the CAPE and EGCG groups to the control group, there was a substantial decrease in the quantitative

determination of PI3K immunohistochemistry staining. The EGCG group's PI3K protein levels were considerably lower than those of the CAPE group.

The CAPE and EGCG groups' p-AKT immunohistochemical staining measures were significantly lower than those of the control group. The EGCG group's p-AKT protein levels were considerably lower than those of the CAPE group.

In comparison to the control group, the quantitative measurement of mTORC1 immunohistochemical staining was considerably lower in the CAPE and EGCG groups. The EGCG group's mTORC1 protein level dramatically dropped, particularly when compared to the CAPE and control groups.

Quantitative determination of mTORC2 immunohistochemical staining was significantly decreased in the CAPE and EGCG groups compared to the control group. There is no difference in mTORC2 protein levels between CAPE and EGCG applied groups.

Docking analysis of CAPE with mTORC1

Ten binding positions of the ligand were chosen on the protein surface, and the docking outcome is presented in Table 1. With a binding affinity of -7.0 kcal/mol for both L.B. and U.B. and an RMSD value of 0.000, indicating a steady binding interaction, the first binding location (ID: 1) had the highest docking score. The other binding positions had docking scores between -6.6 kcal/mol and -5.9 kcal/mol. Detailed images for the ligand-protein interaction concerning affinity, basic hydrogen bonds, and van der Waals interactions of CAPE with mTORC1 protein are provided in Figures 4-A and 4-B. The images describe the most stable binding position and provide information about the task-domain amino acid residues participating in the interaction.

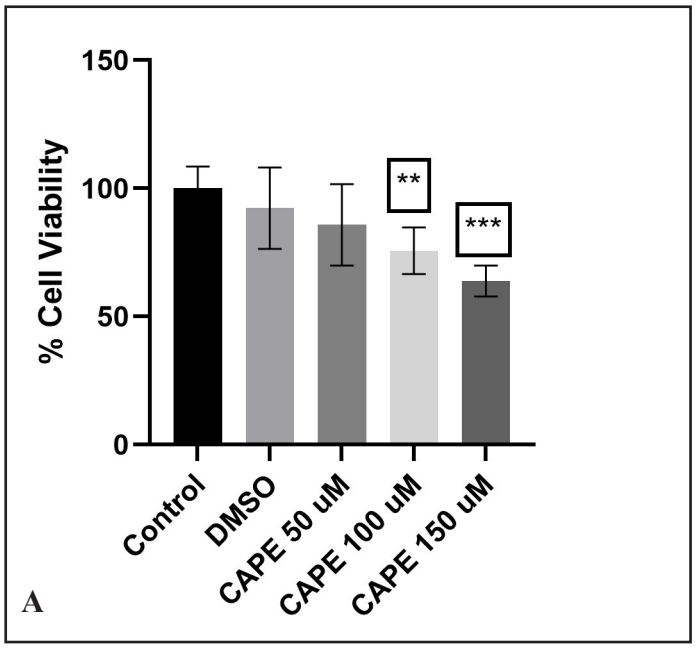

Figure 1-A. Evaluation of cell viability by MTT analysis for 48 hours in ACC-234 cells. Cells treated with Caffeic acid phenethyl ester (CAPE, 50, 100 and 150 µM) and DMSO, dimethyl sulfoxide (1µ/ml). Data are representative of 7-8 separate experiments and values are given as mean ± SD. Statistical analysis was performed by two-way ANOVA and differences between groups were determined by Tukey's multiple comparison test. **, p<0.050, when compared with the control group at the same time periods. ***, p<0.001, when compared with all other groups at the same time period.

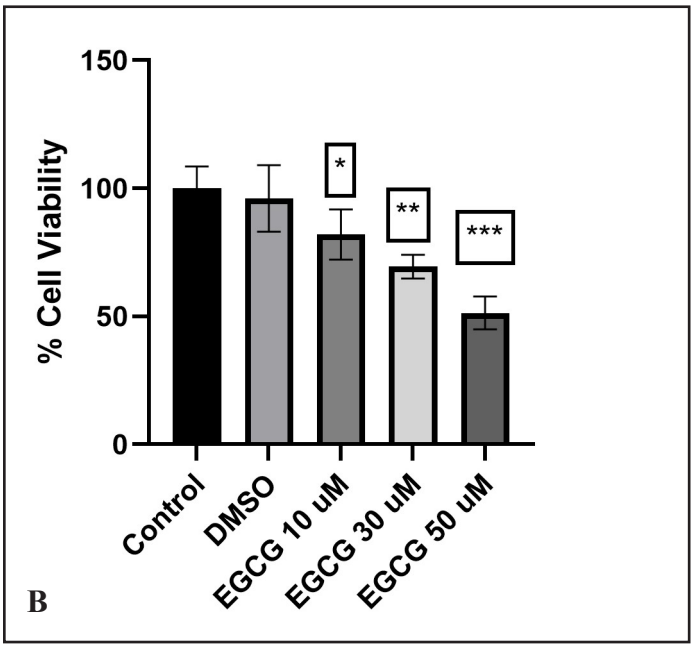

Figure 1-B. Evaluation of cell viability by MTT analysis for 48 hours in ACC-234 cells. Cells treated with Epigallocatechin Gallate (EGCG,10, 30 and 50 µM) and DMSO, dimethyl sulfoxide (1µ/ml). Data are representative of 6-8 separate experiments and values are given as mean ± SD. Statistical analysis was performed by two-way ANOVA and differences between groups were determined by Tukey's multiple comparison test. *, p<0.005, when compared with the control group and DMSO. **, p<0.001, when compared with the control, DMSO and EGCG 10 µM group at the same time period. ***, p<0.0001, when compared with other all group.

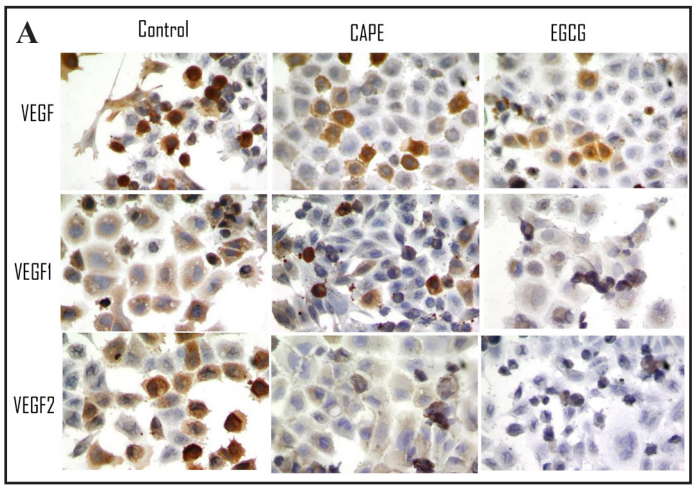

Figure 2.A. Representative immunofluorescent staining of Vascular endothelial growth factor (VEGF), Vascular endothelial growth factor receptor 1(VEGFR1) and Vascular endothelial growth factor receptor 2(VEGFR2) in ACC 234 cells treated with either, CAPE (150 µM) and EGCG (50µM). 20 X magnification. Incubation with 48 hours in the CAPE and EGCG group. Quantitation of VEGF, VEGFR1 and VEGFR2 immunohistochemistry staining in ACC 234 cells by ImageJ software.

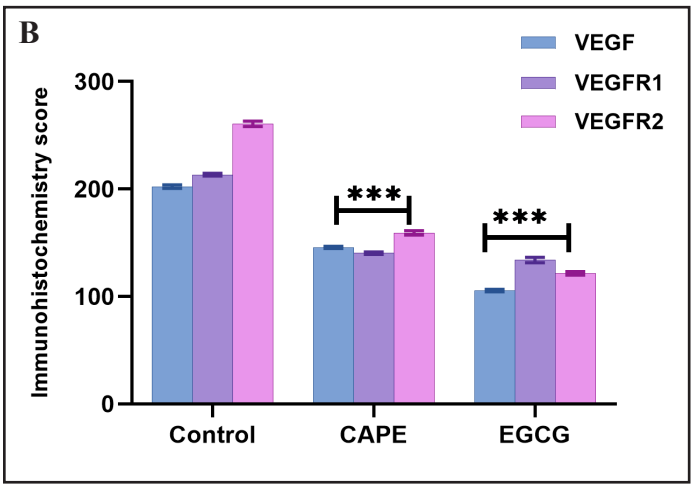

Figure 2-B. Data shown are representative of 8 separate measurements and values are given as mean ± SD. Statistical analysis was performed by one-way ANOVA and differences between groups were determined by Tukey's multiple comparisons analysis. *** $p<0.0001$, when compared control groups.

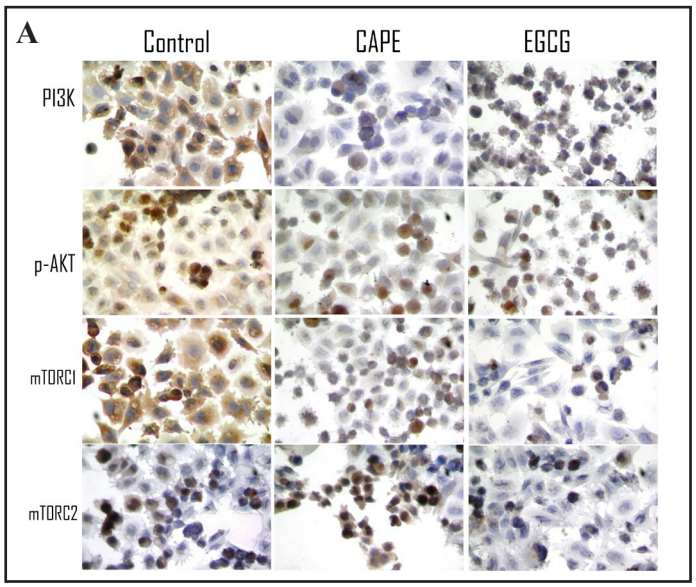

Figure 3-A. Representative immunofluorescent staining of phosphatidylinositol 3-kinase (PI3K), phospho (Ser473) protein kinase Akt (p-AKT) and mammalian target of rapamycin (p-mTOR) in ACC 234 cells treated with either, CAPE (150 µM) and EGCG (50µM). 20 X magnification. Incubation with 48 hours in the CAPE and EGCG group. Quantitation of PI3K, p-Akt, mTORC1 and mTORC2 immunohistochemistry staining in ACC 234 cells by ImageJ software.

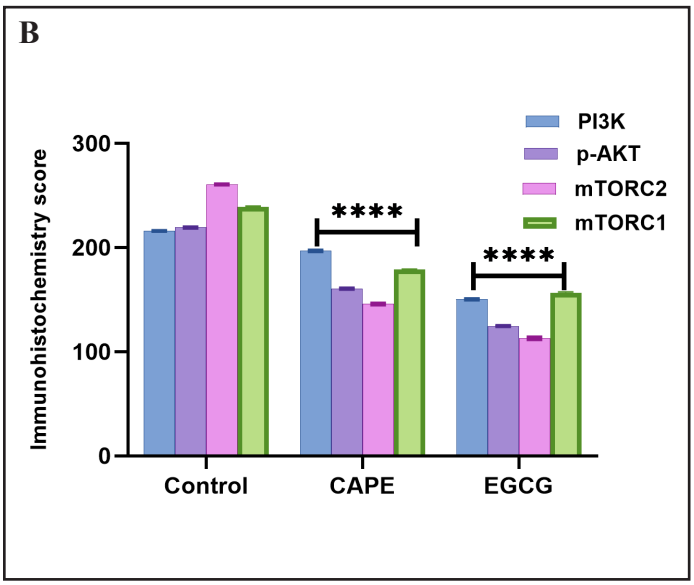

Figure 3-B. Data shown are representative of 8 separate measurements and values are given as mean ± SD. Statistical analysis was performed by one-way ANOVA and differences between groups were determined by Tukey's multiple comparisons analysis. *** $p<0.0001$, when compared control groups.



Figure 4. Binding Mode of the Top-Scoring Docking Model of CAPE with mTORC1.
This figure illustrates the binding configuration of the highest-affinity docking model for CAPE interacting with the mTORC1 protein. A) On the left, the complete 3D structure of mTORC1 is displayed, highlighting the CAPE binding site. B) The inset on the right zooms in on the critical binding interactions. CAPE (in the box) forms multiple interactions with critical residues of mTORC1, including LEU B150, TRP B173, ASN B148,LYS B142, GLU B177 and SER B145. Dark green dashed lines indicate hydrogen bonds, while light green dashed lines represent van der Waals interactions. Dark purple dashed lines indicate Pi-Sigma, while light purple dashed lines represent Pi-Alkyl interactions These interactions depict the most stable conformation of CAPE within the mTORC1 binding pocket, as determined by the docking analysis.

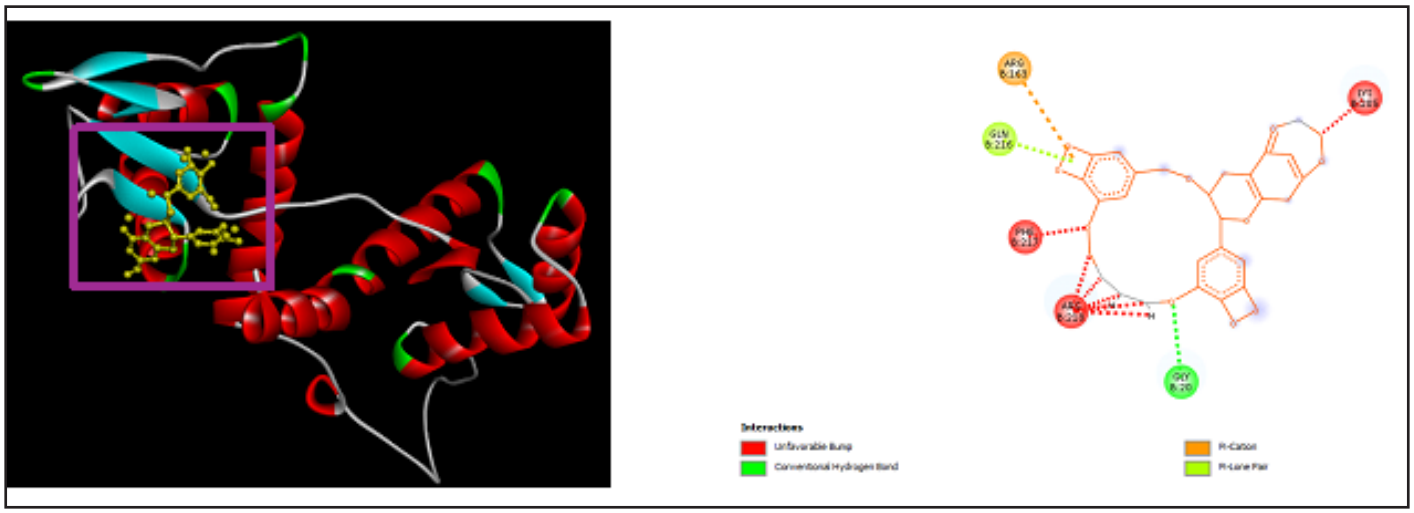

Figure 5. Binding Mode of the Top-Scoring Docking Model of EGCG with mTORC1. This figure illustrates the binding configuration of the highest-affinity docking model for EGCG interacting with the mTORC1 protein. **A)** On the left, the complete 3D structure of mTORC1 is displayed, highlighting the EGCG binding site. **B)** The inset on the right zooms in on the critical binding interactions. EGCG (in the box) forms multiple interactions with critical residues of mTORC1, including ARG B163, GLN B216, PHE B217,ARG B218,GLY B20 and LYS B205.Dark green dashed lines indicate hydrogen bonds, while light green dashed lines represent Pi-Lone pair interactions. Red dashed lines indicate unfavorable bump, while orange dashed lines represent Pi-Cation interactions These interactions depict the most stable conformation of EGCG within the mTORC1 binding pocket, as determined by the docking analysis.

Table 1. Binding Mode of the Top-Scoring Docking Model of CAPE with mTORC1.

Model	Affinity (kcal/mol)	dist from best mode (rmsd l.b.)	dist from best mode (rmsd u.b.)
1	-7.0	0.000	0.000
2	-6.6	20.503	22.178
3	-6.4	2.552	6.935
4	-6.2	17.444	19.987
5	-6.2	5.117	8.426
6	-6.1	20.299	21.291
7	-6.1	4.314	9.457
8	-6.0	1.875	6.756
9	-6.0	11.233	12.002
10	-5.9	10.752	13.690

Docking analysis of EGCG with mTORC1

Ten binding postures in all were produced, and Table 2 summarizes the docking analysis's findings. The initial binding model (ID:1) had the best docking score, ensuring a stable and defined binding interface, with a binding affinity of −5.8 kcal/mol and an RMSD of 0.000 for both lower bound (L.B.) and upper bound (U.B.). Docking values for the other binding models ranged from −5.6 kcal/mol to −5.3 kcal/mol. Figures 5-A and 5-B provide visual representations of the ligand-protein interactions for EGCG and mTORC1, including binding posture, critical hydrogen bonds, and van der Waals interactions.

DISCUSSION

Lung cancer (LC) is still a significant public health concern, especially in underdeveloped nations, despite recent significant advancements in treatment approaches. Our main goal in this work was to assess the in vitro anticancer potential of epigallocatechin gallate (EGCG) and caffeic acid phenethyl ester (CAPE) in LC. A further objective was to elucidate the underlying molecular mechanisms, with specific focus on the complex crosstalk between the PI3K/Akt/mTOR signaling cascade and VEGF-related pathways.

Table 2. Binding Mode of the Top-Scoring Docking Model of EGCG with mTORC1.

Model	Affinity (kcal/mol)	dist from best mode (rmsd l.b.)	dist from best mode (rmsd u.b.)
1	-5.8	0.000	0.000
2	-5.6	1.951	6.309
3	-5.6	2.683	5.825
4	-5.6	2.109	2.718
5	-5.5	3.602	6.990
6	-5.5	12.016	16.491
7	-5.5	3.197	6.610
8	-5.5	4.230	7.840
9	-5.3	10.709	14.767
10	-5.3	3.557	7.420

To achieve these goals, we chose the ACC 234 cell line as our experimental model. This cell line was carefully selected in light of its tumorigenic properties, which make it appropriate for possible application in in vivo research.

Natural therapeutic compounds are known to exert anticancer effects with a comparatively favorable and more tolerable therapeutic index. In this context, CAPE and EGCG were specifically chosen because of their documented bioactive properties and their reported use as potential monotherapeutic agents against lung cancer [23]. Considering CAPE and EGCG have some of those characteristics and have been utilized as possible monotherapeutic drugs against lung cancer, we specifically chose them to be used in our investigation [24, 25].

Previous in vivo studies have demonstrated that CAPE suppresses tumor growth, invasion, and metastasis, inhibits VEGF-stimulated neovascularization, and reduces vascular permeability. Additionally, in mouse models implanted with lung, colon, and melanoma cancer cells, CAPE has been demonstrated to restrict tumor growth and angiogenesis, mostly by inhibition of Cell growth is suppressed when intracellular PI3K/Akt/mTOR signaling is stimulated by VEGF [26]. Accumulating evidence indicates that CAPE exhibits antitumor activity across a wide range of human cancer cell types. Notably, CAPE administration has been shown to overcome cisplatin resistance in lung cancer by inducing G2/M cell cycle arrest and downregulating the PI3K/Akt signaling pathway. VEGF, a key angiogenic mediator,

functions as a potent endothelial cell–specific mitogen that drives pathological angiogenesis [27].

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen that plays a role in pathological endothelial activation during the angiogenesis phase. Recent findings in studies have shown that caffeic acid phenethyl ester (CAPE) actively suppresses tumor cell growth, invasion, and abnormal metastatic spread. CAPE has been shown to prevent VEGF-induced neovascularization by inhibiting plug formation in the vascular matrigel. It has also been shown to reduce vascular filtration in mouse skin capillaries in an in vivo study. Additionally, in C57BL/6 and BALB/c mice models injected with Lewis lung carcinoma, colon carcinoma, and melanoma cells, CAPE dramatically decreased tumor growth and angiogenesis. [28]. This study also showed that CAPE suppresses proliferation by inhibiting the activation of PI3K/Akt/mTOR, one of the intracellular signaling pathways via VEGF.

EGCG has been shown in numerous trials to have inhibitory effects on a variety of cancer types, including triple-negative breast cancer (TNBC). Experimental evidence indicates that EGCG induces cell cycle arrest, promotes apoptotic cell death, and markedly reduces cancer cell proliferation [12]. By activating signaling pathways including AMPK, boosting the effects of chemotherapeutic medications, and modifying autophagy, EGCG has been shown to limit the proliferation and invasion of cancer cells [14]. Previous studies have shown that EGCG induces apoptosis in human pancreatic and colon cancer cells by inhibiting activation of the PI3K/Akt signaling pathway [16].

VEGF, which is secreted by tumor cells, can stimulate cell proliferation and the growth of endothelial cells and form new blood vessel [29]. VEGF also participatesin the promotion of glycolysis in prostate cancer by activating HIF1αand NRP-1 [30]. Wei et al. [31] shown that via inhibiting glycolysis, EGCG is a potent combination partner of gemcitabine that slows the development of pancreatic cancer cells. This study showed that VEGF, VEGFR1, VEGFR2, PI3K, p-AKT, mTORC1 and mTORC2 were significantly decrease in EGCG-treated ACC 234 cells. Together, these data confirmed that EGCG regulates glycolysis in cancer cells through VEGF overexpression.

Activation of the VEGF-mediated PI3K/Akt/mTORC signaling pathway was evident in both the CAPE- and EGCG-treated groups. Notably, cell viability assays and immunohistochemical analyses consistently indicated that CAPE exerted a slightly greater inhibitory sensitivity compared to EGCG.

The phosphoinositide 3-kinase (PI3K)–Akt–mechanistic target of rapamycin (mTOR) signaling axis constitutes a master regulatory network governing a broad spectrum of fundamental cellular processes, including proliferation, survival, metabolic homeostasis, autophagy, and immune regulation. Owing to its pivotal role in sustaining oncogenic growth and cellular adaptability, mTOR has been firmly established as a strategically important therapeutic target, particularly in malignancies driven by genetic aberrations or metabolic dysregulation that result in persistent pathway hyperactivation. Compelling evidence from both in vitro and in vivo models indicates that rapamycin, a well-characterized immunosuppressive agent, potently attenuates mTOR signaling, thereby curtailing tumor cell growth and proliferative capacity. CAPE has been shown to have the highest MAPK protein binding capacity in HT29 colon cancer cells compared to other applied agents [32]. In MDA-MB-231 and A549 cell models, molecular docking analyses have revealed that EGCG exhibits strong binding affinity toward the active site of the PI3K kinase domain, a finding that is consistent with its reported ability to compete with ATP for binding and thereby inhibit kinase activity [33].

There is currently no published evidence addressing the molecular interactions between the mTORC1 complex and CAPE or EGCG in lung cancer cells. To the best of our knowledge, our findings represent the first experimental data to elucidate these potential binding dynamics and provide novel insights into this previously unexplored aspect of mTORC1 regulation.

Limitations

This study has some limitations that should be noted. First, the experiments were limited to a single lung cancer cell line under in vitro conditions, which may not fully reflect the complex in vivo tumor microenvironment. Second, the absence of non-cancerous (normal) cell line controls limits the evaluation of treatment selectivity and systemic cytotoxicity. Finally, while modulation of the PI3K/Akt/mTOR pathway was observed, further validation with advanced biochemical or structural assays is required. Future studies incorporating healthy human cell lines and in vivo models are warranted to enhance the translational relevance and safety profile of CAPE and EGCG.

CONCLUSIONS

In the lung cancer cell line, EGCG and CAPE demonstrated significant regulatory effects on cell proliferation and angiogenic processes through modulation of the VEGF and PI3K/Akt/mTOR signaling pathway. These findings underscore the therapeutic relevance of targeting this axis and highlight the potential contribution of EGCG- or CAPE-based strategies to the development of more effective treatment protocols aimed at disrupting proliferative and pro-angiogenic signaling in lung cancer.

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